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Poison in the Hiking Trail

Role of Ginger Extract on Methomyl

Highlights

Two-Dimensional Nucleation

Properties of Clerodane-Type Diterpenes

Discovering Thoughts, Inventing Future

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Poison in the Hiking Trail

By Alexis Brooks & A Bakarr Kanu

Abstract- An approach combining reverse-phase high-performance liquid chromatography (RP-HPLC) and electrospray ionization mass spectrometry (ESI-MS) was developed to analyze Urushiol congeners in poison ivy extract. The peak signatures detected in poison ivy were separated in 18 min at wavelengths 254 nm, 260 nm, and 280 nm with a gradient elution on the RP-HPLC system. The ESI-MS data confirmed the fragmentation patterns of six Urushiol congeners (C15:0-2 and C17:1-3) detected in the poison ivy extract. Recovery studies conducted with Urushiol (15:2) show recovery within ±2%, well within the recovery efficiency of ±15-20%. The validation data showed that the limit of detection (LOD) and limit of quantitation (LOQ) for Urushiol (15:2) was 0.29 ± 0.03 ppb and 0.97 ± 0.01 ppb, respectively, with a sensitivity of 0.110 ± 0.002 mAU ppb^{-1}. A standard addition calibration approach was used to quantify the Urushiol (15:2) content in the poison ivy extract and reveal one poison ivy leaf may contain 0.674 ± 0.025 mg/g of Urushiol (15:2).Our investigation demonstrates the quantitation of Urushiol congeners in complex mixtures. This same approach can be beneficial for analyzing other chemical components in food and different types of complex matrices.

Keywords: *reverse-phase high-performance liquid chromatography, electrospray ionization mass spectro-metry, urushiol, poison ivy.*

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Poison in the Hiking Trail

Alexis Brooks [α] & A Bakarr Kanu [σ]

Abstract- An approach combining reverse-phase high-performance liquid chromatography (RP-HPLC) and electrospray ionization mass spectrometry (ESI-MS) was developed to analyze Urushiol congeners in poison ivy extract. The peak signatures detected in poison ivy were separated in 18 min at wavelengths 254 nm, 260 nm, and 280 nm with a gradient elution on the RP-HPLC system. The ESI-MS data confirmed the fragmentation patterns of six Urushiol congeners (C15:0-2 and C17:1-3) detected in the poison ivy extract. Recovery studies conducted with Urushiol (15:2) show recovery within ±2%, well within the recovery efficiency of ±15-20%. The validation data showed that the limit of detection (LOD) and limit of quantitation (LOQ) for Urushiol (15:2) was 0.29 ± 0.03 ppb and 0.97 ± 0.01 ppb, respectively, with a sensitivity of 0.110 ± 0.002 mAU ppb^{-1}. A standard addition calibration approach was used to quantify the Urushiol (15:2) content in the poison ivy extract and reveal one poison ivy leaf may contain 0.674 ± 0.025 mg/g of Urushiol (15:2).Our investigation demonstrates the quantitation of Urushiol congeners in complex mixtures. This same approach can be beneficial for analyzing other chemical components in food and different types of complex matrices.

Keywords: reverse-phase high-performance liquid chromatography, electrospray ionization mass spectrometry, urushiol, poison ivy.

I. Introduction

Poison ivy (a plant in the family Anacardiaceae, specifically *Toxicodendron radicans*) is well-known for causing bothersomerash and intense itching in sensitive individuals [1-2]. The allergen in the plant causing the irritation, blistering, and inflammation has been documented as Urushiol (1, 2-benzenediol, 3-pentadactyl-). Touching the stem, root, or leaves of poison ivy results in direct skin contact with Urushiol oil, which causes itching. Urushiol is a lipophilic catechol with a 15 or 17 alkyl side chain either fully saturated or has 1-3 double bonds [3-4].A naming convention is usually adopted depending on the number of carbons and double bonds on the side chain. For example, a 15:0 indicates 15 carbon atoms with zero double bonds, and a 17:3 indicates 17 carbon atoms with three double bonds, etc. Structural activity studies have previously reported that the catechol ring and the side branching may be required for Urushiols' allergenicity. For example, the dimethylether derivative is not allergenic; however, Urushiol congeners with a higher degree of unsaturationin the side chain have higher allergenic potential[5-6]. Sensitivity to Urushiol can develop anytime, and almost all parts of the human body are sensitive to the chemical.

High-performance liquid chromatography (HPLC) has a long history of operation in the reverse phase mode using a C_{18} column and detectors such as UV, DAD, UV-DAD, fluorescence, or electrochemical detectors[7-11].In cases where good separation is required, especially for separation and purification in natural product samples, techniques for the preparation of stationary phases may be required. Some investigations have utilized Urushiol as a stationary phase in an HPLC column to demonstrate good separation performance for studying natural product extracts [12]. Other analyses have used HPLC to separate, identify, and quantify lacquer saps containing catechol lipids [13].An earlier approach by Yamauchi et al. has previously resolved the ten components in Japanese lacquer Urushiol by combining HPLC gel columns that utilize differences in the degree of unsaturation [14].

For identification purposes, mass spectrometry (MS) has long been used to decode organic structures[15]. MS has been the most powerful detector for chromatographic systems, offering qualitative and quantitative information, providing high sensitivity, and distinguishing different substances with the same retention time. Liquid chromatography-mass spectrometry (LC-MS) can be a critical tool for guarding the safety of our food supply by monitoring toxic substances such as pesticide residues[16-17]. The literature shows that the first chromatographic MS Urushiol analysis was a gas chromatography MS (GC-MS) analysis reported in 1975[18]. Draper et al. have employed $HPLC/MS^2$ to determine Urushiol congeners[19]. Urushiol was also identified in poison ivy without any sample preparation using leaf spray MS[20]. MALDI mass spectrometry imaging (MALDI-MSI) was employed to analyze Urushiol in poison ivy stems. The result from the study indicates that the in situ localization of the Urushiol congeners with 15-carbon side chains is distinctly different from those with 17-carbon side chains in the stem tissue[21]. Several other studies have investigated the HPLC-MS approach for analyzing Urushiol in different extracts[22-25].

Our study aims to develop, optimize, and validate an RP-HPLC method for determining Urushiol in poison ivy extract. We also aim to utilize ESI-MS to confirm the fragmentation patterns of Urushiol detected in the poison ivy extract. Our approach will be helpful for quality control authorities seeking to quantify active compounds in nutrition products.

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II. Experimental Section

a) Materials and Reagent

Urushiol (15:2)CAS 83258-37-1 was purchased from MilliporeSigma (Atlanta, GA). HPLC water, LC-MS grade acetonitrile and methanol, and LC-MS optima formic acid were also purchased from Fisher Scientific LLC. The ESI tuning solution for the Advion CMS mass spectrometry was purchased from Agilent Technologies (Santa Clara, CA).

b) Poison Ivy Sample Pretreatment

Dried poison ivy on the train path behind the Wilveria Bass Atkinson Science Building on the Winston-Salem State University campus was ground in a mortar and pestle for approximately one minute, and ~2 g was transferred into a clean 250 mL KIMAX Kimble glass bottle. The sample was soaked in 5 mL of methanol for three days. After the mixture was filtered with filter paper, all solvent was dried with a roto evaporator. The dried extract was weighed and dissolved in 1 mL of methanol. The resulting solutions were then used to prepare 10ppm of the sample. Solid-phase extraction (SPE) was used to clean the sample before analyzing it on an Agilent 1260 HPLC and Advionexpression^{L} CMS mass spectrometry (MS).

c) Urushiol Stock Solutions & Method Validation Studies

Stock solutions of the Urushiol (15:2) were prepared to 1000 ppm by accurately weighing 1 mg of analyte and dissolving in 1 mL ethanol. Subsequent serial dilutions from the stock using 9:1 CH_3OH:H_2O were prepared between concentrations of 0.05 ppm to 150 ppm. Calibration studies were conducted by injecting three replicates of each concentration on the Agilent 1260 HPLC. Data from the calibration studies were used to determine method validation parameters. The method validation studies were conducted at 280 nm wavelength. A standard addition calibration curve corresponding to stock solutions of 0.1 to 150 ppm was generatedto determine the content of Urushiol (15:2) in poison ivy extract.

d) Agilent 1260 LC & Advion CMS MS Instrumental Conditions

We analyze the poison ivyextract dissolved in methanol on an Agilent 1260 HPLC-DAD instrument and an Advion expression^{L} CMS MS. The experimental operating parameters developed on both instrumentations were published elsewhere[7].

HPLC conditions: Freshly prepared mobile phases (Solvent A; 0.1% Formic Acid in Water, Solvent B; 0.1% Formic Acid in Acetonitrile) were placed on the instrument weekly. The injection volume is 5µL, and the column temperature is 45°C. The mobile phase flow rate is 0.400 mL/min. Hold at 90% mobile phase A and 10% mobile phase B for 7.00 min, then ramp to 60% B over 2.00 min, ramp to 95% B over the next 3.10 min, and hold at 95% B for 0.01 min. Return to 90% mobile phase A and 10% mobile phase B over 2.89 min and hold for 3.0 min for re-equilibration. The total gradient program is 18.00 min long.

CMS MS: scan mode; CMS range, start m/z; 10.0 Da, end m/z; 600.0 Da, scan time; 1,000.0 ms, scan delay; 100 µs, delta background start time; 0, delta background end time; 10.0

III. Results and Discussions

a) Agilent 1260 HPLC-DAD

The high-performance liquid chromatography (HPLC) process involves forcing a high pressure through a closed column containing fine particles, resulting in a high-resolution separation[26-28]. Two advantages consistently reported in the literature for HPLC are increased sensitivity and analysis without derivatization[29-30]. The Agilent 1260 HPLC used in these studies consisted of an auto sampler, a solvent delivery system, a high-pressure chromatography column, and a DAD detector[31]. The poison ivy sample was separated with a total run time of 18 min (including 1 min equilibration time), and the peaks were well resolved. Figure 1 shows example chromatograms of a poison ivy sample collected at 254, 260, and 280 nm, respectively. At 254 nm, the peaks detected distinct from the blank occurred at retention times, 4.111 ± 0.101, 6.624 ± 0.036, 6.771 ± 0.024, 7.051 ± 0.007, 7.164 ± 0.027, 7.564 ± 0.057, 12.384 ± 0.045, and 13.904 ± 0.017 minutes. At 260 nm, the peaks detected distinct from the blank occurred at retention times, 4.111 ± 0.098, 6.598 ± 0.049, 6.791 ± 0.044, 7.051 ± 0.011, 7.191 ± 0.032, 7.584 ± 0.052, 12.391 ± 0.067, and 13.911 ± 0.022 minutes. At 280 nm, the peaks detected distinct from the blank occurred at retention times, 4.011 ± 0.077, 6.791 ± 0.033, 7.057 ± 0.027, 7.191 ± 0.032, and 13.918 ± 0.022 minutes. At 280 nm, small signature peaks were seen between 6.138 and 6.558 minutes. That was due to the enhancement of the signal at 280 nm. The chromatographic behavior at the three wavelength studies was different. Above 6 minutes, the chromatographic baseline at 254 nm and 260 nm drifts to higher absorbance. That could be due to the acetonitrile contributing a higher absorbance at the lower wavelength of 254 nm and 260 nm during the gradient run. When the system returns to equilibration, the absorbance of acetonitrile drops back to the baseline. The same effect is seen at 260 nm but to a much lesser extent. At 280 nm, this effect disappears. Figure 1 shows that Urushiol gives a better response at the high wavelength of 280 nm. Most poison ivy containsan oil called Urushiol responsible for the allergic reaction to the plant. Using the Urushiol (15:2) pure standard, we confirmed that the peak at 13.91-13.92 minutes was the Urushiol (15:2) response in the poison ivy extract. With all peaks detected in the poison ivy

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extract fully resolved, the RP-HPLC with acetonitrile as the solvent used in this investigation demonstrated selectivity on conventional C_{18} columns.

b) Advion Expression CMS MS

During the CMS MS studies, the instrument was attentively tuned daily in positive and negative ion detection modes. Masses identified for Urushiol-congenerin the poison ivy extracted with methanol are shown in Table 1.Figure 2 shows the structure of Urushiol (15:0, 15:1, and 15:2), demonstrating possible fragmentation sites. The pure standard we purchased from Millipore Sigma was Urushiol (15:2). The MS data shows several m/z detected for Urushiol (15:2), as shown in Table 1. The most critical masses that correspond to fragmentation from Urushiol (15:2) in the positive ion mode were 317.3 and 339.3, corresponding to the $[M + H]^+$($C_{21}H_{33}O_2^+$) and $[M + Na]^+$ ($C_{21}H_{32}O_2Na^+$) ions, respectively. Other fragmented ions include 123.4 ($C_7H_7O_2^+$ or $C_9H_{15}^+$), 137.2 ($C_8H_9O_2^+$ or $C_{10}H_{17}^+$), 151.1 ($C_9H_{11}O_2^+$ or $C_{11}H_{19}^+$), 165.3 ($C_{10}H_{13}O_2^+$ or $C_{12}H_{21}^+$), 179.3 ($C_{11}H_{15}O_2^+$), 193.3 ($C_{12}H_{17}O_2^+$), 233.3 ($C_{15}H_{21}O_2^+$), 247.1 ($C_{16}H_{23}O_2^+$), 273.1 ($C_{18}H_{25}O_2^+$), 287.3 ($C_{19}H_{27}O_2^+$), 299.3 ($C_{21}H_{31}O^+$) and 301.2 ($C_{20}H_{29}O_2^+$). The fragment observed at m/z 255.1 and 269.5 was attributed to fragments at 287.3 ($C_{19}H_{27}O_2^+$ - H_2O) and 273.1 ($C_{18}H_{25}O_2^+$ - H_2O) losing H_2O. The base peak in the positive ion mode occurred at m/z 397.3, an unnamed peak. In the negative ion mode, the $[M – H]^-$ ion occurred at m/z 315.2 ($C_{21}H_{31}O_2^-$).Four other fragment ions of Urushiol (15:2)were observed in the negative ion mode, and these occurred at m/z 109.3 ($C_6H_5O_2^-$ or $C_8H_{13}^-$), 255.1 ($C_{18}H_{25}O_2^-$ - H_2O), 269.3 ($C_{19}H_{27}O_2^-$- H_2O), and 299.3 ($C_{21}H_{31}O^-$). The base peak in the negative ion mode was the $C_{21}H_{31}O_2^-$, at m/z 315.2. Figure 3 shows the mass spectra of Urushiol (15:2)pure standard in the positive ion mode. In Figure 3(a), we display the full scan mode spectra of the compounds, and in Figures 3(b and C), we display two zoomed-in selected scans that show how the fragments were mined in the data. Figure 4 shows the mass spectra of Urushiol pure standard in the negative ion mode. Identifying these fragments in Urushiol's (15:2) pure standard enabled us to elucidate poison ivy's full scan mode.

Figure 5(a) shows poison ivy's full scan mode mass spectra, whereas Figures 5(b and c) show selected scans of two data-mined spectra in the positive ion mode. Figure 6 shows the mass spectra of poison ivy in the negative ion mode. Note that the poison ivy HPLC chromatogram reveals several chromatographic signatures. This investigation focused on identifying the signature of Urushiol in poison ivy. Previous research reported seven Urushiol congeners in poison ivy ranging from C15:0-3 and C17:1-3 [21]. We thus set out to mine the full scan mode mass spectra to reveal fragments that may be identical to the fragments identified to Urushiol congeners previously reported in poison ivy. In the positive ion mode of poison ivy, a fragment at m/z 321.5 and 343.2 corresponded to the $[M+H]^+$ ($C_{21}H_{37}O_2^+$) and $[M+Na]^+$ ($C_{21}H_{36}O_2Na^+$) ions, respectively, of Urushiol (15:0). The positive ion mode mass spectra reveal other fragments of 137.5 ($C_8H_9O_2^+$), 165.4 ($C_{10}H_{13}O_2^+$), 169.4 ($C_{12}H_{25}^+$), 179.1 ($C_{11}H_{15}O_2^+$), 183.3 ($C_{13}H_{27}^+$), 197.6 ($C_{14}H_{29}^+$), 211.2 ($C_{15}H_{31}^+$), 221.4 ($C_{14}H_{21}O_2^+$), 235.5 ($C_{15}H_{23}O_2^+$), 249.4 ($C_{16}H_{25}O_2^+$), 263.5 ($C_{17}H_{27}O_2^+$), 277.5 ($C_{18}H_{29}O_2^+$), 291.4 ($C_{19}H_{31}O_2^+$), 303.4 ($C_{21}H_{35}O^+$), and 305.5 ($C_{20}H_{33}O_2^+$). A fragment at m/z 273.5 was attributed to m/z 291.4 ($C_{19}H_{31}O_2^+$ - H_2O) losing H_2O. The base peak in the positive ion mode occurred at m/z 104.0, an unnamed peak. In the negative ion mode, the $[M – H]^-$ ion was small and occurred at m/z 319.6 ($C_{21}H_{35}O_2^-$).Four other fragment ions were observed in the negative ion mode of poison ivy, and these occurred at m/z 113.5 ($C_8H_{17}^-$), 182.9 ($C_{13}H_{27}^-$), 291.0 ($C_{19}H_{31}O_2^-$), and 303.3 ($C_{21}H_{35}O^-$). The base peak in the negative ion mode was also the $C_{13}H_{27}$, at m/z 182.9. All these fragments confirmed the presence of Urushiol (15:0) congener in the poison ivy studied.

Mass and fragments for Urushiol (15:1) congener was also seen in the full scan mode mass spectra for poison ivy. In the positive ion mode of poison ivy, a fragment at m/z 319.1 and 341.4 corresponded to the $[M + H]^+$ ($C_{21}H_{35}O_2^+$) and $[M + Na]^+$ ($C_{21}H_{34}O_2Na^+$) ions, respectively, of Urushiol (15:1). The positive ion mode mass spectra reveal other fragments of 123.2 ($C_7H_7O_2^+$), 137.5 ($C_8H_9O_2^+$), 151.2 ($C_9H_{11}O_2^+$), 165.4 ($C_{10}H_{13}O_2^+$), 179.1 ($C_{11}H_{15}O_2^+$), 193.2 ($C_{12}H_{17}O_2^+$), 195.3 ($C_{14}H_{27}^+$), 233.3 ($C_{15}H_{21}O_2^+$), 247.9 ($C_{16}H_{23}O_2^+$), 261.2 ($C_{17}H_{25}O_2^+$), 275.5 ($C_{18}H_{27}O_2^+$), 289.5 ($C_{19}H_{29}O_2^+$), 301.0 ($C_{21}H_{33}O^+$), and 303.2 ($C_{20}H_{31}O_2^+$). A fragment at m/z 229.2 and 215.1 was attributed to fragments at m/z 247.9 ($C_{16}H_{23}O_2^+$ - H_2O) and 233.3 ($C_{15}H_{21}O_2^+$ - H_2O) losing H_2O. In the negative ion mode, the $[M – H]^-$ ion occurred at m/z 317.1 ($C_{21}H_{33}O_2^-$).Two other fragment ions were observed in the poison ivy's negative ion mode spectra, which occurred at m/z 109.2 ($C_6H_5O_2^-$), and 111.2 ($C_8H_{15}^-$). These fragments correspond to fragmentation patterns found in Urushiol (15:1) congener.

The MS of poison ivy also indicates a strong presence of the Urushiol (15:2) congener. In the positive ion mode of poison ivy, a fragment at m/z 317.5 and 339.1 corresponded to the $[M + H]^+$ ($C_{21}H_{33}O_2^+$) and $[M + Na]^+$ ($C_{21}H_{32}O_2Na^+$) ions, respectively, of Urushiol (15:2). Other fragmented ions include 123.2 ($C_7H_7O_2^+$ or $C_9H_{15}^+$), 137.5 ($C_8H_9O_2^+$ or $C_{10}H_{17}^+$), 151.2 ($C_9H_{11}O_2^+$ or $C_{11}H_{19}^+$), 165.4 ($C_{10}H_{13}O_2^+$ or $C_{12}H_{21}^+$), 179.1 ($C_{11}H_{15}O_2^+$), 193.2 ($C_{12}H_{17}O_2^+$), 233.3 ($C_{15}H_{21}O_2^+$), 247.1 ($C_{16}H_{23}O_2^+$), 273.2 ($C_{18}H_{25}O_2^+$), 287.2 ($C_{19}H_{27}O_2^+$), 299.3 ($C_{21}H_{31}O^+$) and 301.0 ($C_{20}H_{29}O_2^+$). The fragment observed at m/z 255.2 and 269.2 was attributed to fragments at 287.2 ($C_{19}H_{27}O_2^+$ - H_2O) and 273.2

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($C_{18}H_{25}O_2^+$ - H_2O) losing H_2O. In the negative ion mode, the $[M - H]^-$ ion occurred at m/z 315.2 ($C_{21}H_{31}O_2^-$).Four other fragment ions of Urushiol (15:2) congener in poison ivy was observed in the negative ion mode, and these occurred at m/z 109.2 ($C_6H_5O_2^-$ or $C_8H_{13}^-$), 255.4 ($C_{18}H_{25}O_2^-$ - H_2O), 269.3 ($C_{19}H_{27}O_2^-$- H_2O), and 299.3 ($C_{21}H_{31}O^-$). From all the fragment signatures identified compared to the pure Urushiol (15:2) standard, we can conclude that the identity of the peak in the HPLC profile between 13.91-13.92 minutes was Urushiol (15:2), one of the congeners responsible for the itching behavior of poison ivy.

Few signatures were observed in the MS that could be assigned to Urushiol (15:3) congener. However, many features exist in the MS, including the $[M + H]^+$ and $[M + Na]^+$, that could confirm the presence of Urushiol (17:1-3) congeners.

c) Method Validation Studies (Agilent 1260 HPLC-DAD)

The Urushiol (15:2) was purchased as a 10 mg solid. Method validation studies typically utilize calibrations involving blanks and known standard concentration preparation. In this investigation, the blank was a solution containing all reagents and solvents used in the analysis with no deliberate added Urushiol (15:2). The blank used in this investigation for preparing all samples has the following ratio: 90:10 methanol: DI H_2O. We initially conducted percent recovery studies to ensure standards were being prepared accurately. A 2 ppm unspiked sample of Urushiol (15:2) was used to design the experiment and calculate standard recoveries. Spikes of 5 ppm (STD-1), 10 ppm (STD-2), 20 ppm (STD-3), and 150 ppm (STD-4) were added to each 2 ppm unspiked sample. The calibration study is shown in Table 2. Triplicate measurements were recorded for each peak area indicated in Table 2; thus, the data shown is the average of the three measurements. The percent recovery for each spiked sample was calculated using eq. 1.

$$\%\ Urushiol\ (15{:}2) recovery = \frac{Peak\ area_{spiked\ \ sample} - Peak\ area_{unspiked\ \ sample}}{Peak\ area_{added}} \times 100\% \quad (1)$$

The data shows an excellent recovery of within ±2% was obtained for each prepared concentration spiked on a 2 ppm Urushiol (15:2) sample, indicating that samples were prepared well and the instrument was functioning correctly.

Table 3 summarizes the calibration response data for Urushiol (15:2) investigated at 280 nm. The calibration plot enables us to determine slope, intercept, correlation coefficient (R^2) values, the limit of detection (LOD), and the limit of quantitation (LOD). We determine the LOD and LOQ by injecting replicate runs of the minimum detectable concentration of Urushiol. Each of the minimum Urushiol concentrations was discernable from the instrument noise. The LOD and LOQ were reported at 0.29 ± 0.03 ppb and 0.97 ± 0.01 ppb, respectively, with a sensitivity of 0.110 ± 0.002 mAU ppb^{-1}. The R^2 value was 0.9998. (see *Table 3*).

The standard addition calibration method was applied to the Urushiol detected in the poison ivy methanol extract. The total content of Urushiol (15:2) detected in ~ 2 g of poison ivy was 1.55 ± 0.03 mg/g of sample. One leaf of typical poison ivy weighs 0.869 g. This weight of poison ivy leaf is expected to contain 0.674 ± 0.025 mg/g of Urushiol (15:2).

IV. Conclusions

We report an improved RP-HPLC method for determining Urushiol (15:2) (1,2-benzenediol, 3-pentadactyl-) in poison ivy extract using a Luna 3u C_{18} column. The HPLC chromatogram revealed other unidentified signature peaks. The mass spectra data show most of the fragmentation patterns of the Urushiol detected in the poison ivy extract. The validation indicates that the HPLC method is repeatable, reproducible, and sensitive. This method showed a successful optimization and validation, and Urushiol can be determined in the matrix of the poison ivy extract using the standard addition calibration method. The approach presents several advantages, including separation, identification, and improved chromate-graphic efficiency. It further shows the quantitation of Urushiol in complex mixtures. This same approach can be beneficial for analyzing other chemical components in food and different complex matrices.

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Table 1: Summary of significant CMS mass spectral characteristics of components found in Urushiol (15:2) and poison ivy [Urushiol (15:0) and Urushiol (15:2)]. The studied scan range was 0-600 m/z.

Urushiol Pure Standard [15:2] (MeOH)	
(+) m/z identified on Advion CMS MS	123.4, 137.2, 151.1, 165.3, 179.3, 193.3, 233.3, 247.1, 255.1, 269.5, 273.1, 287.3, 299.3, 301.2, 317.3, 339.3
(-) m/z identified on Advion CMS MS	109.3, 255.1, 269.3, 299.3, 315.2
Poison Ivy (MeOH), m/z identified for Urushiol 15:0	
(+) m/z identified on Advion CMS MS	137.5, 165.4, 169.4, 179.1, 183.3, 197.6, 211.2, 221.4, 235.5, 249.4, 263.3, 273.5, 277.5, 291.4, 303.4, 305.5, 321.4, 343.2
(-) m/z identified on Advion CMS MS	113.5, 182.9, 291.0, 303.3, 319.6
Poison Ivy (MeOH), m/z identified for Urushiol 15:1	
(+) m/z identified on Advion CMS MS	123.2, 137.5, 151.2, 165.4, 179.1, 193.2, 195.3, 215.1, 229.2, 233.3, 247.9, 261.2, 275.5, 289.5, 301.0, 303.2, 319.1, 341.4
(-) m/z identified on Advion CMS MS	109.2, 111.2, 317.1
Poison Ivy (MeOH), m/z identified for Urushiol 15:2	
(+) m/z identified on Advion CMS MS	123.2, 137.5, 151.2, 165.4, 179.1, 193.2, 233.3, 247.1, 255.2, 269.2, 273.2, 287.2, 299.3, 301.0, 317.5, 339.1
(-) m/z identified on Advion CMS MS	109.2, 255.4, 269.3, 299.3, 315.2

Table 2: Summary of recovery studies for Urushiol (15:2) pure standard investigated using the Agilent 1260 HPLC-DAD.

Standard Name	Peak Area[a] (unspikedsample)[b]	Peak Area (added)	Peak Area (spiked sample)	% Recovery[c]
Blank	0	0	0	NA[d]
STD-1	16.9968	42.1167	58.2645	98.0
STD-2	17.1431	82.4076	98.9049	99.2
STD-3	17.3241	254.8777	271.0746	99.6
STD-4	16.8142	1682.7880	1700.0839	100.0

[a]*Peak areas are in mAU.*
[b]*unspiked sample = 2 ppm.*
[c]*% Recovery calculated using eq. 1.*
[d]*Not applicable.*
STD-1; spiked with 5 ppm Urushiol (15:2), STD-2; spiked with 10 ppm Urushiol (15:2), STD-3; spiked with 20 ppm Urushiol (15:2), STD-4; spiked with 150 ppm Urushiol (15:2).

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Table 3: Summary of method validation parameters for Urushiol (15:2) pure standard investigated using the Agilent 1260 HPLC-DAD.

Analyte	B_1/ppm	B_o	R^2	LOD/ppb	LOQ/ppb
Urushiol	0.110 ± 0.002	0.493 ± 0.003	0.9998	0.29 ± 0.03	0.97 ± 0.01

The following equation gives the calibration summary for the Urushiol response:

$$Peak\ area\ response = B_o(mAU) + B_1(mAU * ppm^{-1}) \times [concentration](ppm)$$

where B_o is the intercept or noise, and B_1 is the sensitivity or slope.

HPLC Solvent A: 0.1% Formic Acid/Water
HPLC Solvent B: 0.1% Formic Acid/ACN
Extraction solvent: Methanol
Sample: Poison Ivy
DAD: 254 nm, 260 nm, 280 nm
Column: Luna 3µ C18(2) 100A (size: 100 × 2.0 mm)

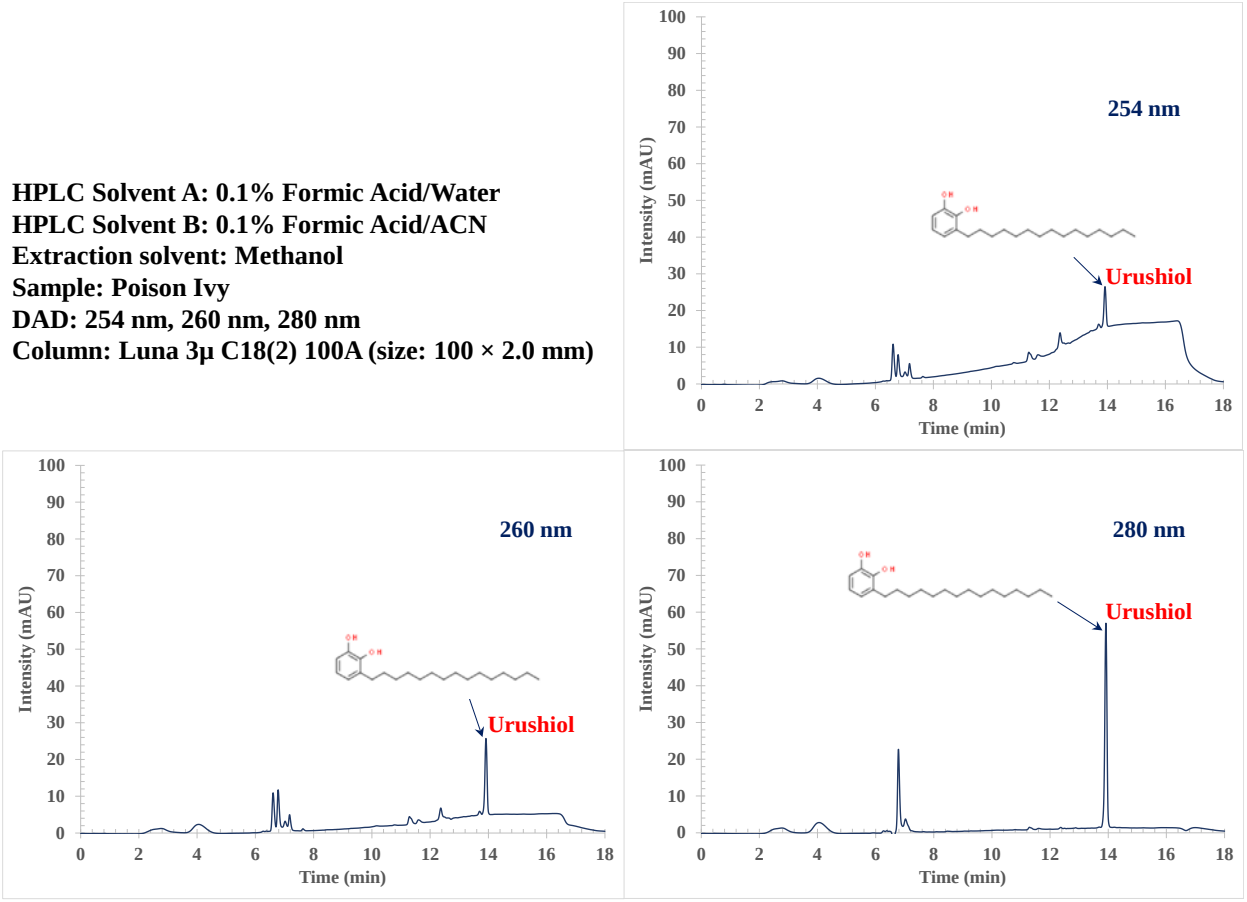
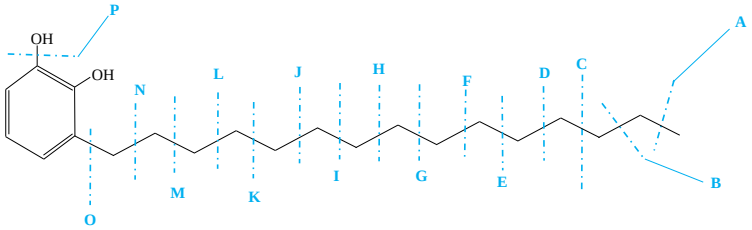


Figure 1: Example RP-HPLC chromatographic extract from poison ivy at 254 nm, 260 nm, and 280 nm wavelengths. The Urushiol (320.51 Da) peak was confirmed with a pure standard. We could see that the acetonitrile contributes a higher absorbance at the lower wavelength of 254 nm during the gradient run. When the system returns to equilibration, the absorbance of acetonitrile drops back to the baseline. The same effect is seen at 260 nm but to a much lesser extent. At 280 nm, this effect disappears. The Urushiol gives a better response at the high wavelength of 280 nm.

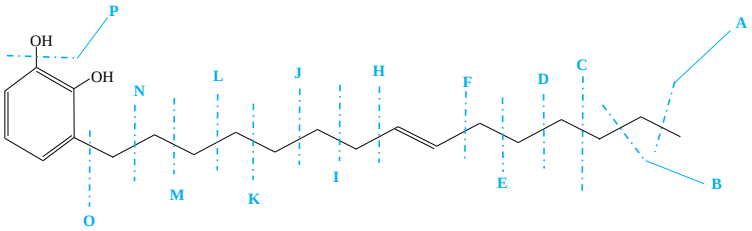
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Urushiol (15:0) [1,2-benzenediol, 3-pentadecyl-] ($C_{21}H_{36}O_2$, 320.51 g/mole)



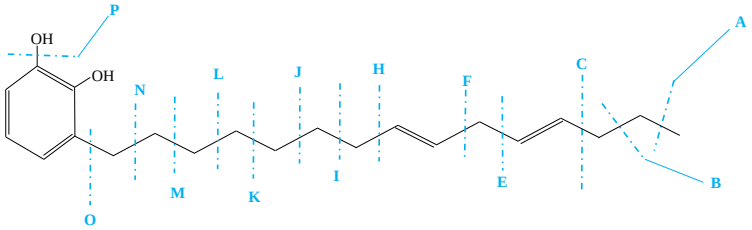
A = $CH_3^{\oplus}$ - 15.1 or $C_{20}H_{33}O_2^{\oplus}$ - 305.4
B = $C_2H_5^{\oplus}$ - 29.1 or $C_{19}H_{31}O_2^{\oplus}$ - 291.4
C = $C_3H_7^{\oplus}$ - 43.1 or $C_{18}H_{29}O_2^{\oplus}$ - 277.4
D = $C_4H_9^{\oplus}$ - 57.1 or $C_{17}H_{27}O_2^{\oplus}$ - 263.4
E = $C_5H_{11}^{\oplus}$ - 71.1 or $C_{16}H_{25}O_2^{\oplus}$ - 249.4
F = $C_6H_{13}^{\oplus}$ - 85.1 or $C_{15}H_{23}O_2^{\oplus}$ - 235.4
G = $C_7H_{15}^{\oplus}$ - 99.1 or $C_{14}H_{21}O_2^{\oplus}$ - 221.4
H = $C_8H_{17}^{\oplus}$ - 113.1 or $C_{13}H_{19}O_2^{\oplus}$ - 207.4
I = $C_9H_{19}^{\oplus}$ - 127.1 or $C_{12}H_{17}O_2^{\oplus}$ - 193.4
J = $C_{10}H_{21}^{\oplus}$ - 141.1 or $C_{11}H_{15}O_2^{\oplus}$ - 179.4
K = $C_{11}H_{23}^{\oplus}$ - 155.1 or $C_{10}H_{13}O_2^{\oplus}$ - 165.4
L = $C_{12}H_{25}^{\oplus}$ - 169.1 or $C_9H_{11}O_2^{\oplus}$ - 151.4
M = $C_{13}H_{27}^{\oplus}$ - 183.1 or $C_8H_9O_2^{\oplus}$ - 137.4
N = $C_{14}H_{29}^{\oplus}$ - 197.1 or $C_7H_7O_2^{\oplus}$ - 123.4
O = $C_{15}H_{31}^{\oplus}$ - 211.1 or $C_6H_5O_2^{\oplus}$ - 109.4
P = $HO^{\oplus}$ - 17.1 or $C_{21}H_{35}O^{\oplus}$ - 303.4

Urushiol (15:1) [3-(8Z-pentadecyl)-1,2-benzenediol] ($C_{21}H_{34}O_2$, 318.26 g/mole)



A = $CH_3^{\oplus}$ - 15.1 or $C_{20}H_{31}O_2^{\oplus}$ - 303.3
B = $C_2H_5^{\oplus}$ - 29.1 or $C_{19}H_{29}O_2^{\oplus}$ - 289.3
C = $C_3H_7^{\oplus}$ - 43.1 or $C_{18}H_{27}O_2^{\oplus}$ - 275.3
D = $C_4H_9^{\oplus}$ - 57.1 or $C_{17}H_{25}O_2^{\oplus}$ - 261.4
E = $C_5H_{11}^{\oplus}$ - 71.1 or $C_{16}H_{23}O_2^{\oplus}$ - 247.3
F = $C_6H_{13}^{\oplus}$ - 85.1 or $C_{15}H_{21}O_2^{\oplus}$ - 233.3
H = $C_8H_{15}^{\oplus}$ - 111.1 or $C_{13}H_{19}O_2^{\oplus}$ - 207.3
I = $C_9H_{17}^{\oplus}$ - 121.1 or $C_{12}H_{17}O_2^{\oplus}$ - 193.3
J = $C_{10}H_{19}^{\oplus}$ - 139.1 or $C_{11}H_{15}O_2^{\oplus}$ - 179.3
K = $C_{11}H_{21}^{\oplus}$ - 153.1 or $C_{10}H_{13}O_2^{\oplus}$ - 165.3
L = $C_{12}H_{23}^{\oplus}$ - 167.1 or $C_9H_{11}O_2^{\oplus}$ - 151.3
M = $C_{13}H_{25}^{\oplus}$ - 181.1 or $C_8H_9O_2^{\oplus}$ - 137.3
N = $C_{14}H_{27}^{\oplus}$ - 195.1 or $C_7H_7O_2^{\oplus}$ - 123.3
O = $C_{15}H_{29}^{\oplus}$ - 209.1 or $C_6H_5O_2^{\oplus}$ - 109.3
P = $HO^{\oplus}$ - 17.1 or $C_{21}H_{33}O^{\oplus}$ - 301.3

Urushiol (15:2) [3-(8Z-11Z-pentadecyl)-1,2-benzenediol] ($C_{21}H_{32}O_2$, 316.48 g/mole)



A = $CH_3^{\oplus}$ - 15.1 or $C_{20}H_{29}O_2^{\oplus}$ - 301.3
B = $C_2H_5^{\oplus}$ - 29.1 or $C_{19}H_{27}O_2^{\oplus}$ - 287.3
C = $C_3H_7^{\oplus}$ - 43.1 or $C_{18}H_{25}O_2^{\oplus}$ - 273.3
E = $C_5H_9^{\oplus}$ - 69.1 or $C_{16}H_{23}O_2^{\oplus}$ - 247.3
F = $C_6H_{11}^{\oplus}$ - 83.1 or $C_{15}H_{21}O_2^{\oplus}$ - 233.3
H = $C_8H_{13}^{\oplus}$ - 109.1 or $C_{13}H_{19}O_2^{\oplus}$ - 207.3
I = $C_9H_{15}^{\oplus}$ - 123.1 or $C_{12}H_{17}O_2^{\oplus}$ - 193.3
J = $C_{10}H_{17}^{\oplus}$ - 137.1 or $C_{11}H_{15}O_2^{\oplus}$ - 179.3
K = $C_{11}H_{19}^{\oplus}$ - 151.1 or $C_{10}H_{13}O_2^{\oplus}$ - 165.3
L = $C_{12}H_{21}^{\oplus}$ - 165.1 or $C_9H_{11}O_2^{\oplus}$ - 151.3
M = $C_{13}H_{23}^{\oplus}$ - 179.1 or $C_8H_9O_2^{\oplus}$ - 137.3
N = $C_{14}H_{25}^{\oplus}$ - 193.1 or $C_7H_7O_2^{\oplus}$ - 123.3
O = $C_{15}H_{27}^{\oplus}$ - 207.1 or $C_6H_5O_2^{\oplus}$ - 109.3
P = $HO^{\oplus}$ - 17.1 or $C_{21}H_{31}O^{\oplus}$ - 299.3

Figure 2: Illustrative fragmentation patterns of Urushiol (15:0 and 15:2)

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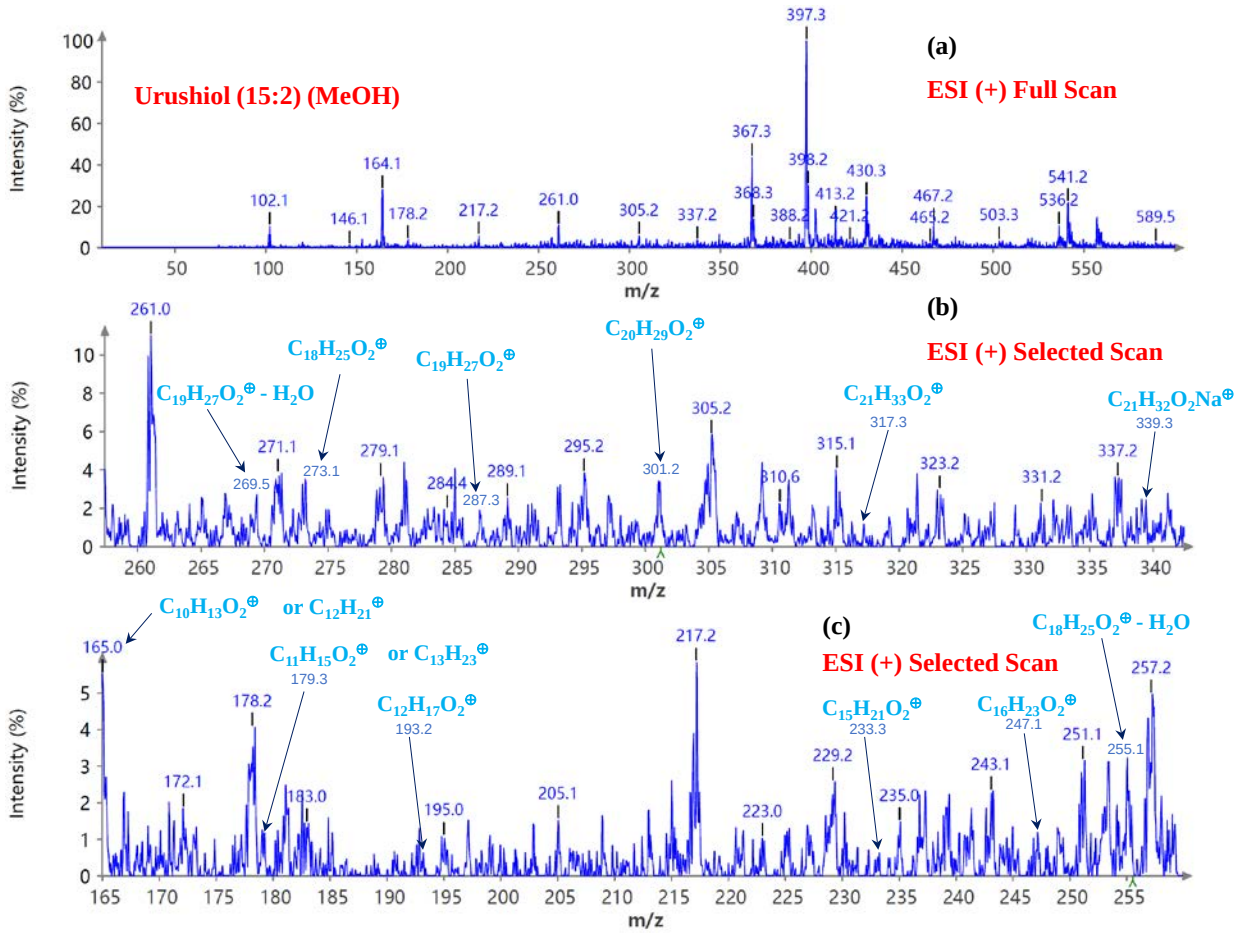


Figure 3: Example MS spectra of pure Urushiol (15:2) standard in the positive ion mode for (a) full scan, (b) selected scan for m/z 269.5, 273.1, 287.3, 301.2, 317.3, 339.3, and (c) selected scan for m/z 165.0, 179.3, 193.2, 233.3, 247.1, and 255.1.

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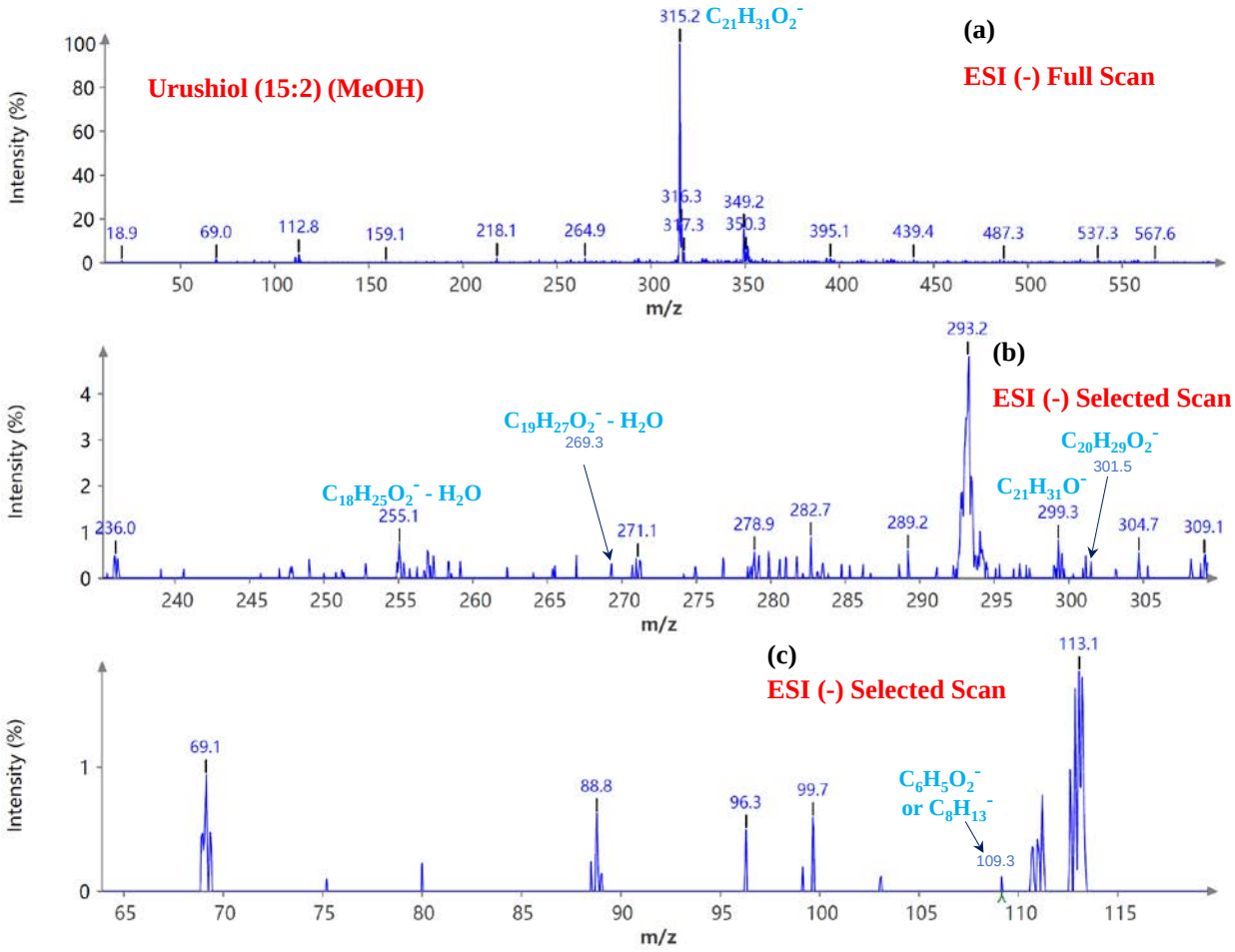


Figure 4: Example MS spectra of pure Urushiol (15:2) standard in the negative ion mode for (a) full scan showing m/z 315.2, (b) selected scan for m/z 255.1, 269.3, 299.3, and 301.5, and (c) selected scan for m/z 109.3.

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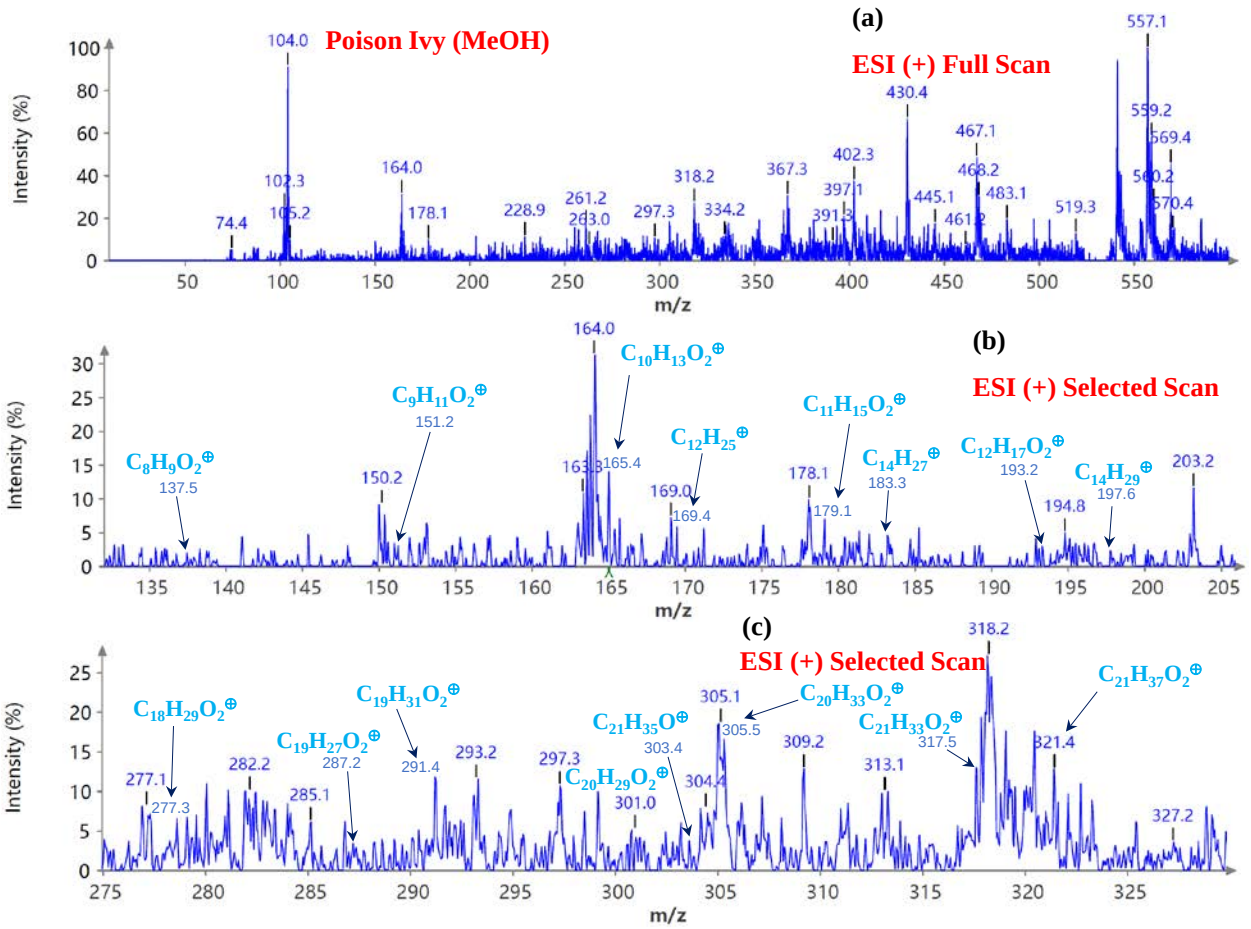


Figure 5: Example MS spectra of poison ivy extract in the positive ion mode for (a) full scan, (b) selected scan for m/z 137.5, 151.2, 165.4, 169.4, 179.1, 183.3, 193.2, 197.6, and (c) selected scan for m/z 277.3, 287.2, 291.4, 301.0, 303.4, 305.4, 305.5, 317.5, and 321.4.

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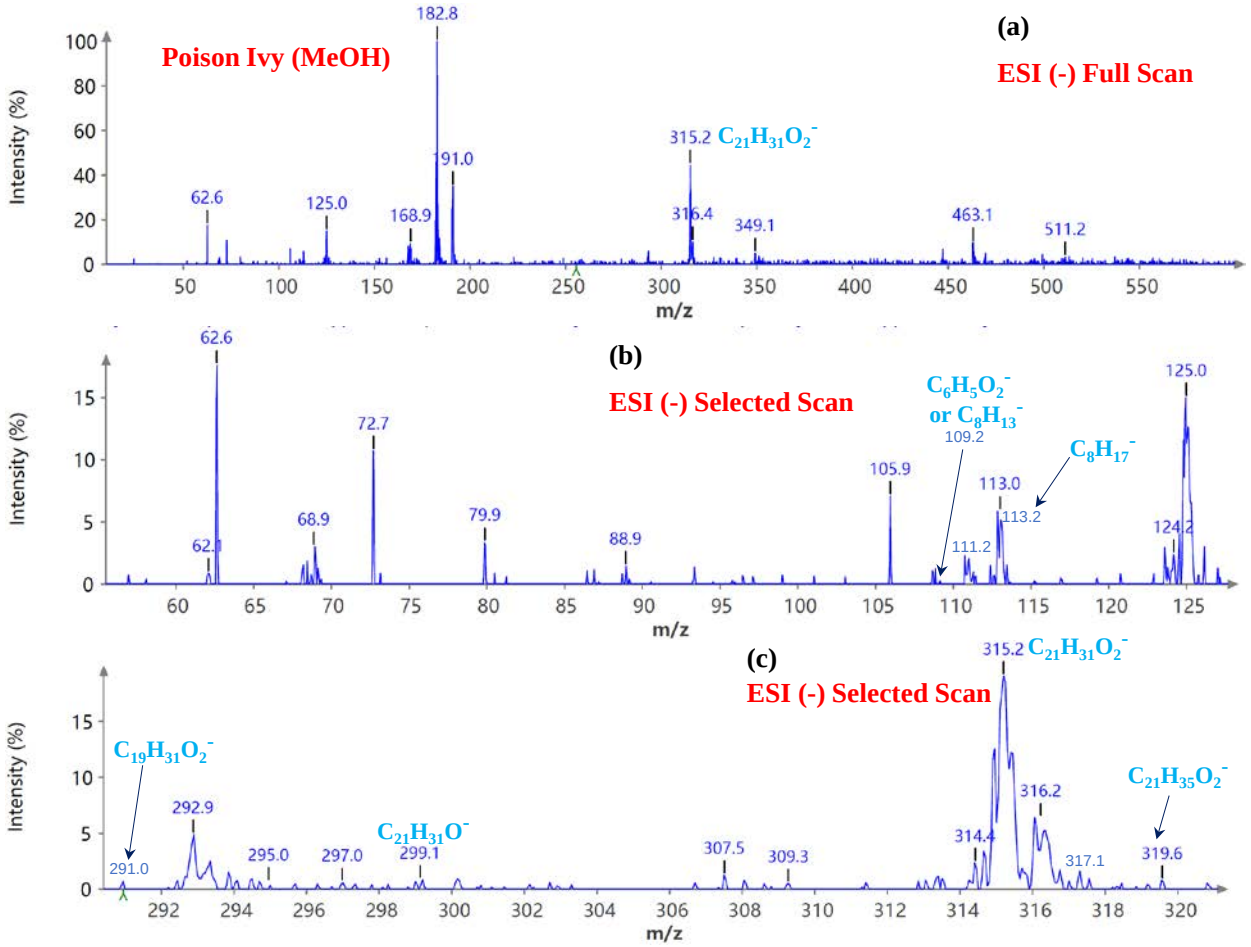


Figure 6: Example MS spectra of poison ivy extract in the negative ion mode for (a) full scan, (b) selected scan for m/z 109.2 and 113.5, and (c) selected scan for m/z 291.0, 299.1, and 319.6.

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Possible Protective Role of Ginger Extract on Methomyl Induced Hepatotoxicity in Adult Male Albino Rats

By Marwa. M. Zaki, Amir. E. Abo Elhassan, Eman. S. M. El Zahid, Ahmed, M. K. A, Ahmed. M. I. Hejab, Ayman. H. Kamel, Nedal M. Fahmy, Tarek. R. Amin & Saad. S.M. Hassan

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Abstract- This study aimed to evaluate the possible histological and ultrastructural changes of liver induced by methomyl pesticide exposure and estimation the possible protective effect of ginger extract for hepatic damage in rats. Ginger is used worldwide primarily as a spicy condiment and of the herbal sources of natural protection from contamination and oxidative stress thus play an important role in chemoprevention of liver diseases. Methomyl is one of the most frequently prescribed pesticides, that are used as a pesticide. It is characterized as a highly toxic compound and has been reported to cause multiple organs damage. Fifteen male albino rats were allocated into 5 main groups. (3 rats in each); one served as control and the four remained groups were for different treatments. GC-MS analysis of ginger extract revealed the content of gingerol, quercetin (3.20%), limonene and zingiberene. Treatment of methomyl treated rats with ginger extract maintained serum ALP, AST& ALT levels and reduced the damage effect with protective efficacy against pesticide induced hepatotoxicity, which appears also more effective than its therapeutic application.

Keywords: *methomyl, hepatotoxicity and ginger extract.*

GJSFR-B Classification: *DDC Code: 363.7384 LCC Code: QH545.P4*



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Possible Protective Role of Ginger Extract on Methomyl Induced Hepatotoxicity in Adult Male Albino Rats

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Abstract- This study aimed to evaluate the possible histological and ultrastructural changes of liver induced by methomyl pesticide exposure and estimation the possible protective effect of ginger extract for hepatic damage in rats. Ginger is used worldwide primarily as a spicy condiment and of the herbal sources of natural protection from contamination and oxidative stress thus play an important role in chemoprevention of liver diseases. Methomyl is one of the most frequently prescribed pesticides, that are used as a pesticide. It is characterized as a highly toxic compound and has been reported to cause multiple organs damage. Fifteen male albino rats were allocated into 5 main groups. (3 rats in each); one served as control and the four remained groups were for different treatments. GC-MS analysis of ginger extract revealed the content of gingerol, quercetin (3.20%), limonene and zingiberene. Treatment of methomyl treated rats with ginger extract maintained serum ALP, AST& ALT levels and reduced the damage effect with protective efficacy against pesticide induced hepatotoxicity, which appears also more effective than its therapeutic application. Coadministration of methomyl with ginger extract showed a slight improvement in some hepatocytes that looked normal in the examination but still markedly affected and showing signs of degeneration. Results obtained in this study demonstrated that high doses of methomyl induced histological and ultrastructural changes in the liver and the levels of enzymes were raised. due to oxidative stress and the use of ginger extract had partially improved the toxic effect of methomyl.

Keywords: methomyl, hepatotoxicity and ginger extract.

I. Introduction

Plants have been the major source of drugs for the treatment of various diseases in many ancient systems of medicine in the world. Ginger is an underground rhizomes of plant Zingiber officinale belonging to the family Zingiberaceae which is widely consumed as spice for the flavoring of foods *(Ajith et al., 2007)*. It has been reported that ginger and its extracts possess some pharmacological activities including hypoglycemic, insulinotropic and hypolipidemic in human and in experimental animals *(Kondeit et al., 2005)*.

The antioxidants in ginger include gingerols, shogaols, monoterpenes, sequiterpenes, some phenolic derivatives and other phytochemicals which are responsible for their pharmacological activities *(Li et al., 2001)*.

Many previous studies investigated the hepatoprotective effects of ginger extract against liver toxicity induced by ethanol, carbon tetrachloride, bromobenzene and acetaminophen with significant decrease in the level of ALT and AST *(Mallikarjuna et al., 2008; El-Sharaky et al., 2009)*.

The present work was conducted to study the effect of ginger on the liver tissues& serum profile enzymes.

II. Materials & Methods Experimental Design

Fifteen male albino rats were allocated into 5 main groups. 3 rats in each,

Group 1: served as control were administered 1ml once daily of tween 80, vegetable oil and distilled water.

Group 2: received ginger extract in a dose 1/10 LD_{50} (1250 mg/kg /day orally) *Zaki et al (2022).*

Group 3: animals received methomyl orally in a dose 1/10 LD_{50} (1.25 mg/kg /dayorally) Araki et al (1982).

Groups 4: animals received ginger extract simultaneously with methomyl after two hours in the previous doses.

Group 5: (treatment) received ginger extract for 2 hours after methomyl exposure

The treatments were given for rats for 28 days, then, rats were sacrificed by ether anesthesia and specimens from liver were taken for light and electron microscopic examination. Gas chromatography–mass spectrometry (GC-MS)analysis. The chemical composition of water extract of ginger roots was performed using Trace_ GC Ultra-ISQ mass spectrometer(Thermo-Scientific, Waltham, MA) with a

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direct capillary column TG–5MS (30m_0.25mm_0.25 mm film thickness). The column oven temperature was initially held at 40_C, and then increased by 5_C/min to 280_C. The injector and detector (MS transfer line) temperatures were kept at 250_C. Helium was used as a carrier gas at a constant flow rate of 1 ml/min. Extract derivatization was done using BSTFA/TMCS (80:20, v:v) for 1 h at 70_C, after evaporation to dryness of dichloromethane/methanol mixture. The resulting solution was dried and then dissolved in hexane. The solvent delay was 2 min and diluted samples of 1 ml were injected automatically using Auto sampler AS3000 (Thermo-Scientific, Waltham, MA) coupled with GC in the split less mode. EI mass spectra were collected at 70 eV ionization voltages over the range of m/z 50–650 in full scan mode. The ion source and quadrupole temperatures were set at 200_C and 150_C, respectively. The components were identified by comparison of their retention times (Rt) and mass spectra with those of WILEY 09 (Flavor & Fragrance Natural & Synthetic Compounds) and NIST 11 (National Institute of Standards and Technology, Gaithersburg, MD) Mass Spectral databases.

a) Plant material

The ginger rhizomes were collected from the Faculty of Pharmacy, Heliopolis University for Sustained Development, Medicinal Plant Reserve. It was identified and authenticated by, Medicinal plants branch at the national research center (Latin name: *Zingiber officinale*; plant part: *Rhizome*).

b) Methomyl

Technical grade methomyl (90% active ingredient) was obtained from (Tabouk) Pesticide Company, Egypt.

c) Animals used

Healthy adult male albino rats (120-180 gm) were obtained from laboratory animal breeding unit (Faculty of Medicine, Zagazig University). The rats were kept in metal cages during the whole experimental period under hygienic condition, fed on well balanced ration and provided water ad-libitum, through the experiment. The light system was 12/12 hrs. dark/night cycle. The rats were accommodated to laboratory conditions for two weeks before the experiment.

Aqueous ginger extract was prepared from available ginger (Zingiber officinale) roots. The ginger roots were peeled on crushed ice, and (100 gm) of ginger was cut into small pieces and homogenized in 75 ml of cold, sterile 0.9% NaCl in the presence of crushed ice. The homogenization was carried out in a blender at high-speed bursts for total 15 minutes.

i. *Blood samples collection and preparation of plasma*

The blood was collected by the retina and allowed to clot for 30 min. at room temperature. The clotted blood was then centrifuged at 3500 RCF for 30 min. The serum was separated and stored at -25°C until protein and enzyme analyses were performed.

a. *Determination of alanine amino transferase (ALT) and aspartate amino transferase (AST)*

ALT and AST were determined colorimetrically according to the method of *Reitman, (1957).* The reaction mixture consisted of 1 ml of a mixture of phosphate buffer (pH 7.2), 0.2 mM α-ketoglutaric and 200 mM L-aspartate. Incubate for exactly 30 min., add 1 ml of 0.001 M 2,4-dinitrophenyl hydrazine, wait for at least 30 min., and then 10 ml of 0.4 N NaOH were added. The optical density of the produced brown color is measured after 5 minutes, using a spectrophotometer at 520 nm.

b. *Determination of acid and alkaline phosphatases (ALP)*

The activities of acid and alkaline phosphatases were determined using the method of *Powell and Smith, (1954).*

In this method, the phenol released by enzymatic hydrolysis of disodium phenylphosphate reacts with 4-aminoantipyrine,and by the addition of potassium ferricyanide, the characteristic brown color is produced. The reaction mixture consisted of 1 ml carbonate buffer (pH 4.5 & 10.4),1ml of 0.01 M disodium phenyl phosphate (substrate), and 0.1 ml sample, and then incubate for exactly 30 min. at 37°C. At the end of incubation period 0.8 ml of 0.5 N NaOH was added to stop the reaction. Then add 1.2 ml of 0.5 N $NaHCO_3$, followed by the addition of 1 ml of 4-aminoantipyrine solution (1 %) and 1 ml potassium ferricyanid (0.5%). The produced color was measured immediately at 510 nm. The enzyme activity is expressed by unit (U),where 1 unit hydrolyze 1.0μ mole of p-nitrophenyl phosphate per minute at 37°C, and pH 10.4.

d) Statistical analysis

The significance differences were determined by analysis of variance (ANOVA). The significance of various treatments was evaluated by Duncan's multiple range tests ($p < 0.05$). Data were subjected to statistical analyses using the software package *Costat® Statistical Software (2005)* a product of Cohort Software, Monterey, California, USA, The values of each measurement of the tested parameters were recorded as mean of five readings i standard error. Statistical analysis was carried out using simple one-way analysis of variance (ANOVA) test, using spss software windows version 17 (SAS, 2001). A probability of $P < 0.05$; and $P< 0.0$ as the level of significance unless stated otherwise. Statistical significant differences among all treatments were carried out by least significant differences (LSD).

III. Results

Chemical composition of GE The GC-MS analysis of GE revealed that it contains phenolics, alkaloids, flavonoids, tannins, anthraquinones,

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terpenoid, and steroids. The identified bioactive components of GE are listed in Table 1, with their respective RT and percent composition (area %), where the most important substances are gingerol, quercetin, DL-limonene, ar-curcumene, zingiberene, b-sesquiphellandrene, linalool, pyrazine, and 1,8-terpin hydrate. The major identified compound was the gingerol (7.09%), while from total ion chromatogram (TIC), we note that the flavonoid quercetin has been appeared at multiple RTs with total percent composition of 3.2%.

Table (1): Bioactive components of aqueous extract of ginger roots as determined by using gas chromatography-mass spectroscopy (GC-MS)

Compound name	Molecular formula	Molecular weight	Retention time	Area%
Nerolidol	$C_{15}H_{26}O$	222	29.3	0.05
Lucenin	$C_{27}H_{30}O_{16}$	610	33.75	0.06
Clionasterol	$C_{40}H_{56}$	414	33.55	0.09
Curcumene	$C_{15}H_{22}$	202	28.38	0.09
Cineole	$C_{10}H_{18}O$	154	24.86	0.10
Phenol	$C_9H_{12}O_2$	152	22.88	0.11
Sesquiphellandrene	$C_{15}H_{24}$	204	29.39	0.12
Zingiberene	$C_{15}H_{24}$	204	28.69	0.18
Isochiapin	$C_{19}H_{22}O_6$	348	41.21	0.18
Dihydrostillbene	$C_{16}H_{18}O_4$	275	41.97	0.20
Quercetin	$C_{18}H_{16}O_7$	344	43.03	3.41
Pyrazine	$C_5H_6N_2$	95	8.35	12.71
Hydroxylinalool	$C_{10}H_{18}O_2$	170	23.52	1.15
Terpinhydrate	$C_{10}H_{20}O_2$	171	24.29	1.09
Rosifoliol	$C_{15}H_{24}O$	220	36.34	0.74
Limonene	$C_{10}H_{16}$	140	15.17	0.27
Gingerol	$C_{17}H_{26}O_4$	294	45.17	0.79

Table (10): Effect of tested materials on the liver enzymes esterase of albino rats

	G1 Control	G2 Ginger	G3 Methomyl	G4 Ginger& methomyl	G5 Methomyl & ginger
ALT (U/L)	134.16 ±1.211	124.12 ±5.21	152.15 ±1.379*	142.4 ±1.455**	144.27 ±1.205
AST (U/L)	35.45 ±0.684	40.56* ±5.96	73.32 ±1.215*	43.77 ±0.997**	65.55 ±1.990*
ALP (U/L)	375.16 ±0.921	395.22 ±11.54*	655.65 ±1.655*	511.28 ±1.568*	579.52 ±4.403*

*The values were given as means ± SEM (Standard Error of Mean); n=5, Significant difference levels versus control: *p≤0.05; ** p≤0.01, compared with control.*

The serum enzyme activities in rats fed supplemented with ginger extract; hot and cold for 28 days are given in Table 3. All treated groups showed significant decrease of serum aspartate and alanine aminotransferase, gamma glutamyl transferase and alkaline transferase activities as compared with control group.

The liver enzymes levels were reduced by the same amount after pesticide withdrawal with or without GE treatment, suggesting that GE does not exhibit metal-chelating ability and does not provide hepato protection against methomyl accumulation. Additionally, the markers of liver function remained persistently high, particularly AST, at early exposure periods in rats treated with methomyl, which may be indicative of hepatic damage *(Omobowale et al., 2014)*. GE initially improved transaminases activities (Farag et al., 2010) but failed to restore their activity to normal towards the end of the exposure period. Thus, GE could protect against the early methomyl induced hepatotoxicity. Administration of ginger improved liver function s as it reduced liver enzymatic activities. These results were in accordance with that of *Mallikarjuna et al. (2008)* who showed that administration of ginger ethanolic extract (200 mg/kg) orally from day 15 to day 21 along with country-made Liquor (CML) produced significant lowering of AST, ALT, ALP and tissue lipid peroxide levels. Treatment of ginger significantly decreased serum urea and increased serum creatinine concentration since, ginger contain polyphenols and flavonoids that influence removing certain waste products from plasma. These results agree with *Ajith et al., (2007)* who reported that the

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presence of polyphenols and flavonoids in ginger extract might be responsible for the antioxidant nephroprotective activities and the reduction of serum urea and creatinine levels.

a) Histopathological Work Up

Fixation and tissue processing: The formalin preserved liver, specimen was processed in an automated tissue processor. The processing consisted of an initial 2 step fixation and dehydration. Fixation comprising tissue immersion in 10% buffered formalin for 48 hours, followed by removal of fixative in distilled water for 30 minutes. Dehydration was then carried out by running the tissues through a graded series of alcohol (70% , 90% ,and 100%). The tissue was initially exposed to 70% alcohol for 120 minutes followed by 90% alcohol for 90 minutes and then two cycles of absolute alcohol, each for one hour. Dehydration was then followed by clearing the samples in several changes of xylene. It consisted of tissue immersion for an hour in a mixture comprising 50% alcohol and 50% xylene, followed by pure xylene for one and a half hour. Samples were then impregnated with molten paraffin wax, then embedded and blocked out. Paraffin sections (4–5 um) were stained with hematoxylin and eosin, *(Suvarna et al., 2012)* Stained sections were examined for circulatory disturbances, inflammation, degenerations, apoptosis, necrosis, and any other pathological changes in the examined tissues.

b) Histopathologic Finding

i. *Group 1. (Control free)*

Liver: Examined serial sections from liver revealed normal hepatic parenchyma with preserved lobular pattern, portal triades and associated structures (portal vein, hepatic artery, hepatic vein, bilsductiles and lymphatics). Central vein sinusoids and hepatic cords were apparently normal. Hepaticlobules were separated by fine fibrous stromal connective tissue.(Fig.1).

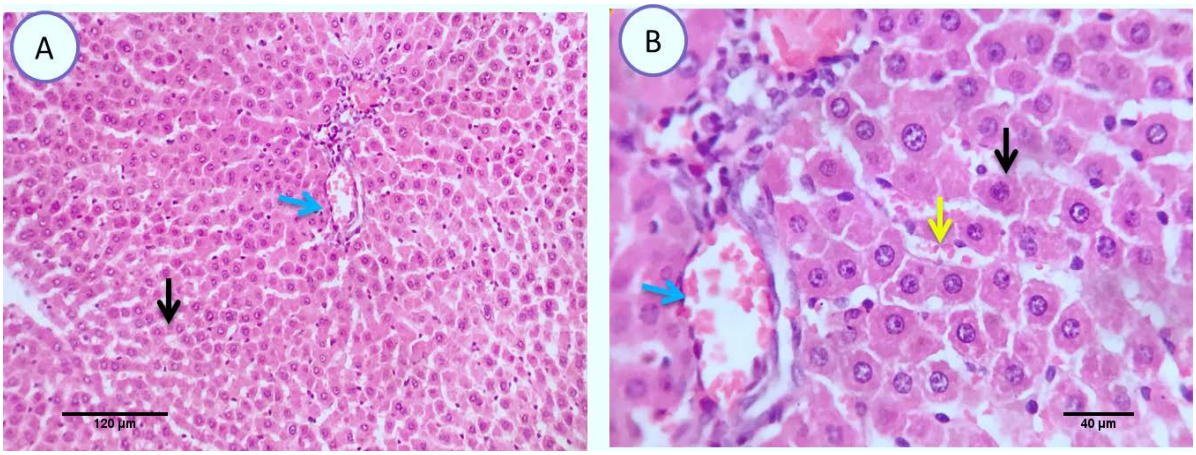


Fig. 1: Photo-micrograph from liver, control group, showing normal hepatic parenchyma with preserved lobular pattern, central veins(blue arrow), Sinusoids and hepatic cords(black arrow) . Scale bars 120, 40 um

ii. *Group 2 Ginger administration*

Liver: Examined serial sections denoted apparently normal hepatic parenchyma, lobular arrangement, portal triads structures, vascular tributaries and biliary tree, however some sections showed vascular dilatation and mild portal round cells aggregation. (Fig.4).

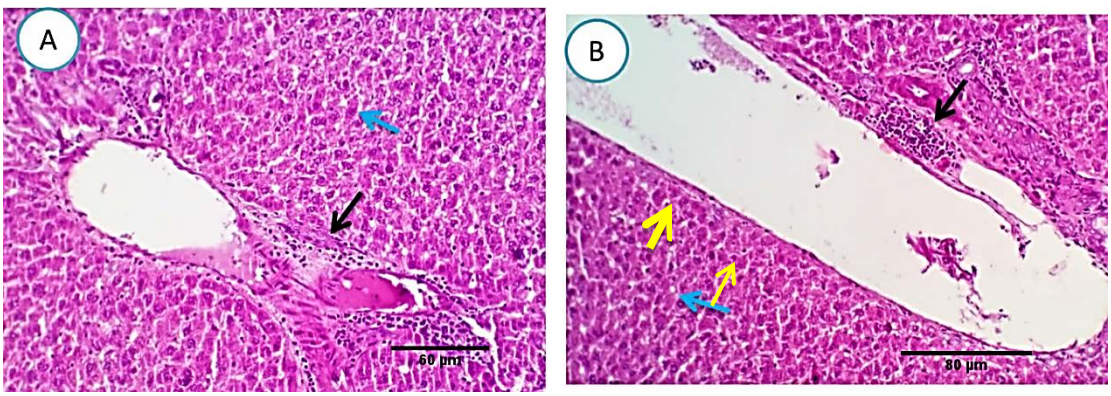


Fig. 2: Photomicrograph of liver (Group .G) showing normal hepatic parenchyma(blue arrows), vascular dilatation(yellow arrows)and mild portal round cells aggregation(black arrows). Scale bars 60, 80 um

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Here, as a proof of concept, we used a mouse model to show that orally from ginger extracts resulted in protecting mice against methomyl-induced liver damage.

c) *Group3 (Methomyl pesticide)*

Liver: Examined sections from liver of this group denoted moderate portal biliary proliferation, congestion of portal blood vessels, round cell infiltration, multifocal interstitial lymphocytic and macrophages aggregations replacing previous necrotic patches beside degenerative changes in a few hepatocytes. (Fig.4).

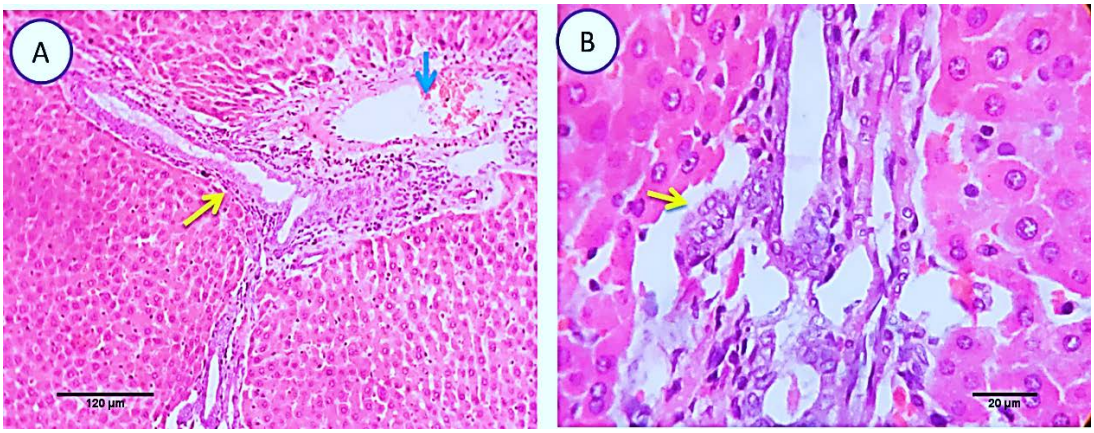


Fig. 3: Photo-micrograph from liver, group (2), showing, portal biliary proliferation (yellow arrow), congestions of portal blood vessels (blue arrow) and round cell infiltration beside degenerative changes in a few hepatocytes. Scale bars 120, 20 um.

d) *Group 4 (Ginger protection followed by methomyl administration)*

Liver: Sections from liver of this group denoted moderate portal biliary proliferation, congestion of portal blood vessels, round cell infiltration, multifocal interstitial lymphocytic and macrophages aggregations replacing previous necrotic patches beside degenerative changes in a few hepatocytes. (Fig.10)

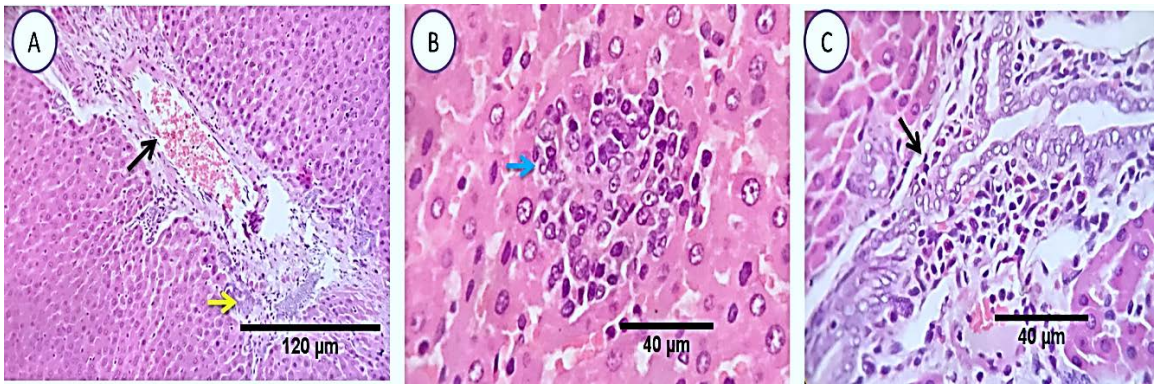


Fig. 4: Photo-micrograph from liver, group (4), showing, portal biliary proliferation (A, yellow arrow, B, black arrow), congestions of portal blood vessels(A, black arrow), round cell infiltration and interstitial lymphocytic and macrophages aggregations replacing previous necrotic patches (B, blue arrow). Scale bars 120, 40, 40 um.

e) *Group 5 (Ginger treatment after methomyl administration)*

Liver: Examined sections from liver of this group denoted moderate portal biliary proliferation, congestion of portal blood vessels, round cell infiltration, multifocal interstitial lymphocytic and macrophages aggregations replacing previous necrotic patches beside degenerative changes in a few hepatocytes.(Fig.7).

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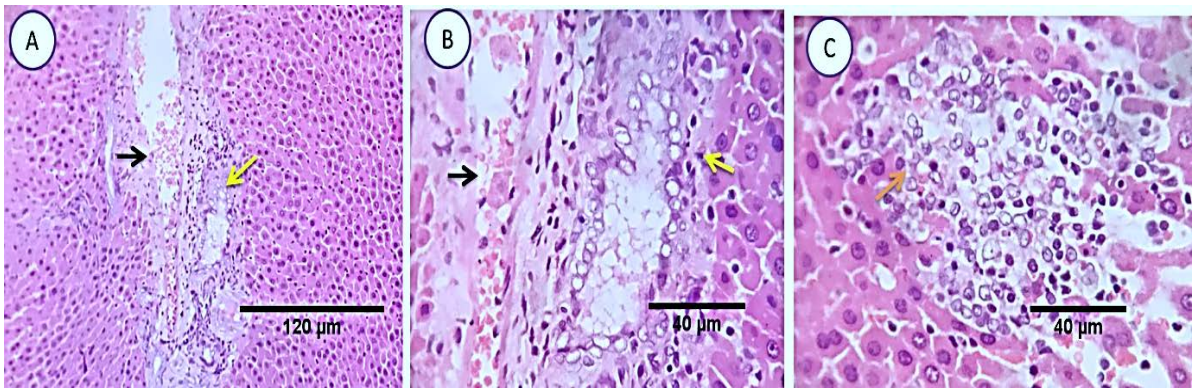


Fig. 5: Photo-micrograph from liver, group (3), showing, portal biliary proliferation (yellow arrow), congestions of portal blood vessels (black arrow) and round cell infiltration and interstitial lymphocytic and macro-phages aggregations replacing previous necrotic patches (orange arrow). Scale bars 120, 40, 40 um.

IV. Conclusion

Ginger extract appear to be highly effective in improving the toxic effects caused by pesticide, and the use of ginger was beneficial in lowering liver enzymes and has hepato protective effect.

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Two-Dimensional Nucleation in the Dislocation Model of Crystal Growth

By V. I. Rakin

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TWODIMENSIONALNUCLEATIONINTHEDISLOCATIONMODELOFCRYSTALGROWTH

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Two-Dimensional Nucleation in the Dislocation Model of Crystal Growth

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Abstract- A combined mechanism of nonequilibrium dislocation growth of crystal faces combined with equilibrium formation of a two-dimensional nucleation is presented. The binding energy of atoms in the crystal near the helical dislocation has been calculated based on the Lennard-Jones potential. The study substantiates thermodynamic conditions for the occurrence of hollow dislocation nuclei detected earlier in AFM observations of crystal growth. Conditions for the linear Onsager approximation in response to non-linear kinetics of crystal growth are described. The three values of solution supersaturation, the relationships between which are highly variable, are controlling the growth process of the crystal face. The supersaturation and their interrelations depend on the peculiarities of the defective crystal structure, the of the crystal-solution interaction, and the peculiarities of the crystallization medium hydrodynamics.

I. Introduction

It is known that any flat crystal surface is characterized by a certain density of free bonds possessing a fixed heat of adsorption and, on this basis, is a crystal defect. If the crystal is in a multicomponent medium, then, according to the second principle of thermodynamics, impurity atoms and molecules that lower the surface energy are deposited on such centers. Then it can be confidently argued that a limiting variant of Langmuir theory is always realized: the adsorption value approaches the monolayer capacity [1]. This state of the crystal surface in a multicomponent crystal-forming medium is characteristic of both equilibrium and nonequilibrium conditions. However, certain free energy thermodynamic fluctuations [2], irregularly and for a short period, are able to clear surface areas of adsorbate. Note that the distribution function of fluctuations on a homogeneous face surface can be described by Gibbs equilibrium theory [3]. The growth of the crystal face is possible only in these short intervals between desorption and secondary adsorption of impurities.

Crystal growth as a macroscopic phenomenon should be described by the equations of nonequilibrium thermodynamics. The mechanical-statistical solution to the problem of measuring macroscopic physical variables of the thermodynamic phase is well known [4]). Suppose the body is made up of *N* atoms. Then the size of the system can always be estimated at best to the accuracy of one atom. The relative error of measurements of any intensive thermodynamic variables cannot be less than $1/\sqrt{N}$. Thus, the locality limit for thermodynamic variables is at least the first tens of nanometers and partially overlapped by the field of thermodynamic fluctuations.

At the atomic level, some mechanisms of building particles embedding into the structure are implemented at the impurity desorption site, and objects with atomic scale in at least one dimension should be discussed. These are helical and combined dislocations, elementary steps, kinks of steps, two-dimensional nuclei, etc. After theoretical description of these mechanisms in the language of classical or quantum mechanics, a transition to thermodynamic description is necessary. But at this transition the problem of time inevitably arises – time reversibility in the laws of classical physics (CPT–invariance in quantum mechanics) and time irreversibility in thermodynamics. Note that the problem of time, as well as the problem of total adsorption of impurities on the growing face did not attract serious attention of the researchers of crystal growth [5, 6]. And, besides, the kinetic theories of growth widely used the Arrhenius equation [7], which has no strict theoretical justification for a nonequilibrium system. As a result, classical growth theories are internally inconsistent and abound in numerous empirical formulas and coefficients. However, the equations and phenomenological coefficients of nonequilibrium thermodynamics, in contrast to empirical coefficients, have a reliable justification in the principle of local equilibrium and the three principles of thermodynamics [3]

II. Dislocation in a Kossel Crystal

Let us discuss the helical dislocation as a defect of crystal structure, without the participation of which the growth of most crystals in nature does not occur [4, 5]. Consider a Kossel crystal [8] with a primitive cubic cell in which each particle inside the crystal contacts six neighbors and 20 more particles on the diagonals and use the Lennard-Jones potential:

$$U(r) = 4\varepsilon[(\chi/r)^{12} - (\chi/r)^{6}]. \qquad (1)$$

The equilibrium distance between particles in pairwise interaction is well known:

$r_{min} = \chi\sqrt[6]{2} = 1{,}122\chi$. However, the minimum of interaction energy (1) of a surface particle is reached at

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a smaller distance $r_{min} = 1{,}085\chi$ from the nearest neighbor in the crystal. Inside the crystal, the bonds between particles become stronger and the internal energy decreases. In an ideal Kossel cubic crystal, the particles are at fixed distances r/r_{min} from each other [8]. For a surface particle, the first coordination hemisphere contains 17 neighbors and the second contains 57.

The Lennard-Jones potential serves only to illustrate model representations, since it has the necessary properties of the interatomic forces of attraction and repulsion.

It is easy to see that the main contribution to the bonding energy of a surface particle is made by the first coordination sphere. Taking into account interaction with particles of the second coordination sphere makes a correction not exceeding 8%, but always enhancing the binding energy of the particle with a crystal. To simplify further calculations, we will limit ourselves to the first coordination sphere only. We will also disregard the effect of adsorbed impurity, which additionally weakens the bonding strength of all surface atoms to the crystal. These two factors have a systematic but opposite effect.

Consider the model of a helical dislocation exiting to the crystal surface along the OZ axis at the point with coordinates x=0, y=0 (Fig. 1). The dislocation has a unit Burgers vector. Assigning the zero value of the z-coordinate to the upper boundary of the first atom – (½.½.0), we obtain coordinates of other three atoms located around dislocation: (–½.½.¼), (–½.–½.½), (½.–½.¾).

Suppose that the z-coordinate of any surface atom depends linearly on the angle of rotation of the radius-vector drawn from the dislocation axis to the center of the atom. Then at tetragonal symmetry of the crystal face the position z of surface atoms in the first quarter of the (x,y) plane can be described by the formula

$$z = \operatorname{arctg}(x/y)/2\pi - 1/8. \qquad (2)$$

In other quarters, if the coordinate system is rotated, z has to be further increased by ¼.

Using this expression in other quarters when rotating the coordinate system, z should be further increased by ¼. It should be noted that expression (2) does not lead to a minimum of the free energy of the crystal, but at x,$y\to\infty$, the dissymmetry of the nearest surroundings of any surface atom, expressed in values of Δz, rapidly decreases and the dislocation effect disappears. This property of the formula reflects the physical phenomenon and allows the use of expression (2) for model constructions.

As a result the bonding energy of surface atoms in the first quarter is described by law 1 (Fig. 2). In the remaining quarters the bonding energy differs only for the first four atoms in the narrow region (Fig. 2) immediately adjacent to the dislocation axis at the given arrangement of atoms (Fig. 1).

It is obvious that the atoms located in the first quarter of the coordinate system are most strongly bonded, but the atoms in the fourth quarter are weakly bonded to the crystal surface (Fig. 1, Fig. 2). For an ideal surface without dislocation, the binding energy of any surface atom is about $U/4\varepsilon = -7.94$, which corresponds to a certain value of the equilibrium concentration of the surrounding crystal-forming solution.

For atoms 4 (Fig. 1), the relative reduction of the chemical potential is a rather large value $\Delta\mu_c/\mu_c = 0.28$. In the thermodynamic equilibrium state, the chemical potential of the crystal substance is equal to the chemical potential of the substance in solution [3] $\mu_c = \mu_s$, and the latter is defined by the Lewis formula:

$$\mu_s = \mu_0 + RT\ln(a), \qquad (3)$$

where μ_0 is the chemical potential of the building particles in the standard state in the saturated solution, a is their activity in the solution. If the chemical potential of the substance in the crystal decreases, the equilibrium solution corresponding to it will have a different (lower) concentration. Converting the relative change in chemical potential per molar number of atoms of type 4 we get:

$$\frac{\Delta\mu_c}{\mu_c} = \frac{\Delta\mu_s}{\mu_c} = \frac{RT\ln(a'/a_0)}{\mu_c} \approx \frac{RT\ln(c'/c_0)}{\mu_c} = \frac{RT\ln(\sigma'+1)}{\mu_c}, \qquad (4)$$

where $\sigma' = (c' - c_0)/c_0$ is the relative change in the equilibrium concentration of the solution for atom 4 (Fig. 1). Expanding the logarithm into Taylor's series and using standard Gibbs free energy of potassium sulfate formation under normal conditions ($\mu_c = -1321\,J/(mol\cdot K)$) as an example, we obtain $\sigma' \approx -0{,}15$ for equilibrium conditions.

Thus, the equilibrium solution for the part of the crystal surface composed of atoms 4 should be considered as undersaturated, and atom 4, which is on the edge of the step, will be definitely removed from the crystal surface as a result of entropy fluctuations in the state of equilibrium. It will be followed by removal of atom 5, for which bond strength will immediately decrease after removal of atom 4, and then, clockwise, by other atoms (Fig. 1), for which the solution will also become undersaturated. However, the magnitude of the relative change in the chemical potential will decrease, as the distance to the dislocation axis grows, and eventually the process will cease. However, as the dislocation axis deviates, the magnitude of the relative change in the chemical potential will decrease and the process will stop. As a result, a funnel is formed around the dislocation – a "hollow core" (Fig. 3), theoretically

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predicted back in the 1950s [9, 10] and later observed in atomic force microscopes [11, 12].

The linear dimensions of such a formation cannot be established in the Lennard-Jones model, but one can use the thermodynamic model of a negative two-dimensional disk [9].

The random formation of empty space around a dislocation in a Kossel crystal, which is an open hollow cut disc of diameter r_1 and unit height coinciding with the size of the building particle b can be described using free energy fluctuations:

$$\Delta\Psi' = \frac{2\pi r_1 E}{b} - \frac{\pi r_1^2}{b^2}\Delta G, \qquad (5)$$

in which the first term is responsible for the emergence of an additional crystal surface along the side wall of the single-layer disk, and the second term is the change in internal energy due to the loss of particles that formed the crystal substance in the disk body, ΔG is the average change in the chemical potential of the crystal when one building particle is embedded in its surface structure, E is the bond energy between two surface particles. The bonding energy in the macroscopic sense determines the specific surface energy of the crystal, but in this case reflects the bonding between only two adjacent particles. Although formula (4) makes thermodynamic sense, all variables in it are not macroscopic. In addition, the variable ΔG per molecule is not the same as that for building particles inside the crystal, because it depends on a smaller number of bonds between the particles.

The conservation of the hollow disk on the crystal in equilibrium with the surrounding solution is determined by the known variation extremum condition

$$\delta(\Delta\Psi') = 0. \qquad (6)$$

The chemical potential of the equilibrium solution, but undersaturated with respect to the atoms on the edge of the step near the dislocation axis (Fig. 1, atom 4) differs by the value

$$\Delta G' = kT\ln(\sigma' + 1), \qquad (7)$$

and takes a negative value $\sigma' < 0$. As a result of the solution of the variational equation (5) we obtain the negative radius of curvature of the side walls of the equilibrium disk at the helical dislocation site (Fig. 3)

$$r'_1 = \frac{bE}{kT\ln(\sigma'+1)} \approx \frac{bE}{kT\sigma'} < 0. \qquad (8)$$

So, the value of the free energy as a result of the formation of the hollow disk is also negative and is

$$\Delta\Psi'_1 = \frac{\pi r_1 h_0 E}{b^2} < 0, \qquad (9)$$

which at thermodynamic equilibrium in the system indicates a natural process. In F.C. Frank [9] the sign of r'_1 is not discussed, but for the conditions of thermodynamic equilibrium the non-fluctuational nature of this disk and the sign are of great importance.

Let us note two opposing factors. On the one hand, the adsorbed impurity additionally weakens the connection of the atoms located at the edge of the step with the lower lying atoms. It occurs in the intervals between the fluctuations. So, the undersaturation value σ' for the atoms nearest to the dislocation certainly increases. But on the other hand, the lattice distortions quickly weaken and the value of the under-saturation decreases in response to digressing from the helical dislocation (Fig. 2). At the edge of the disk the undersaturation $\sigma' \to 0$. Thus, formula (6) represents some average value of the underdesorption index within the disk area, which is realized at the moments of impurities desorption.

Simultaneously, in deeper layers of crystalline matter, the second and subsequent hollow disks are formed in the dislocation core, but with a smaller radius (Fig. 3), because the binding force of atoms located at the edge of the non-growing step increases with depth. As a result, the equilibrium cone-shaped dislocation core at constant pressure and temperature, in accordance with the second law of thermodynamics, reduces the free Gibbs energy of the crystal by the value depending on chemical bonds of atoms, molecules and complexes in the crystal structure, the helical dislocation structure and on the impurity composition of the equilibrium crystallization medium by the value:

$$\Delta\Psi' = \sum\frac{\pi r_i h_0 E}{b^2} < 0. \qquad (10)$$

It is important to note that the dislocation core can persist only up to a certain supersaturation degree. So, in reality, the dislocation core can only be observed at minor supersaturation, for example, in AFM growth studies [11, 12].

III. Crystal Growth Rate

Consider the growth of a crystal face with uniformly distributed growth steps running away from the dislocation core (Fig. 3). Evidence has been produced to prove the stability of uniformly distributed rectilinear elementary steps on the face of an equilibrium crystal [13]. The calculation is based on the Van der Waals interaction energy. Since most of the time the crystal face covered with adsorbate is in thermodynamic equilibrium with the solution [14], the structure of the surface even during crystal growth corresponds to the case described in [13]. Uniformly alternating steps on the flat face of the growing crystal are displayed through the surface interferometry (Fig. 4). The angle between the base surface of the octahedron face of potassium

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alum and the inclined flat face of the growing pyramid, which is a simple crystallographic form of tetragontrioctahedron, changes usually in the range from 10 to 20 angular minutes [14].

Analysis of the events occurring in the region of impurity desorption *X* (Fig. 3) as a result of average free energy fluctuations at the crystal face leads in the first approximation to a formula determining the "instantaneous", on the macroscopic scale of measurements, normal growth rate of the crystal face region [14]:

$$v = \frac{\beta h_0 \sigma_f}{\kappa \mu_1 \mu_2 (1-q)\tau} = B\sigma_f. \qquad (7)$$

Here: κ and β are kinetic coefficients determining the rate of settlement of kinks by adsorbate molecules and building particles. These coefficients depend on temperature and concentrations of impurity and building particles in the environment, but not on supersaturation (under-saturation) of the crystallization medium; $\sigma_f = (c_f - c_0)/c_0$ is the relative supersaturation of the solution at the surface of the desorbed face (away from the dislocation); $\mu_1 = l_1/b$ is the relative distance between kinks along one elementary step; $\mu_2 = l_2/b$ is the relative distance between steps.

To solve the time problem [15], the transfer theorem [16] was used. As a result, there are two additional macroscopic parameters in expression (7). Over the elementary, macroscopic time of stationary growth τ, an average of $1/(1-q)$ independent fluctuations occur (high dispersion value is $q/(1-q)^2$). *q* is a statistical parameter. Thus, the known fluctuations of the growth rate of the face can be easily explained by the large variability in the number of fluctuations for a fixed elementary time of stationary crystal growth.

The kinetic coefficients β and κ are close in magnitude because they reflect the competitive struggle of similarly sized building and impurity particles for the free kink.

Stationary crystal growth, while preserving the macroscopic flat face and the dynamic stepped surface topography at the molecular level (Fig. 3), entails additional conditions that were taken into account when deriving formula (7):

1. The number of fractures per unit face area at a given fixed supersaturation should be constant in time. That implies continuous generation of new rows of construction particles at the stage, compensating the phenomenon of fracture annihilation. The mechanism of this process is realized at the equilibrium transformation stage of the surface, represented by uniformly distributed elementary growth steps [13], as a result of small but frequent fluctuations of the free energy.
2. It is known that the mean value of the effective fluctuations of the free energy, relevant to the model outlined, depends only on the temperature of the thermodynamic system and is constant over time. Therefore, regardless of the nature of the surface topography, the number of kinks within the desorption section can be assumed to be constant. Hence, it follows that the size of the surface area affected by the average fluctuation is proportional to the surface density of fractures under the given stationary growth conditions.
3. If the average linear size of the desorption region is less than the distance between stages l_2, then, according to the solution of the problem similar to the Buffon problem in the probability theory, all kinks will be concentrated on a section of one step length within area *X*. Thus, as the distance between steps increases, the frequency of kinks will as you might expect grow to the natural limit $\mu_1 = 1$.

The frequency of steps μ_2 in the desorption region depends on the critical curvature of the elementary step adjacent to the helical dislocation r_2 (Fig. 3) [6]. To solve such a problem, let us again turn to the two-dimensional nucleus model (4). Suppose the crystal face is subject to the solution oversaturation σ_f. We obtain a positive value of the radius of curvature of the elementary step:

$$r_2 = \frac{bE}{kT\ln(\sigma_f+1)} \approx \frac{bE}{kT\sigma_f} > 0. \qquad (8)$$

As a rule, at the effective Burgers vector of dislocation h_0, an initial step quickly disintegrates into elementary steps at distances of the order of 100 nm from the dislocation [11, 12]. Therefore, the critical curvature can be calculated using the elementary step model (4).

Taking into account the natural limitation of the angular speed of rotation of the lower elementary step after the disintegration of the step height in the Burgers vector [6] in the first approximation we obtain the frequency of steps on the side face:

$$\mu_2 = \frac{2\pi r_2 h_0}{b^2} = \frac{2\pi h_0 E}{bkT\sigma_f}. \qquad (9)$$

Thus, a quadratic dependence of the growth rate on supersaturation near the face surface appears when dislocation steps alternate uniformly:

$$v = \frac{\beta bkT}{2\pi\kappa\mu_1 E(1-q)\tau_0}\sigma_f^2 = A\sigma_f^2. \qquad (10)$$

If the Burgers vector exceeds the elementary step twice, the step frequency also doubles (Fig. 4). Thus, as a result of impurity desorption at the given section of the face, the unit step (the lower step of the package of the Burgers vector) present at distances up to 100 nm from the dislocation exit will have a radius of curvature corresponding to the parameter of a two-

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dimensional nucleus. This case reflects the requirements of equilibrium thermodynamics at the moment of impurity desorption and ensures the safety of the step. In a series of fluctuations, the radius of curvature, r_2, may vary slightly near the mean value (8), which controls the stationary macroscopic growth rate (10).

Note that observations of crystal growth lead to the well-known nonlinear dependence of the growth rate on the magnitude of supersaturation in the stirred solution (Fig. 5). For a well-stirred solution, a linear dependence between the supersaturation of the solution away from the crystal and near the surface of the face is usually assumed:

$$\sigma_f = \vartheta\sigma. \tag{11}$$

Within this assumption, let us discuss the empirical data (Fig. 5.). At the initial stage the dependence of normal face growth rates on supersaturation is close to quadratic, which follows from formula (10), but following some supersaturation ($\sigma > 0.06$) it becomes linear (Fig. 5, linear trend for alumina hexahedron).

This transition to linear dependence is caused by the face relief rearrangement. When supersaturation increases up to the mentioned transition, numerous macro steps are formed on the crystal faces. According to (8), the frequency of steps increases. In an isothermal stationary process, the average fluctuation entropy jump value remains unchanged, so the area affected by desorption phenomena *X*, which is accounted for by a macro step, also decreases. Thus, at high kink density and growth of supersaturation, the relief parameters μ_1 and μ_2 simultaneously tend to reach 1 and the limiting case comes – their dependence on supersaturation disappears. Thus, linear formula (7) in which B=*const* will be fulfilled (Fig. 5). For different simple crystallographic forms this transition occurs at different supersaturation values, which is caused by the structure of dislocations prevailing on the face.

IV. Linear Onsager Regime

At small supersaturations the justification of the linear Onsager regime becomes an important problem. In the stationary mode of growth of an open thermodynamic system, a linear dependence between the coupled thermodynamic forces and thermodynamic fluxes should be observed [3]. Thus, the normal velocity of the face and the supersaturation of the solution near its surface at small deviations from equilibrium should be related by a linear function. But the experimental data (Fig. 5) and formula (10) obviously contradict this. This discrepancy is well known and is often mentioned in scientific literature [6].

Note that in the macroscopic description of the growing face, such as (111) (Fig. 4.), the echelon of elementary steps on it represents a new crystallographic form with a different Miller index. In Fig. 4, the faces of the trigonal pyramid of the right lower dislocation in the circled region represent a simple tetragon trioctahedron shape with Miller indices {45.45.46}. As the supersaturation increases, the step frequency (9) grows and the face indices continuously decrease (the frequency of bands in the interferogram should increase). Therefore, the boundaries of the nonequilibrium thermodynamic system change with increasing supersaturation. Onsager's postulates formally cannot be applied to such system. However, it is possible to change the physical model by fixing the boundaries of the system by a certain simple tetragontrioctahedron shape in a small oversaturation interval by the condition μ_2=*const*. Then, instead of equation (10), formula (7) with the constant coefficient *B* should be used in the thermodynamic analysis, and the linear Onsager regime comes into force.

V. Supersaturations of Solution

In the described model we used values of relative changes of solution concentration: σ', σ_f and σ. The first two of which are difficult to measure in the experiment.

Note that even when the viscous crystallization medium is actively stirred, the relationship between σ_f and σ cannot be expressed as a linear relationship. However, assuming that all of the building substance diffusing through the viscous Newtonian boundary layer to the growing face of the crystal in the stationary growth regime is deposited on its surface, we obtain an additional equation for the growth rate:

$$v = \frac{D}{\rho}\frac{dc_f}{dx}, \tag{12}$$

where *D* is the diffusion coefficient of the substance in solution, ρ is the density of the crystal.

From expressions (10) and (11) in linear approximation of the boundary layer we obtain the relationship between supersaturations:

$$\sigma_f = \frac{1}{2\omega}\left(\sqrt{4\omega\sigma + 1} - 1\right), \tag{13}$$

where $\omega = (A\delta\rho)/(Dc_0)$ is a dimensionless parameter, δ is the thickness of the boundary layer. From the data in Fig. 5, it is difficult to establish the value of the parameter ω. However, it is clear that at low supersaturation the boundary growth mode always tends to the kinetic mode ($\sigma_f \to \sigma$), while at high supersaturation – to the diffusion mode ($\sigma_f \to \sqrt{\sigma/\omega}$).

The situation is different when the growth rate depends linearly on supersaturation $0.06 < \sigma < 0.15$

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(Fig. 5). Based on formulas (7) and (11), the growth mode will always be determined only by the value of the ϑ coefficient, which can be established empirically [14].

The case of natural convection is described in the model of the stationary boundary layer of the solution [17], when calculating the entropy production by the unit area of the growing face in the stationary mode:

$$\frac{d_i S}{dt} = Kv, \qquad (14)$$

where K is the thermodynamic coefficient, linearly depending on supersaturation σ at stationary growth in a small deviation from equilibrium. In the extended version of the thermodynamic coefficient K, gravitation also provides its contribution. But the main component in K is always the first term $-\rho R \ln(\sigma + 1)$.

Expression (12) is obtained for the stationary regime of growth, at which the structure of the laminar boundary layer can be considered as linear. In this case, it is impossible to derive the dependence of entropy production density by the growing face of the crystal on supersaturation of the solution. However, it is noted that the thickness of the boundary layer is always a free parameter, which is determined only by the solution of the hydrodynamic problem of substance transfer in solution.

VI. Conclusions

It has been shown by the method of molecular dynamics that the equilibrium state in a gas is formed after an average of ten particle collisions [3]. In solutions, local equilibrium occurs in time of the order of 10^{-11} – 10^{-12} s. Thus, a interval of 10^{-2} – 10^{-3} s [14] between fluctuations leading to growth, is in stable thermodynamic equilibrium. During this time, the so-called "equilibrium" processes of alignment of the step distribution density on the dislocation pyramid [13] and the kink density on each step are realized as a result of minor fluctuations. It is important that entropy fluctuations differ in varieties – fluctuations of temperature, number of moles of a chemical component, volume, polarizability, magnetization, potential energy of the center of mass, etc. These varieties of fluctuations can also have different signs, are independent, equally probable, and are the source of such equilibrium surface transformations. Due to these numerous fluctuations, a stable stationary macroscopic structure of the growing face surface is formed under constant crystallization conditions on a large time scale (Fig. 4). Due to the equilibrium state of the face completely covered by adsorbate and "equilibrium" fluctuations of free energy, the habit of the stationary nonequilibrium and equilibrium crystal forms coincide, which allows a continuous transition from growth to equilibrium [18] and further to dissolution. But as dissolution begins, the macro structure of the dislocation pyramid changes [14].

An important feature of the approach to crystal growth through fluctuations of free energy and entropy is the separation of events occurring on a time scale of the order of 10^{-4} s during relaxation and "equilibrium" processes and events on a scale of 1 minute. Each separate fluctuation of free energy should be considered as an independent random event, the relaxation consequences of which are described by classical dynamics [14]. But the macroscopic kinetics of crystal growth is influenced only by the integral result of multiple relaxation processes. Such a model makes it possible, let us emphasize, to bypass, but not to solve the problem of time – its reversibility in Newtonian dynamics and irreversibility in macroscopic phenomena of the growth process.

Within such a two-level theory of crystal growth, it is possible to reliably justify equilibrium two-dimensional nucleation of two types as an integral part of the combined nonequilibrium dislocation mechanism of crystal growth. However, in contrast to the conclusions of the authors of [9-12], hollow dislocation nuclei exist in a limited range of supersaturation due to the equilibrium mechanism and have little effect on the growth kinetics of the face. Based on the combined growth mechanism, it is easy to explain the complex behavior of the crystal growth rate given an increasing degree of deviation of the crystal-forming system from equilibrium, and the occurrence of a hollow dislocation nucleus resulting from minor deviations from equilibrium, which do not contradict the equilibrium and nonequilibrium thermodynamics and circumvent the problem of time.

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Figures

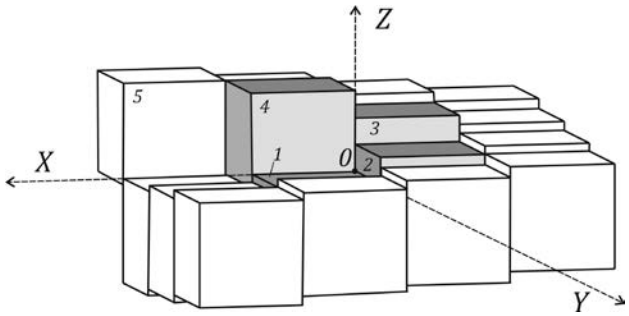


Fig. 1: Location of surface atoms on the edge of tetragonal symmetry around the helical dislocation. The first layer of the nearest 16 atoms is shown. Atoms directly in contact with the dislocation are highlighted in color.

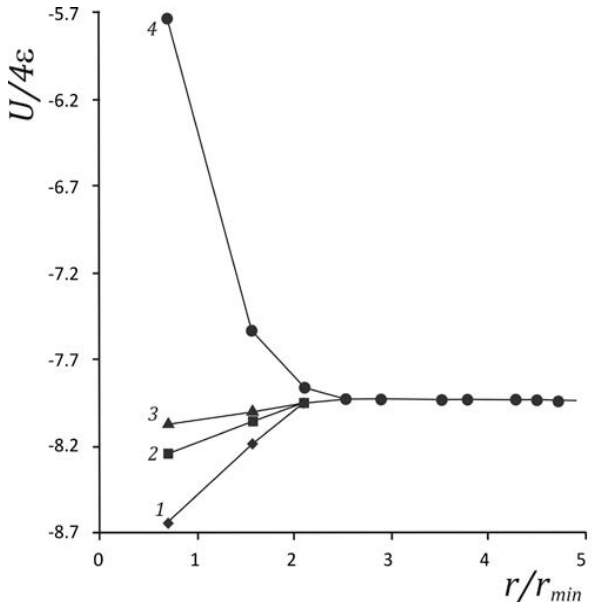


Fig. 2: Dependence of atom binding energy on the distance to the dislocation. The number indicates the atoms closest to the dislocation, located in the corresponding quarter of the coordinate system (Fig. 1)

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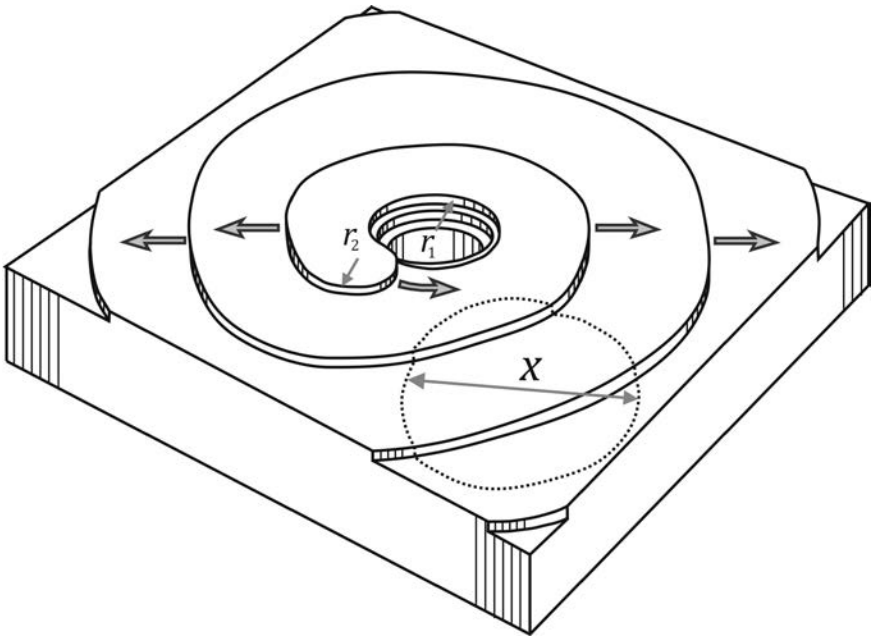


Fig. 3: Structure of the dislocation pyramid on the growing crystal face

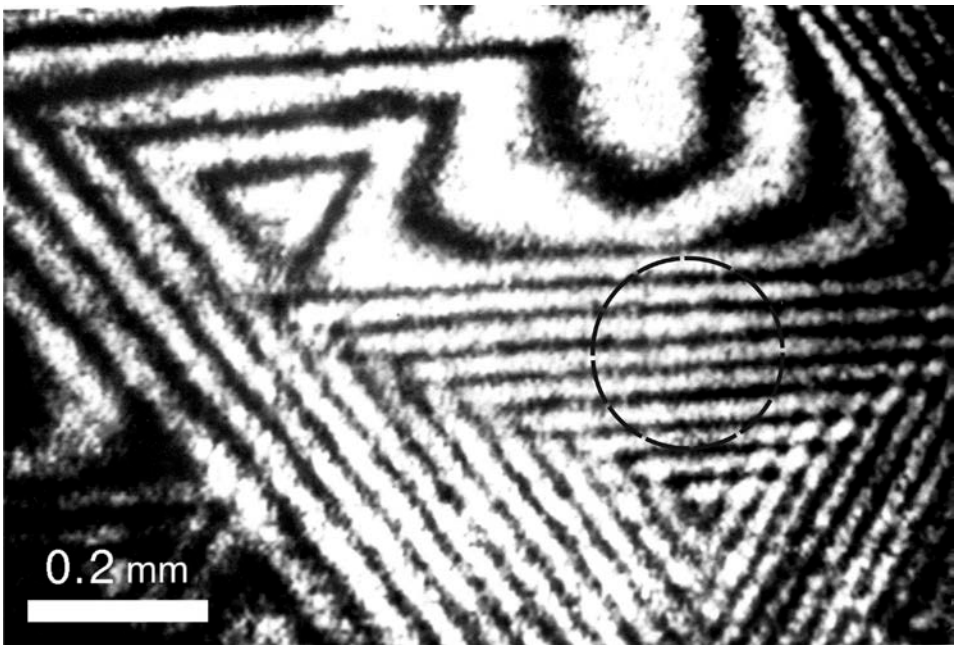


Fig. 4: Interferogram of the growth pyramids of two helical dislocations differing twice by the Buergers vector. The frequency of interference fringes is proportional to the frequency of elementary steps on the surface of the face (111) of alumina. The more active lower pyramid absorbs the upper one.

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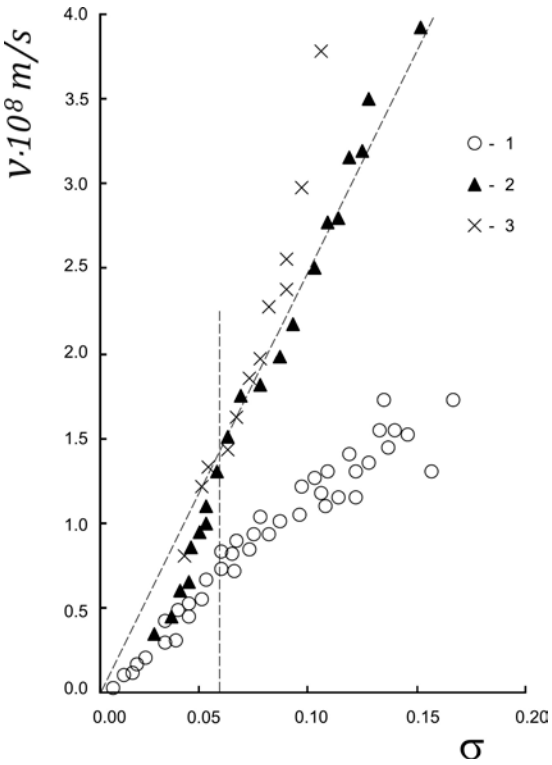


Fig. 5: Kinetics of growth of the crystal faces of alum-potassium alum in aqueous solution at active agitation, obtained with a Michelson interferometer. T = 20°C. 1 – faces {111}, 2 – {100}, 3 – {110}.

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Microbial Polymers, Natural Pesticides, and Environmental Protection from Chemical Pollutants

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Abstract- Through this article, we offer you to highlight the important and vital role of microbial polymers by identifying them, their types, and their different uses in industry and agriculture, and how to extract them from microbial environments in different ways. The interest in this topic comes from the global concern based on preserving the environment and not using chemicals represented in pesticides and chemical fertilizers and their harmful effects on the environment, climate changes and global warming, and among them comes the interest in using natural materials produced by microbes, which have a good effect on the environment and at the same time disposal Security from harmful pests using environmentally safe natural pesticides.

Keywords: microbial polymers, natural pesticides and environmental protection.

GJSFR-B Classification: DDC Code: 344.046 LCC Code: K3585



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Microbial Polymers, Natural Pesticides, and Environmental Protection from Chemical Pollutants

Amany M. Basuny [α], Moustafa A. Aboel-Ainin [σ] & Esraa Hassan [ρ]

Abstract- **Through this article, we offer you to highlight the important and vital role of microbial polymers by identifying them, their types, and their different uses in industry and agriculture, and how to extract them from microbial environments in different ways. The interest in this topic comes from the global concern based on preserving the environment and not using chemicals represented in pesticides and chemical fertilizers and their harmful effects on the environment, climate changes and global warming, and among them comes the interest in using natural materials produced by microbes, which have a good effect on the environment and at the same time disposal Security from harmful pests using environmentally safe natural pesticides.**

Keywords: microbial polymers, natural pesticides and environmental protection.

I. Polymers Definition

Polymer, any of a class of natural or synthetic substances composed of very large molecules, called macromolecules, that are multiples of simpler chemical units called monomers. Polymers make up many of the materials in living organisms, including, for example, proteins, cellulose, and nucleic acids. Moreover, they constitute the basis of such minerals as diamond, quartz, and feldspar and such man-made materials as concrete, glass, paper, plastics, and rubbers.

The word polymer designates an unspecified number of monomer units. When the number of monomers is very large, the compound is sometimes called a high polymer. Polymers are not restricted to monomers of the same chemical composition or molecular weight and structure. Some natural polymers are composed of one kind of monomer. Most natural and synthetic polymers, however, are made up of two or more different types of monomers; such polymers are known as copolymers.

II. Types of Polymers

There are several types of polymers. Among the main ones are: natural, synthetic, addition, condensation and rearrangement. For more detailed information about each, check out the descriptions below!

a) Natural polymers

Natural polymers are all those found in nature. Among the main examples are rubber, polysaccharides, starch, glycogen and proteins.

b) Synthetic polymers

Synthetic or artificial polymers are manufactured in the laboratory and generally have petroleum-derived ingredients. The best-known examples of this option are: polystyrene, methyl polymethacrylate (acrylic), polypropylene, polyethylene and polyvinyl chloride (PVC).

c) Addition polymers

This compound is obtained by successively adding monomers. As examples of these polymers, we have polysaccharides, which are formed by monomers of monosaccharides, and proteins, which are produced by amino acid monomers.

d) Condensing polymers

The condensing polymers are obtained by adding two different monomers with the elimination of a molecule of acid, alcohol or water during the polymerization process.

e) Rearrangement polymers

The rearrangement polymers are the result of the reaction between the monomers that undergo rearrangement and their chemical structures throughout polymerization. An example of this is polyurethane.

f) Biodegradable polymers

Finally, biodegradable polymers degrade into biomass, water and carbon dioxide as a result of the action of enzymes or living organisms. Under favorable conditions, they can be degraded in a few weeks.

III. History of Bacterial Polymers

The first discovery of a bacterial polymer dates back to the mid nineteenth century, when Louis Pasteur discovered dextran as a microbial product in wine124. Van Tieghem125 then identified the bacterium (Leuconostoc mesenteriodes) that is responsible for dextran formation. This discovery was followed by the

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finding, in 1886, that cellulose is produced by bacteria126. Shortly after the discovery of these exopolysaccharides, the first intracellular reserve polymers were discovered, such as the polyamide cyanophycin in cyanobacteria127 and, 40 years later, the polyester polyhydroxybutyrate in Bacillus megaterium128. Most other industrially and medically relevant bacterial polymers were found in the early to mid twentieth century, such as alginate129, xanthan130, poly-g-glutamate131 and polyphosphate132. Shortly after the discovery of the various biopolymers, the activities of their biosynthesis enzymes (either purified or in cell extracts) were described, and radioisotope-labelled precursors were also used to elucidate some details about the metabolic pathways for biopolymer formation133–140.

Between 1970 and 2000, the advent of gene-cloning techniques and DNA-sequencing methods enabled the identification of biosynthesis genes, such as the cyanophycin synthetase gene (cphA) 58, and gene clusters, such as those found in the Pseudomonas aeruginosa genome76,77,141–144. It is striking that around two decades after the identification of genes and gene clusters involved in the biosynthesis of well-established polymers (for example, cellulose and alginate) the functional assignment of essential genes is still lacking10,145. Moreover, the reaction mechanisms of key enzymes, including various synthases, synthetases and polymerases, as well as the functions of co-polymerases and polymerase subunits and of proteins involved in polymer export and secretion (such as polysaccharide transporters, secretins and translocons) are still poorly understood.

IV. Production of Microbial Polymers for Food Industry

Natural polymers can be classified as microbial-, plant-, and animal-derived based on their sources. High cost of downstream processes of plant and algal gums drives the polymer industry toward microbial derived polymers. Furthermore, microbial polysaccharides generally have higher molecular weights than plants, which affect their properties (Oner et al. 2016).

Current discoveries in microbial polymer biosynthesis have initiated new areas for medical and industrial applications. Novel molecular mechanisms and production processes have been discovered. These molecular mechanisms have formed important tools for process engineering and applications, which are getting popular in pharmaceutical, agriculture, and particularly, in food industry.

Microbial polymers are long-chained, natural, biodegradable, biocompatible, nontoxic materials and are easy to handle compared to synthetic polymers. Xanthan gum, dextran, alginate, bacterial cellulose, gellan, curdlan, levan, pullulan, glycogen are important microbial polysaccharides that can be of bacterial or fungal origin (Vijayendra and Shamala 2014).

Generally, water-soluble polymers are used as suspending, gelling, and thickening agents in food industry. Polymers can also add characteristics such as sweetening, cryoprotection, antioxidant, anticaking, flavoring, antifoaming, chelating, stabilizing, preservative, and coating (Rosalam and England 2006).

a) Production Processes for Levan

Levan is an unusual fructan homopolysaccharide composed of β-(2,6)-fructo-furanosyl residues with a terminal d-glucopyranosyl unit. Levan is synthesized from various bacteria such as Acetobacter, Aerobacter, Azotobacter, Bacillus, Erwinia, Gluconobacter, Pseudomonas, Streptococcus, Zymomonas and Halomonas sp. (Kazak Sarilmiser et al. 2015). Extremophilic and gram- negative Halomonas sp. was reported as first levan producer in 2009 by Poli et al. This system is very promising compared to mesophilic producers because it enables unsterile, low-cost production (Oner et al. 2016). Halomonas levan and its derivatives can be used as bioflocculating agent (Sam et al. 2011), adhesive nanostructured multilayer films (Costa et al. 2013), heparin-mimetic bioactive material (Erginer et al. 2016), and temperature sensitive hydrogels for drug-releasing systems with pNIPA (Osman et al. 2017) among many others.

b) Production Processes of Pullulan

Pullulan is a natural, water soluble, linear homopolysaccharide composed out of maltotriose units. Maltose molecules are linked by α (1→4) glycosidic bonds, while consecutive maltotriose units are linked by α (1→6) glycosidic bonds. Pullulan was discovered in the late 1950s and isolated froma polymorphic fungus called Aureobasidium pullulans. It has been commercially produced since 1976. This homopoly-saccharide has been used in many studies and applications involving cosmetics, pharmaceuticals, and food industries.

Pullulan is a biopolymer that is synthesized within the cell and then excreted on the outer layer after production. Like many biopolymers, the main disadvantage is a high production cost. Therefore, the research has been shifted to the use of for the production process (Prajapati et al. 2013; Wang et al. 2015; Wu et al. 2016).

Characteristics of pullulan are highly dependent on fermentation parameters, fungal strain, and its morphology. Even though many studies have been carried out to find a relationship between the morphology of the fungus and the characteristics of pullulan, no definitive evidence has been found yet. The content of the fermentation medium is crucial for the optimal polymer yield. Commercial fermentation media are composed of peptone, phosphate, and basal salts.

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c) Production Processes for Alginate

Alginate is a polysaccharide composed of β-D-mannuronate and α-L-guluronate linked by 1,4 glycosidic bonds. Alginate was initially collected from brown seaweeds and has been commercially available since the beginning of the twentieth century. Alginate is produced by several different species of brown seaweed and two different species of bacteria; Pseudomonas and Azotobacter.

Microbial production has benefits over algal production such as low cost, ability of production in small scales and applied in different fields. As mentioned previously, bacterial alginate can be obtained using Pseudomonas and Azotobacter; for commercial alginate production, human pathogen Pseudomonas aeruginosa and soil bacteria Azotobacter vinelandii are most widely preferred (Sabra and Zeng 2009; Hay et al. 2013; Ahmad et al. 2015).

Microbial production of alginate can be obtained through batch, fed-batch, and continuous fermentation. Epimerases lyases and acetyase enzymes are the important alginate modifying enzymes that were reported previously (Høidal et al. 2000).

d) Production Processes for Curdlan

Curdlan is a linear bacterial exopolysaccharide and classified as (1, 3) β-glucans. Curdlan is a special polysaccharide due to its rheological properties, solubility, and biomedical characteristics. Curdlan is named after its "curdle" competence when heated. Parameters such as pH, nitrogen, carbon, oxygen, and phosphate levels affect the production yields of curdlan. Curdlan is an extracellular polymer and biosynthesis occurs in three different steps. Substrate utilization, followed by intracellular metabolism of utilized substrate and finally excretion of polymer out of the cell membrane (Sutherland 1977, Zhang and Edgar 2014).

e) Production Processes for Gellan Gum

Gellan is a bacterial polysaccharide produced by Sphingomonas elodea. It belongs to a group of polysaccharides called sphingans, named by the bacteria from which it is produced. This biopolymer is an anionic, linear polysaccharide with high molecular weight composed out of D-glucose, L-rhamnose, and D-glucuronic acid in molar ratios of 2: 1: 1 (Tako 2015).

Production of gellan begins with the isolation of the bacterium from the surface of a plant belonging to Elodea genus. Gellan production is accomplished via fermentation with immersion method. The medium used for incubation contains nitrogen, carbon sources, and some crucial trace minerals.

f) Production Processes of Xanthan Gum

Xanthan is a complex exopolysaccharide synthesized by plant-pathogenic bacterium Xanthomonas campestris. Exopolysaccharides produced by these pathogenic bacteria have a characteristic feature of protection against adverse environmental conditions such as drying, temperature oscillations, radiation, and adhesion (Luvielmo et al. 2016).

Xanthan gum is commonly applied as a thickening and stabilizing agent in different types of food and industrial products. The process of production of xanthan includes several steps. First, the chosen microbial is grown on solid media or in liquid media and used to inoculate the culture in large bioreactors. The mode of operation, medium composition, type of bioreactor, temperature, pH, and dissolved oxygen concentration influence both the microorganism growth and xanthan production. At the end of the fermentation, cells are usually removed via filtration or centrifugation operations from the culture broth that contains xanthan, bacterial cells, and numerous other chemicals. Next step is purification, where precipitation can also be included by using water-miscible nonsolvent, followed by the addition of certain salts and pH adjustments. The product is then mechanically dewatered and dried. The dried product is milled and packed into containers with a low permeability to water.

g) Production Processes of Dextran

Dextrans are a group of homopolysaccharides composed of a linear chain of α-(1, 6) glycosidic linkages that may form branches on the main chain. It was first observed by Louis Pasteur, but this bioploymer's potential in food industry was not investigated until the 1950s. Dextran is one of the oldest bacterial polysaccharides with a multitude of functions.

Dextran is an exopolysaccharide synthesized by Streptococcus, Lactobacillus, and some Weisella species and is very sensitive to environmental conditions like substrate concentration, pH, temperature, and salinity. Because different strains of bacteria belonging to the same species can produce dextran with varying structures, it is, in theory, possible to produce dextran according to specific needs. For example, keeping the substrate levels low tends to give dextran a higher molecular weight (Das and Goyal 2014; Zannini et al. 2016; Baruah et al. 2017).

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Adoption of Occupational Safety and Health Measures in the Informal Manufacturing Sector in Kampala, Uganda

By Stephen Aurice Wekoye, Wilkister Nyaora Moturi & Stanley Makindi

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Abstract- Globalization has facilitated the rapid increase in informal employment and has been associated with the "generation of employment that is flexible, precarious and insecure". Many informal jobs are not only "flexible, precarious and insecure but are also hazardous and take place in unhealthy and unsafe environments. Informal sector workers operate in inhumane conditions and makeshift places without sanitary facilities. The cost in human terms of the existence of the informal sector and ways in which it is sustained is tragic. Enforcement and compliance with safety and health standards are unknown. There are high and tragic incidences of occupational related accidents and injuries that go unabated in Kampala. The purpose of the study was to assess compliance levels of occupational safety and health (OSH) measures among informal manufacturing sector workers in Kampala, Uganda. A cross sectional survey design was used, both qualitative and quantitative data were collected. Three hudred and eighty eight (388) firms were sampled among the manufacture of metal products, furniture, textiles and clothing, concrete and brick, paper and paper recycling, repair of machinery and other manufacturing sectors of the informal sector.

Keywords: *adoption; compliance, occupational safety health measures; informal sector.*

GJSFR-B Classification: *DDC Code: 621 LCC Code: TJ151*



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Stephen Aurice Wekoye [α], Wilkister Nyaora Moturi [σ] & Stanley Makindi [ρ]

Abstract- Globalization has facilitated the rapid increase in informal employment and has been associated with the "generation of employment that is flexible, precarious and insecure". Many informal jobs are not only "flexible, precarious and insecure but are also hazardous and take place in unhealthy and unsafe environments. Informal sector workers operate in inhumane conditions and makeshift places without sanitary facilities. The cost in human terms of the existence of the informal sector and ways in which it is sustained is tragic. Enforcement and compliance with safety and health standards are unknown. There are high and tragic incidences of occupational related accidents and injuries that go unabated in Kampala. The purpose of the study was to assess compliance levels of occupational safety and health (OSH) measures among informal manufacturing sector workers in Kampala, Uganda. A cross sectional survey design was used, both qualitative and quantitative data were collected. Three hudred and eighty eight (388) firms were sampled among the manufacture of metal products, furniture, textiles and clothing, concrete and brick, paper and paper recycling, repair of machinery and other manufacturing sectors of the informal sector. Data on hazards and practices were obtained using questionnaires and International Labour Organization (ILO) adapted workplace checklistwith a response rate of 92%, secondary data got fromOSH Conventions, Acts, textbooks and government reports. Data was analyzed into frequencies and percentages. Various types of hazards were identified; inadequate ventilation (50.4%), optical radiation (44%), extreme weather (37.4%), extreme heat (34.3%), extreme noise (27.9%)in manufacture of metal products. Noxious gases (26.7%) and paints (19.5%) in furniture and metal products while sharps (21.3%) were in manufacture of metal products. Ergonomic hazards, heavy lifting (19.5%) in metallic products and psychosocial hazards such as stress accounted for 30.5% in metal products. Compliance with OSH control measures was low, however Personal Protective Equipment (PPE) usage was 65.4% and 61.6% applying OSH control measures in the informal sector. Creation of awareness through mass media, training and awareness, provision of OSH regulations and regulation by government were recommended.

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I. Introduction

Globalization has facilitated the rapid increase in informal employment and has been associated with the "generation of employment that is flexible, precarious and insecure" (Lund and Nicholson, 2003).A large number of workers worldwide work informally, yet the discipline and practice of OSH covers largely only formal workers in the formal workplaces (Lund, Alfers and Santana, 2016).It has been pointed out that the cost in human terms of the existence of the informal sector and ways in which it is sustained is tragic. Workers in the informal sector activities often toil for low wages, under poor and inhumane conditions and unhealthy environments. Enforcement and compliance with safety and health standards are unknown (Karanja, Muchiri and Muruka, 2003).

Many informal jobs are not only "flexible, precarious and insecure but are also hazardous and take place in unhealthy and unsafe environments (Burton, 2010). Informal sector workers operate in inhumane conditions and makeshift places without sanitary facilities. Examples of such environments include road reserves, informal market places, wetlands/ marginal lands and poorly serviced homes, all of which can expose workers to environmental hazards, disease, traffic accidents, fire hazards, crime, assault as well as weather related discomfort and musculoskeletal injuries. Despite the risks involved, due to unconventional nature and location, informal workers in most African countries are not protected by institutions mandated to protect them. Conventional OSH institutions have been designed to protect formal workers in the formal sector environments (Alfers, Draft, Joronen, Oluranti, Surienty, Sains and Tse, 2012). Informal workers operate in typically non-standard workplaces excluded by definition from OSH protection measures. The study will provide data in Uganda; unregulated informal sector for redress by providing data to policymakers and duty bearers to increase adoption levels within the sector. The purpose of the study was to assess the compliance of OSH measures in the informal manufacturing sector and identify interventions for mitigation in Kampala City.

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II. Literature Review

Promoting safety begins with having a published company safety policy. The policy should make it clear that safe work practices are expected of all employees at all times. It serves as a foundation on which other promotional efforts are hinged. One of the keys to safety promotion at work is to involve the employees. They usually know better than anyone else where the hazards exist. In addition, they are the ones who must follow and implement safety rules. Safety training is one of the best ways to promote safety in workplaces. Initial safety training should be part of the orientation process for new employees, which should be aimed at developing new, specific in-depth knowledge and at reviewing or updating existing knowledge. Another way to promote safety at workplaces is through the use of safety committees. These provide a formal structure through which employees and management can channel concerns and suggestions about safety and health issues. Uganda's Occupational Safety and Health Act (OSH Act, 2006). requires firms with five and above employees in a workplace to set up safety committees and only requires a safety policy when the firm or company has a staff of more than 20 workers, yet most of the enterprises in the informal sector have less than five at the workplace.

There are three main approaches that have emerged to manage hazards at workplaces and these include; safe person, safe place and safe systems. Safe person strategies involve techniques that focus on equipping the person with knowledge and skills to avoid creating dangerous scenarios in the first instance or with the ability to deal with unsafe situations should they arise; communicating awareness of situations that have the potential to cause harm or with the recovery of the person after an illness or injury experience whether it being physical or psychological. Due to the complexity of issues associated with the human factor, a wide range of treatment options can be listed like; pre-employment screening, training needs analysis for competency, awareness and refresher training, continous education, networking, awareness of fatigue, employee assistance programs, health promotion and vaccination, use of PPE and application of behavioural based safety (Makin and Wnders, 2008).

Safe place strategies are underpinned by risk assessment process and the application of the hierarchy of controls up to the point where alterations are made to the existing physical environment. They include arrangements for abnormal emergency situations, monitoring and supervision. Safety system strategies refer to situations of leadership and direction in putting up safety systems in place that may include setting OSH policy, safe procurement criteria, incident investigation as well as having preventive and reactive mechanisms in place (McSween, 1995). Safety system strategies may require regular feedback and open communication(Makin and Wnders, 2008). Setting work place policies and carrying out incident investigations as well as feedbacks and open communication may not be feasible in the informal sector.

A country needs to develop a national OSH policy. Such a policy should aim at promoting and advancing at all levels the rights of workers to a safe and healthy environment; assessing and combating occupational risks and hazards at source and developing a national preventative safety and health culture. This can be followed by a national OSH system which comprises of all the infrastructure, mechanisms and specialized human resources required to translate the principles and goals defined in the national OSH policy. Lastly, the country should develop and implement a national OSH program. The national OSHprogram is the strategic program with predetermined time frame, which focuses on specific national priorities for OSH, identified through the analysis of the National OSH System and upto date national profile. The aim of this programme is to promote, develop and maintain a preventative OSH culture and bring about continuous improvements to the weak or ineffective elements of national OSH system(Makin and Wnders, 2008).

Practice of safety and health measures is affected by the barriers to good standards of OSH which include complexity- a situation where employees usually become unhappy with the amount of information available on safety and health. This may not be tailored to them including the red tape procedures to perform simple jobs. Regulation requirements can become overwhelmingly difficult to understand when poorly communicated. Competing demands to meet production targets or keep within budgets may compromise safety and health of workers while behavioural issues particularly changing workers' attitudes and behaviour to work safely is one of the biggest challenges in safety and health at work. Therefore, to achieve high levels of safety and health requires a safety and health culture. These can be through proper and competence recruitment, training, supervision, monitoring and evaluation of workers supplemented by a system of accountability and enforcement where institutional failures at workplaces are addressed (Hughes and Ferrett, 2011).

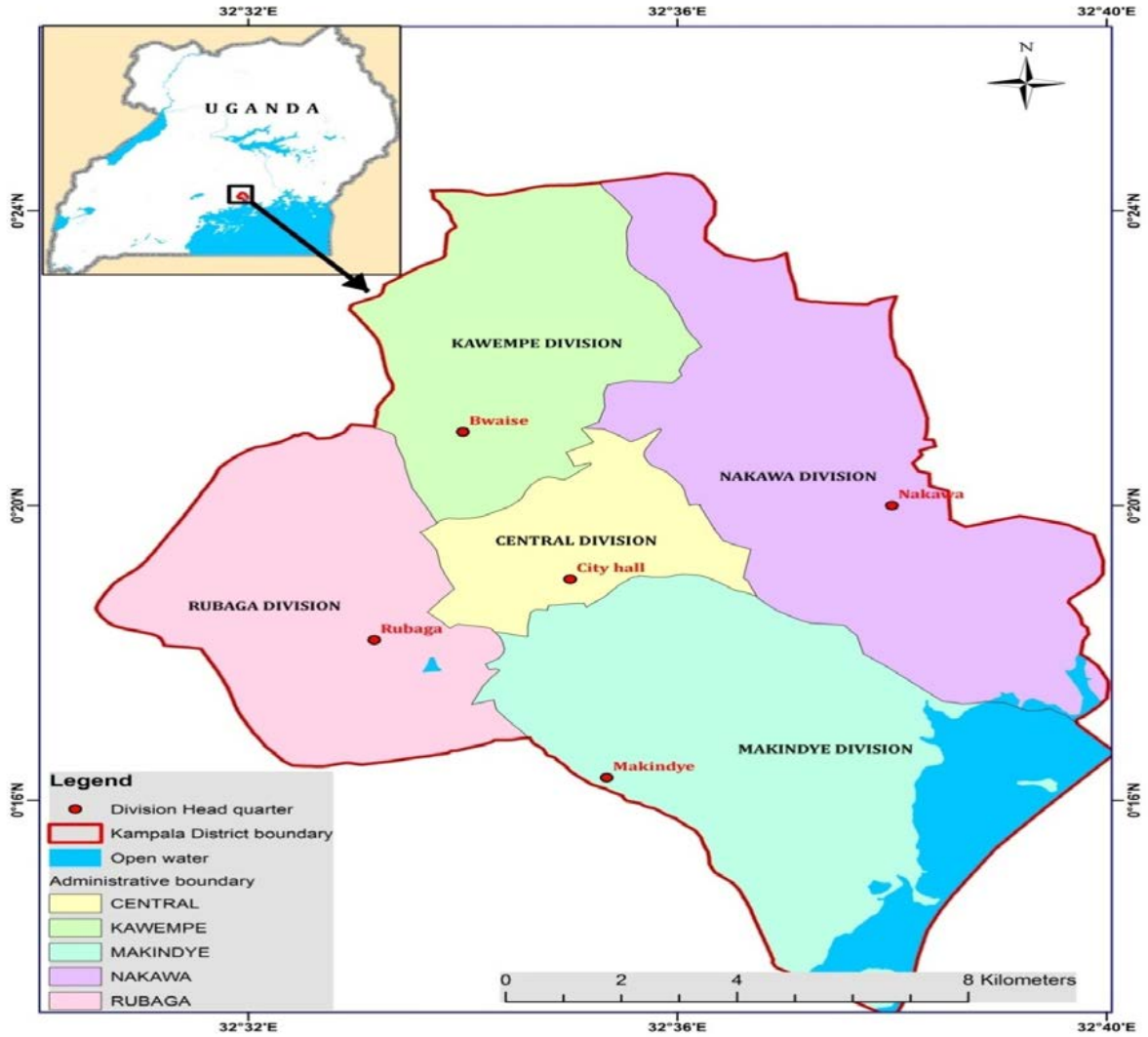
III. Materials and Methods

a) Study design and setting

The study employed a cross-sectional survey designin Kampala City. Kampala City lies on Latitudes 00° 18' 49" North of the Equator and Longitudes 32° 34' 52" East of Greenwich. It is bordered by Wakiso district in the south, west and north, Kira Municipal Council in the east and Lake Victoria in the South. Administratively

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Kampala is divided into 5 Municipalities which include; Kampala Central, Nakawa, Kawempe, Rubaga and Makindye, It covers a total surface area of 189 Km^2 of which 169 Km^2 island and 19 Km^2 water (Kampala Capital City Authority, 2016). Figure 1) below shows the study area boundaries comprising all the divisions of Kampala City. Inset is the map of Uganda.



(Source: KCCA GIS Dept., Uganda)

Fig. 1: Adminisrative map of Kampala City showing the study areas

b) Sampling

The study population comprised of 8,652 enterprises in the key sectors (clusters) based on the Census of Business Establishments (COBE) for Uganda Uganda Bureau of Statistics (UBOS, 2014).Cluster sampling technique was used to select the enterprises among the clusters on which simple random sampling was done to get the study enterprises. These included; the manufacture of metal products, textile and clothing, bricks and concrete products, repair of equipment and machinery, recycling of paper and paper products and other manufacturing. The actual enterprises were selected proportional to size at the enterprise level.(those which are many gave more respondents).

The sample size of the study was determined using the formula that yielded a representative sample meant for large populations(Singh, and Masuku, (2014).

$$n = \frac{Z^2 pq}{e^2}$$

Where *n* is the sample size

Z^2 is the abscissa of the normal curve that cuts off an area **α** at the tails (1- **α** equals the desired confidence level is 95% (1.96)

e is the desired level of precision (0.05)

p is the estimated portion of an attribute that is present in the population equal to 0.5 and *q* is the *1-* p

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Therefore the sample size n $= \frac{Z^2pq}{e^2} = \frac{(1.96)^2(0.5)(0.5)}{(0.05)^2} = 388$ enterprises.

Inclusion criteria consisted of those enterprises with less than 5 employees and willing to participate in the survey. The owner of the enterprise was the target for interview. Where both the owner and employee was available then one employee was selected using simple random sampling and interviewed by the research assistants using the questionnaires The study excluded workplaces that were not involved in some sort of manufacturing products from raw materials. People who were not employed in the sector like students and apprentices, who had worked for less than one month and those who declined to participate in the study. all participants signed a consent form.

c) Data collection

Relevant information for the study was obtained from primary and secondary sources. Secondary data were obtained from relevant literature such as Scholarly articles, OSH Conventions, Annual reports, Acts of Parliament and textbooks. The primary data was obtained through the field survey using the interviewer-administered piloted questionnaires due to the ability to generate reliable and valid data from a population within a short period at minimum cost. A walk-through survey using the ILO adapted checklist was applied on the sampled firms to identify types of hazards and control measures at work places as well as environmental hygiene conditions (Kogi, 2012). Checklists are practical instruments for investigating and improving policy for workplace safety and health as well as ideal for rapid risk assessemt when inspecting important arears of a workplace for purposes of determining planned measures. Compliance of OSH measures was measured on Likert scale. 1= Never, 2= Rarely, 3= Sometimes, 4= Often, and 5= Always. Answers 1-3 were summed up to indicate no or unsatisfactory compliance while 4-5 indicated yes or satisfactory compliance of OSH measures at work Obstacles to OSH were assessed using 5 questions. On a scale of 1-5 (1= Not a problem, 2= Minor problem, 3= Moderate barrier, 4= Serious barrier and 5= Very serious barrier) for the severity of the obstacles. The respondents were asked to suggest the possible solutions to improve OSH at work. The variables were; use PPE, always comply with safety practices at work, always comply with hazard control measures, ask for OSH information, comply with reporting of incidents and unsafe practices, and comply with audit exercises. Additional information was got from key informants in the Ministry of Gender, Labour and Social Development, Kampala Capital City Authority (KCCA), National Organization Trade Unions (NOTU) and Federation of Uganda Employers (FUE). Field checking of questionnaires was done after the field interviews, errors were immediately verified and corrected daily.The study duration was 4 months from May to August 2018 and comprised of a sample size of 388 enterprises.

d) Statistical Analysis

Descriptive statistics were generated using statistical software for social sciences (SPSS) Windows, Version 20.0 (Armonk, NY: IBM Corp) for the demographic variables. Percentages and frequencies were reported in tables and graphical forms. Other variables were tabulated and presented in percentages.
Ethical approval of the study protocol was done by the Makerere University School of Social Sciences Research Ethics Committee, Ref No. MAKSS REC 11.17.09 and Uganda National Council for Science and Technology. Permission was also sought from the Ministry of Gender, Labour and Social Development and Kampala Capital City Authority. Participation of the study population was voluntary and each research participant signed a written informed consent form.

IV. Results

a) Socio-demographic characteristics of the respondents

Table 1 below, shows the demographic characteristics of espondents in the study area. It shows the, gender, age, marital status, education of respondents, number of eployees, the period of work, the working hours per day and working hours per week. A total of 228 businees owners and 160 employees were interviewed with a response rate of 100%, majority of the respondents were male (67.8%) compared to their female counterparts (32.2%). A considerable proportion of the respondents (70.9%) were reported married followed by singles (26%).

Results also showed that 40.7% of the respondents were aged 30 years and below while slightly less than a quarter (20.6%) were aged 31-40 years.The average age was 31 years old. In terms of education, a half of respondents (50.5%) had attained at least some level of education equivalent to secondary level. About 26.5% had acquired primary level whereas those who possessed higher education (tertiary/ vocational) were 14.2%. The largest segment was youth 30 years and below.

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Table 1: Socio-demographic characteristics of respondents

Variable	Variable category	Number of enterprises (n=388) Frequency	Percent (%)
Gender	Male	263	67.8%
	Female	125	32.2%
Age category	30 Years & Below	158	40.7%
	31-40 Years	80	20.6%
	41-50 Years	79	20.4%
	51 years & Above	71	18.3%
	Mean age	71	30 ±2.16
Marital Status	Single	101	26.0%
	Married	275	70.9%
	Divorced	08	2.1%
	Widowed	04	1.0%
Education	None	14	3.6%
	Primary	103	26.5%
	Secondary	196	50.5%
	Tertiary/Vocational	55	14.2%
	Degree level	20	5.2%
Period working in informal sector	1-5 Years	133	34.3%
	6-10 Years	123	31.7%
	10 Years & Above	132	34.0%
No. of employees of work	0 Self / owner	228	58.8%
	1-3 Employees	82	21.1%
	4 or 5 Employees	78	20.1%
Work hours per day	1-8 Hours	61	15.7%
	9 Hours & Above	327	84.3%
Work days per week	1-5 Days	45	11.6%
	6 Days & Above	343	88.4%

More than a quarter of respondents (34.3%) had spent 1-5 and (31.7%) had 5-10 years working in the informal sector, while a related proportion of 34.0% had spent above 10 years of work in informal sector. Furthermore, most employers engaged themselves in their businesses thereby not employing workers(self employed) (58.8%) whereas employers who employed 1-3 employees were only (21.1%). Most of them (84.3%) worked for 9 hours per day. The average number of hours worked being 8±1.86 hours per day averaging 40 hours per week and 6 days (88.4%) per week with a mean working rate of 5±1.88 days per week.

b) *Environmental and industrial hygiene in the sampled premises*

About 56.2% of the workplaces were in the open operating in hot sun (no roof, no walls/ no structure housing the workers), 33.2% closed (enclosed in a structure with both roof and walls), 9.8% partially enclosed (only roof but no walls). About 77.8% operational/working space (floors) surrounding the work stations were littered with rubbish and full of waste materials, 87.1% did not regularly empty their waste containers while 83.5% of the working environment around the workplace was not swept. A situation that exposes workers to physical and biological hazards. Only 24.7% had appropriate roofs, 23.2% had appropriate walls and 6.8% had emergency exits free from obstruction.

In terms of welfare, only 4.9% had sanitary facilities, 3.6% had resting facilities and 2.1% running water. Fire precautions were almost non-existent with only 6.4% having fire provisions.

c) *Occupational safety and health hazards identified in the study area*

Table 2, below analyses the with types of the premises agnaist physcical hazards. The specific physical hazards include the extreme heat, extreme weather, extreme noise, excessive optical radiation, unsuitable lighting, inadequate ventilation body vibration and slippery floors in the informal manufacturing sector in Kampala City.

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Table 2: Physical hazards in the sampled premises in the informal sector n=388

Type of premises	Physical hazards							
	Extreme heat % (n)	Extreme weather % (n)	Extreme noise % (n)	Excessive optical radiation	Unsuitable lighting % (n)	Inadequate ventilation % (n)	Body vibration % (n)	Slippery floors % (n)
Metal fabrication and welding	34.3% (71)	37.4% (88)	27.9% (79)	44% (55)	39.3% (48)	50.4% (66)	5.5% (15)	12.6% (36)
Manufacture of Furniture	27.1% (56)	18.7% (44)	21.6% (61)	32.8% (41)	15.6% (19)	39.7% (52)	9.1% (25)	4.9% (14)
Textiles and clothing	15% (31)	11.5% (27)	14.1% (40)	18.4% (23)	18% (22)	7.6%(10)	7.3% (20)	11.9% (34)
Concrete and brick making	18.4% (38)	8.1% (19)	25.4% (72)	37.6% (47)	0%	0%	6.9% (19)	5.6% (16)
Paper making and recycling	5.3% (11)	9.8%(23)	21.6% (61)	24% (30)	33.6% (41)	18.3% (24)	12% (33)	7% (20)
Repair of machinery and equipment	0%	6.9% (19)	0%	0%	0%	0.0%	0%	0%
Other manufacturing	14% (29)	8.1% (19)	11.7% (33)	8.8% (11)	17.2% (21)	10.7% (14)	2.9% (08)	12.9% (37)

Source: Field data

The most hazardous subsector was manufacture of metal products with inadequate ventilation and excessive optical radiation (50.4% and 44.0) of the sampled premises. Other rampant physical hazards were; extreme weather (37.4%) of the metal production and inadequate ventilation (39.7%) in the manufacture of furniture.

Table 3, below gives an analysis of the major forms of chemical hazards in the types of premises. thse include the metal fumes, solvents used, chemical paints and gase generated. in the sampled premises in the informal manufacturing sector in Kampala City.

Table 3: Chemical hazards in the sampled premises n=388

Types of premises	Metal fumes % (n)	Solvents used % (n)	Chemical hazards Chemicals /paints used % (n)	Gases generated % (n)
Manufacture of Metal products and welding	13.8% (41)	8.6% (27)	15% (51)	26.7% (91)
Manufacture of Furniture	16.2% (48)	16.9% (53)	19.5% (66)	21.1% (72)
Textiles and clothing	4.7% (14)	2.9% (09)	3.8% (13)	11.7% (40)
Manufacture of Concrete and brick Products	8.8% (26)	4.8% (15)	5% (17)	23.5% (80)
Paper making and recycling	0%	10.5% (33)	14.5% (49)	7.9% (27)
Repair of machinery and equipment	5% (17)	0.0%	0.0%	2.6%(10)
Other manufacturing	13.8% (41)	5.4% (17)	7.7% (26)	6.5% (22)

Source: Field data

Chemical hazards included hazards mainly in the furniture industry comprised of chemicals/paints (19.5%) in maunufacture of furniture and fumes 23.5% in brick making. Chemical hazards prevalent in the manufacture and fabrication of metal products, gases generated from welding were 26.7%, while concrete and brick making was 23.5% of the sampled enterprises.

Tables 4, below addresses the types of premises agnaist the mechanical hazards prevalent in the informal sector. These comprised majorly of mechanical sharps or mechinalcal edges and high powered force

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Table 4: Mechanical hazards in the sampled premises n-388

Types of premises	Mechanical hazards	
	Mechanical sharps/ edges % (n)	High powered force % (n)
Metal fabrication and welding	18.5% (67)	25% (86)
Manufacture of Furniture	21.3% (77)	19.2% (66)
Textiles and clothing	6.6% (24)	13.1% (45)
Concrete and brick making	13.8% (50)	4.4% (15)
Paper making and recycling	5.8% (21)	10.2% (35)
Repair of machinery and equip	15.0%	0.0
Other manufacturing	5.2% (17)	5.5% (20)

Source: Field data

Mechanical hazards were prevalent in metal fabrication and welding sector with high powered force (25%) and mechanical sharps /edges (18.5%) of the sampled premises.

d) Compliance of occupational safety and health practices in the study area

Figure 2, below looked at the levels of compliance with occupational safety and health measures at work in the sampled premises in the informal manufacturing sector in Kampala City. Cpmliance was assessed on the use of PP, application of safety measures, asking for OSH information and carrying out of audit exercises in the informal manufacturing sector in Kampala City.

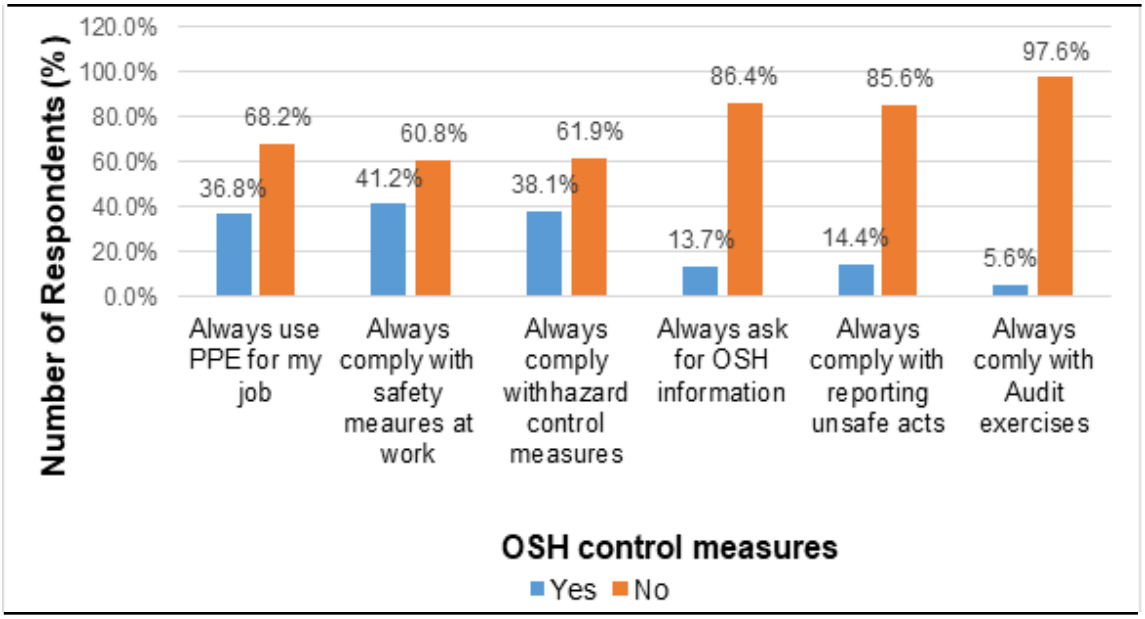


Figure 2: Respondents' compliance withOSH measures

Compliance of occupational safety and health measures was generally poor with below 50%. A high proportion of respondents (60.8 %) did not comply with safe work practices, and 85.6% did not comply with reporting of incidents and unsafe acts.

e) Suggestions on possible solutions to improve occupational safety and health at workplaces in the study area

Figure 3, below summarises the possible solutions to improve occupational safety and health in the sampled premises in the informal manufacturing secotor in Kampala City. Using a scale of 1-5, (1= Not a priority, 2= Low priority, 3 = Medium priority, 4= High priority and 5= Essential), employers listed the possible solutions to improve the glaring occupational safety and health situation being marred by the obstacles aforementioned. The response on solutions to occupational safety and health included; training, provision of PPE, collaboration with government, upgrading of equipment and technology and vocation training.

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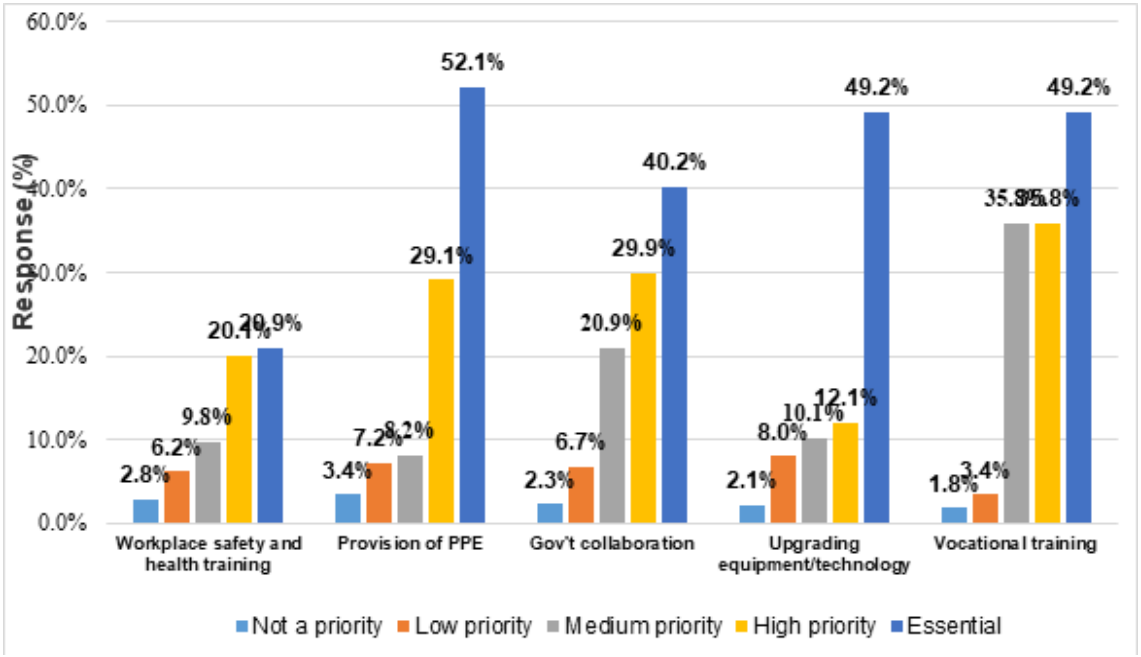


Figure 3: Possible solutions for improvement of occupational safety and health at workplaces in the study area

Respondents revealed that provision and use of more PPE (52.1%) was essential in the control of hazards of occupational safety and health at workplaces in the informal manufacturing sector in Kampala City. They also agreed that upgrading equipment/technology and vocational training and government collaraboration respectively.

V. Discussion

The socio demographic results compare with similar study where a number of males being higher than females is consistent with a Lagos study in Nigeria where the majority of respondents (90%) were male and 10% were females (Adebola, 2014). a disparity that could primarily be a reflection of the economic gender disparity in the developing countries with the fact that informal sector manufacturing is risk averse and male dominated. Women are more likely to be outworkers, unpaid contributing family workers and less likely to be employers. Females are more likely to work at homes or on streets rather than workshops or factories (Chen, 2016).Working hours were found to be 40 hours per week which was similar to the formal sector. This was however lower than those found in that operated at 55hours per week in Kenya. In a similar study in Kamukunji, Kenya, (Keitany, 2014) found the same age for the workers below 35 years in a small scale metal industry. The high number of respondents aged below 30 years is expected as it is the point of career life (youth stage) that most workers start entering the job market. These are mainly the unemployed youth who comprise the biggest segment of the population, cannot find jobs in the formal sector due to lack of prerequisite skills and vocational training.

The findings concur with the Auditor General's Report in Uganda, where an audit of the Department of Occupational Safety and Health (DOSH) in 50 sampled enterprises in Uganda found out that 80% of the enterprises were non-compliant with written OSH policy, 82% were without clear fire exits, 72% had no fire alarms in place, 84% did not carry out fire drills to their staff and 80% without First Aid boxes on the premises. The general situation was that only 20% of the enterprise having adequate occupational safety and health services in Uganda (Government of Uganda, 2016). Thisis attributed poor adopted on inadequate staffing, inadequate awareness and sensitization, limited logistics, absence of national OSH policy and lack of OSH Laboratory to analyze exposure measurements and test PPE equipment.The results on occupational hazards in the informal sector are also consistent with a similar study by the National Institute of Labour Protection in Vietnam. (Nguyen, 2010).

[17]that showed 70% of production workshops being unsafe and 80-90% dirty. This is however, in contrast with a study in Nairobi that found adequate drinking water (97.7%) at the workplace, food cafeteria available (98.5%) and resting space inadequate (38.6%) at workplaces. While toilets were also adequate (98.1%) but waste disposal was inadequate (only 8%) and drainage (12.3%) (Keitany, 2014). The situation in Nairobi was in a regulated formal setup with enforcement and supervision of OSH services as compared to the unregulated informal sector in Uganda.

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It was evident that most of the enterprises operate in the open and do not have the necessary OSH services and facilities. In most developing countries the informal sector is found in marginal lands usually not regulated and without municipal services, this exposes workers to unhealthy and unsafe environments. Lack of regulations mean that the informal sector operates in the dark without OSH monitoring and supervision. The sector is poor and cannot access funding from government and other agencies making them to have poor or no facilities which in turn affect OSH. A study in Accra and Takoradi, Nigeria by Alfers (2009), found out that market fires, poor sanitation, lack of sufficient storage facilities, physical and psychological effects were among the major hazards affecting the informal economy.

On compliance with OSH measures, the results are in contrast to a study in Lagos, Nigeria (Adebola, 2014), found high compliance with safe practices among respondents (92.3%), while 79.6% complied with the use of personal protective equipment. The contrast is attributed to informal sector in Uganda being unregulated with no enforcement to encourage high adoption of OSH measures compared to the formal sector. A study in Oyo State Nigeria revealed that knowledge, attitude and compliance with preventive measures were good among those who were more recently employed in the industry. This was however contrary to findings in the same study which showed 93.7% of those who had spent more than 6 years having good adopted with OSH measures(Onajole, Odeyemi, Ogunowo, Onwetuelo and Oridota, 2004).

Compliance with safety and health measures is affected by the barriers to good standards of OSH which include complexity- a situation where employees usually become unhappy with the amount of information available on safety and health which may not be tailored to them and the red tape procedures to perform simple jobs. Regulation requirements can become overwhelmingly difficult to understand and poorly communicated. Competing demands to meet production targets or keep within the budgets may compromise safety and health of workers, while behavioural issues particularly changing workers' attitudes and behaviour to work safely is one of the biggest challenges in the safety and health at work. Therefore, to achieve high levels of safety and health requires a safety and health culture and enforcement. These can be through proper and competence recruitment, training, supervision, monitoring and evaluation of workers supplemented by a system of accountability and enforcement where institutional failures at the workplaces are addressed [9].This compares with a similar study in the United Arab Emirates that revealed that despite the workers knowledge of occupational hazards, the use of personal protective equipment was very low [13]. In the textiles and clothing manufacture, workers engaged in garment manufacturing sectors had high knowledge of health problems related to their occupation, had good knowledge on the importance and use of personal protective equipment and their benefits but very few workers complied to such measures (Parimalam, Kamalamma and Ganguli, 2007).

OSH legislation sets out specific standards on government policies regarding practices in work places and determines the extent of the punishment meted out against offenders. The author concludes that government laws and regulations have a strong influence on the extent to which firms implement OSH programmes (Ndegwa, Guyo, Orwa, Ng'an'ga, and Murigi, 2014). However in contrast in other studies it has been urged that legalizing health and safety standards in the informal workplace has little relevance because most workers in this sector are either self-employed or work within small bands with little additional resources to meet legislative demands. They suggest that policy frameworks should instead focus on raising awareness, providing technical expertise in hazard management, providing resources for control measures and providing medical expertise for medical surveillance, diseases diagnosis and management (Naidoo, Kessy, Mlingi, Peterson, and Mirembo, 2009).

Small businesses do not consider OSH a priority. This means that safe practices do not depend on knowledge and attitude alone but related to availability of appropriate personal protective clothing and equipment, being constantly informed about safety precautions coupled with effective supervision for their use. The lack of supervision and auditing, non-seeking of information makes implementation to be latent and a time bomb since there is lack of enforcement both on the employer and the regulatory agency.

The informal sector adoption results concur with a survey carried out in small enterprises in Canada comprising of 103 manufacturing metal products and 120 in the garment sector, 37% of the employers considered the cost to be an obstacle while 30% thought that lack of training, prioritizing of production than safety and lack of time to be barriers to safety(Champux, and Brun, 2003). Investing in the safety and health of workers will try to minimize its internal production costs as the provision of safety gadgets and decent work environment involves costs that must be paid by the firm with expectations of receiving benefits of such investments in form of higher productivity (Ogunrinola, Fadayomi, Amoo and Sodipe, 2012). The equilibrium level of safety is the point where the rising marginal cost of job safety intersects the downward sloping marginal benefits from job safety(McConnell, Brue and Macpherson, 2010). However, evidence in most countries has shown that the level of safety attained is affected by the low level of investment in safe working environment by private firms and hence has

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motivated the public to intervene to reverse the trend. This is the actual situation in the informal manufacturing sector which has low investments and cannot easily invest in OSH and hence requires public intervention.

Cost of OSH preventive measures for instance in terms of buying Fire extinguishers, training, First aid, welfare facilities, decent work environments is the most important obstacle affecting the implementation of OSH measures at work in Kampala due to the fact that the informal sector does not have access to external funding in government and financial institutions. Lack of information and lack of government intervention and guidance are the major may affect the sector. Government needs to intervene by regulation of the sector, providing information and training to the informal sector.

The essential measures listed in the informal sector to improve OSH are internal to the organization and can easily be achieved by creation of awareness in the informal sector. Management should demonstrate in words and actions, through policies, procedures and financial incentives, that it is committed to workers' safety and health, then supervisors and workers will respond by ensuring that work is performed safely throughout the enterprise. Total commitment on the part of management to making safety and health a priority is essential to successful OSH program(Alli, 2009). However, the situation is different in small enterprise where management is not clear, policies and procedures are non-existent and no regulation is done by government.

Similarly, it is has been urged that OSH administration requires that employers protect their workplace depending on the dangers or work place settings. It recommends the use of manufacturing or work practice control to handle or reduce risks to the minimum level possible. Personal protective equipment is usually required to be worn to minimize exposure to a variety of hazards. Although this can be feasibly enforced in formal enterprises, it is a myth in the informal sector in developing countries(Amir, Hashim, Qandee, Ishtiaq and Anam, 2017).

A number of OSH management strategies were fronted during the study to improve the situation in the informal manufacturing sector. These suggestions among other included workplace safety and health training, provision and use of personal protective equipment as well as vocational training of the workers in the informal sector. Other suggestions were upgrading the technology in the informal sector.

VI. Conclusion

Most worklaces were in the open operating in hot sun (no roof, no walls/ structure housing the workers), The state of openness exposes workers to weather extremes. Working space surrounding the work stations were littered with rubbish and full of waste materials. Welfare facilities were almost non existant. The informal manufacturing sector is affected with physical, chemical, and mechanical hazards with various risk magnitudes. Various types of hazards identified were; inadequate ventilation, optical radiation, extreme weather, extreme heat, extreme noise in manufacture of metal products. Noxious gases and paints in furniture and metal products while sharps were in manufacture of metal products. Compliance of OSH measures were very low due to lack of regulation in the sector, although use of PPE was rated high as a control measure for hazards in the workplace, the type and appropriateness was in question. However PPE alone can notgaurantee good OSH in the workplace it must be supported by other measures like safe work practices, safe person and safety culture. The challenges to adoption of OSH by workers included; lack of government guidance and support, lack of information and lack of adequate funding to invest in safety programmes and equipment.

Possible solutions put forward to control the occupational hazards were; provision of PPE, upgrading equipment, technology for example getting new and latest machinery, vocational and technical training to acquire more skills in OSH and collaboration with government and partners. There is need to initiate an intensive mass media campaign by government and partners for creation of awareness, advocacy and sensitization on OSH hazards and control measures in the informal manufacturing sector targeting employers and employees, registration of all informal manufacturing enterprises for OSH monitoring and inspection, and development of relevant OSH regulations by government. Kampala City should regulate the secotrand all companies tobe registered and inspected regularly. Workplace policies and appropriate PPE to all employees at work should be provided.

VII. Ethical Considerations

Ehtical approval of the study protocol was done by the Makerere University School of social Sciences Research and Entics Committee and the National Council of Science and Technology. Permision was sought from the Ministry of Gender, Labour and Social Development and KampalaCapital City Authority in Kampala , Ugand.

Competing interest

All the authors declare that they have no competing interests.

Disclaimer

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Availability of Data and Materials

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All the authors read and approved the final manuscript.

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The Impact of Rosemary on Cardiovascular and Hypertension Diseases: A Mini-Review

By Abdessamad Benabbou, Abdelhamid Bitari, Adyl Oussaid, Abdelouhed Oussaid, Rachid Touzani, Belkheir Hammouti & Savas Kaya

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Abstract- An estimated 17.7 million deaths are attributable to cardiovascular disease, or 31% of total global mortality, cardiovascular diseases (Hypertension) represent one of the most significant health problems of modern civilization. Stroke; and heart attack often lead to lethal outcomes; the essential problem underneath is thrombus formation. Prophylactic approaches include acetylsalicylic acid and clopidogrel therapy on the level of primary hemostasis, i.e., primary clot formation.Thus, the application of plant species, medicinal plants rich in rosmarinic acid and polyphenols, and flavonoids in the prevention of thrombus formation is of interest. The rationale of the present review is to analyse the activity of Rosmarinus officinalis in the cardiovascular system. Pre-clinical; studies under experimental conditions show that Rosemary has a marked effect on Hypertension.

Keywords: rosemary; rosmarinus officinalisl.; hypertension; cardiovascular diseases; rosmarinic acid; flavonoids.

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The Impact of Rosemary on Cardiovascular and Hypertension Diseases: A Mini-Review

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I. Introduction

Low levels of blood pressure are not considered a significant disease as they do not imply a risk to a patient's life. Nonetheless, people suffering from Hypotension, mainly chronic or constitutive hypotension, suffer from physical and psychological symptoms such as temporary fatigue and sensation of weakness that usually affect their daily life and health-related quality of life [1, 2]. Several herbal remedies have been traditionally used to treat hypotension, such as those plants rich in purine bases (i.e., caffeine, theobromine [3]), likecoffee tea (Camellia Sinensis [4]) or cola (Cola nitida or Cola acuminata [5]), or different essential oil-containing plants. Rosemary (*Rosmarinus officinalis L.*) is a spontaneous shrub growing in the Mediterranean. It; belongs to the Lamiaceae family and has been used because of its medicinal properties in the earliest times The first references cited the traditional use of rosemary oil as a tonic for asthenia relief, blood circulation, and the nervous system, chronic weakness, as then and, peripheral vascular disorders. For medicinal purposes, Rosemary oil was distilled during the Middle Ages and used as a tonic, stimulant, and carminative for dyspepsia, headache, and nervous tension, as described in the Dioscorides Materia Medica in 1555 [6,7]; as a bath additive, it has been traditionally used in conditions of exhaustion and for stimulation of circulation [8]. And, Indian Materia Medica [9], described it as having carminative and stimulant actions. The; British Herbal Pharmacopoeia (1983) lists the specific indications of "Depressive states with general debility and indications of cardio-vascular weakness" for Rosemary oil. Nowadays; rosemary essential oil has used as a brain and nerve tonic, and as a remedy for mental fatigue [10]. Several; other activities are reported in the literature: antiseptic, diuretic, antidepressant, and antispasmodic; it is also used to treat colds, influenza, and rheumatic pain [11,12] and has proved to enhance the performance for overall quality of memory and secondary memory factors [13]. Rosemary leaves contain no less than 12 ml/kg of essential oil, whose composition may vary according to the plant chemotype or other factors such as climatic conditions, geographic origin, or time of collection [14-17]. This work aims is to study *Rosmarinus officinalis* essential oil's effect on primary hypotension and so, its positive impact on the HRQOL of patients. To determine the relationship between the two types of variables in the study and to assess the effectiveness of Rosemary essential oil, statistical methods were used as a key tool. Quantitative variables (SBP and DBP), and scores from physical and mental summary measures were obtained from the SF-36 Health Survey.

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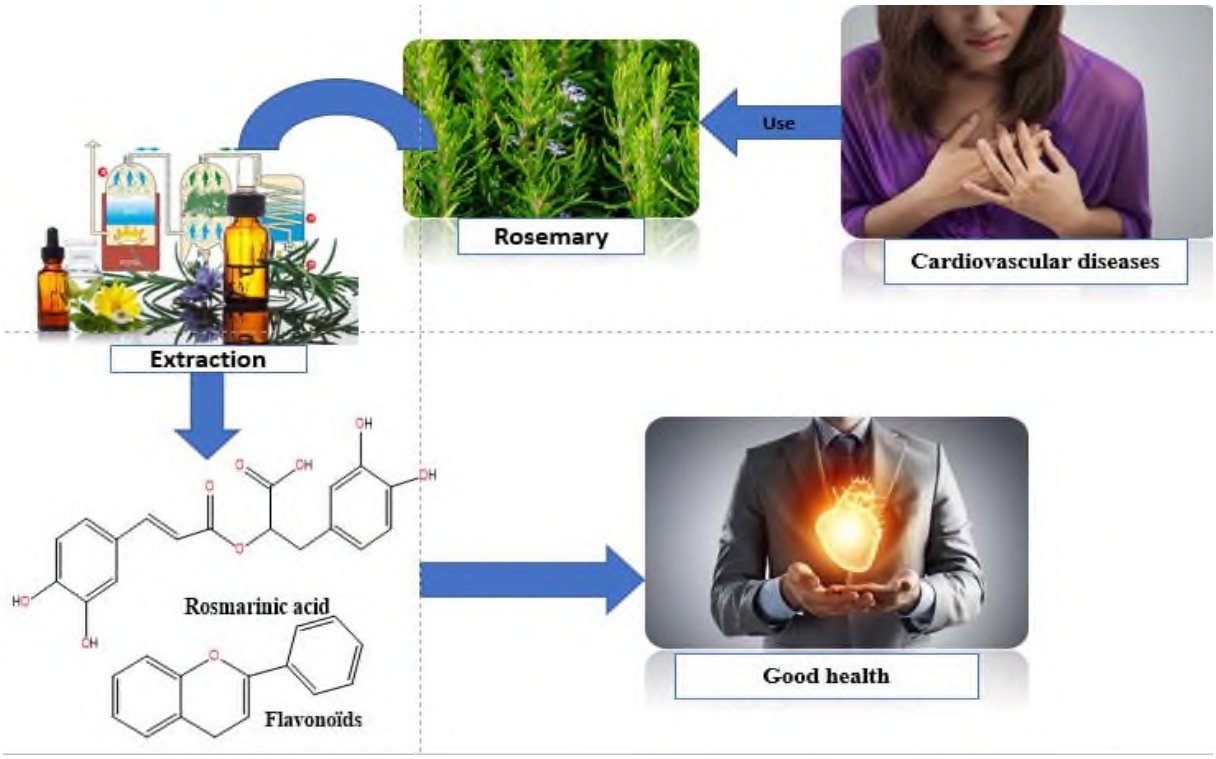


Figure 1: The Effect of Rosemary on Cardiovascular Diseases.

II. Cardiovascular Diseases

Cardiovascular diseases (CVD) [18] are disorders of the heart and blood vessels and include conditions such as Hypertension, hyperlipidemia, thromboembolism, coronary heart disease, and heart failure. The most prevalent of these is hypertension, which is a major factor in the development of CVDs [19]. According to tong, World Health Organization (WHO), over 17 million people die per year (31% of all global deaths) from CVDs). Before 2030, it is predicted that mortality will reach 23.3 million due to the rise in CVD diagnoses [20].

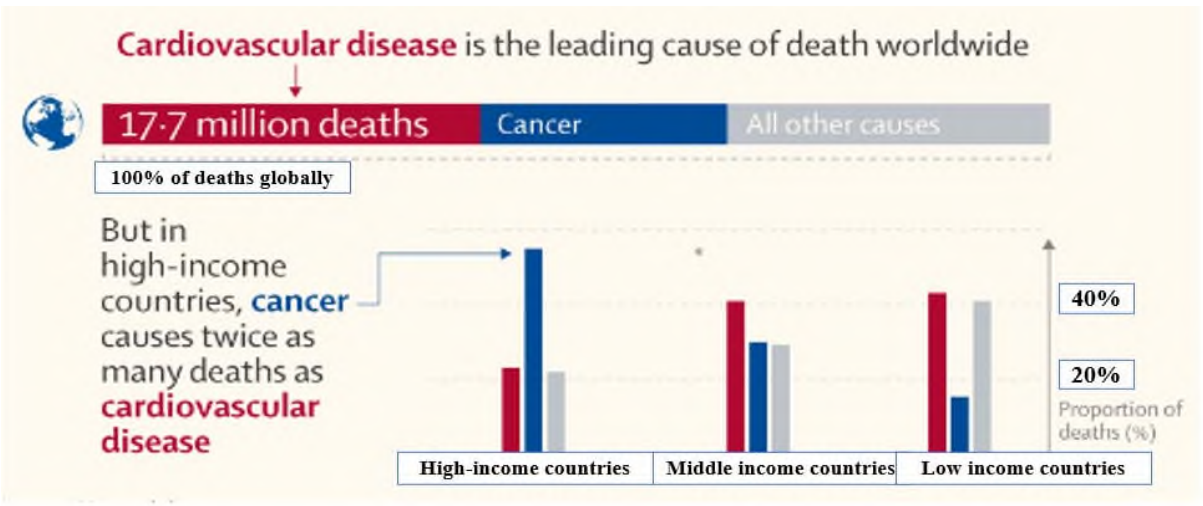


Figure 2: Cardiovascular Diseases Risk Factors and Mortality Around The World (WHO).

For instance, the most common cause of death in Morocco is cardiovascular disease. These illnesses can be divided into various categories., namely, valvulopathy, heart failure, arterial Hypertension, ischemic heart disease, cardiomyopathy, and arterial diseases. This project's goal is to describe the prevalence of cardiovascular diseases according to the age and sex of patients and to study the association between sex, age, and the appearance of different types of cardiovascular diseases [21].

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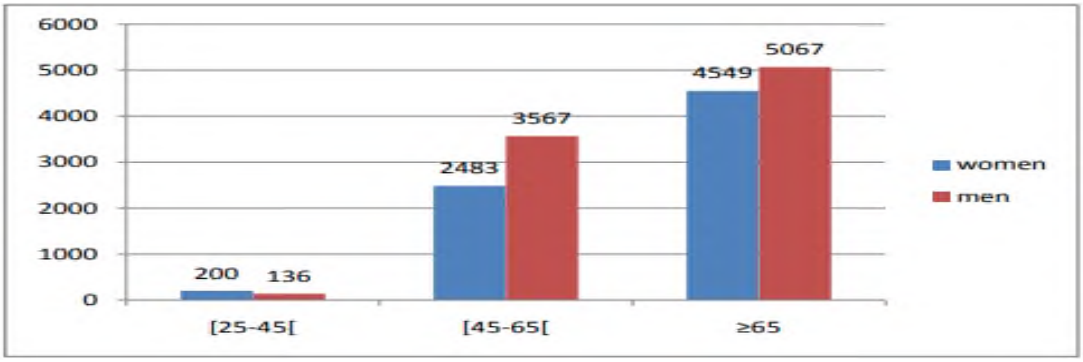


Figure 3: Distribution of Patients by Age and Sex [21]

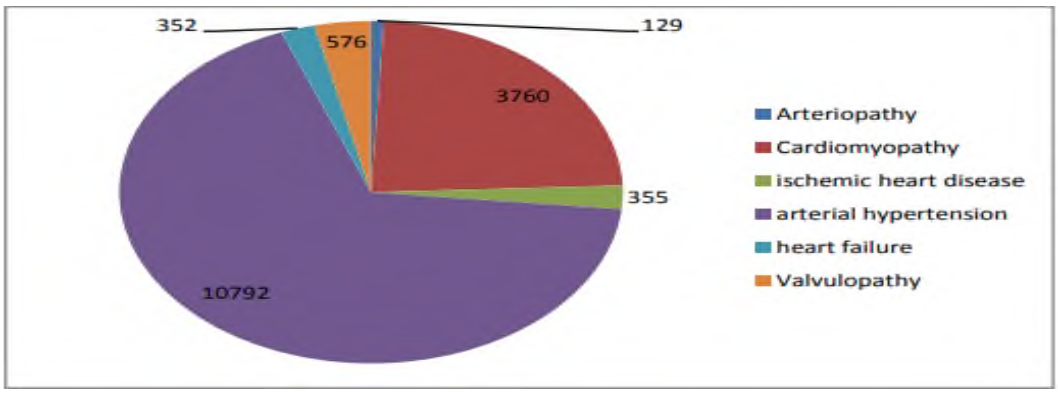


Figure 4: Prevalence of Cardiovascular Diseases during the Study Period [21]

The survey involved 16002 patients, including 7232 women (45.2%) and 8770 men (54.8%) with a slight male predominance (sex ratio = 1.21, chi-square test = 87.3, $p \leq 0.0001$). It was 70.27 16.5 years on average. Women are most affected in young adults, while men outnumber women in adults and seniors (Figure 3). Cardiovascular disease prevalence was as follows: Hypertension (67.4%), cardiomyopathies (23.2%), valvulopathies (3.6%), ischemic heart disease (2.2%), cardiac insufficiency (2%), and arterial diseases (1%) (Figure4) [21].

Cardiovascular diseases include pathologies that affect the heart and all blood vessels, such as (WHO):

- Stroke
- Atherosclerosis
- Congenital heart disease
- Myocardial infarction
- Heart failure
- Diseases of the vessels
- Heart rhythm disorders
- High blood pressure (Hypertension)

III. Hypertension

Blood pressure is the force exerted by circulating blood on the walls of the arteries, that is to say, the main vessels that allow blood circulation in the body. We speak of hypertension when this pressure is too high (WHO).

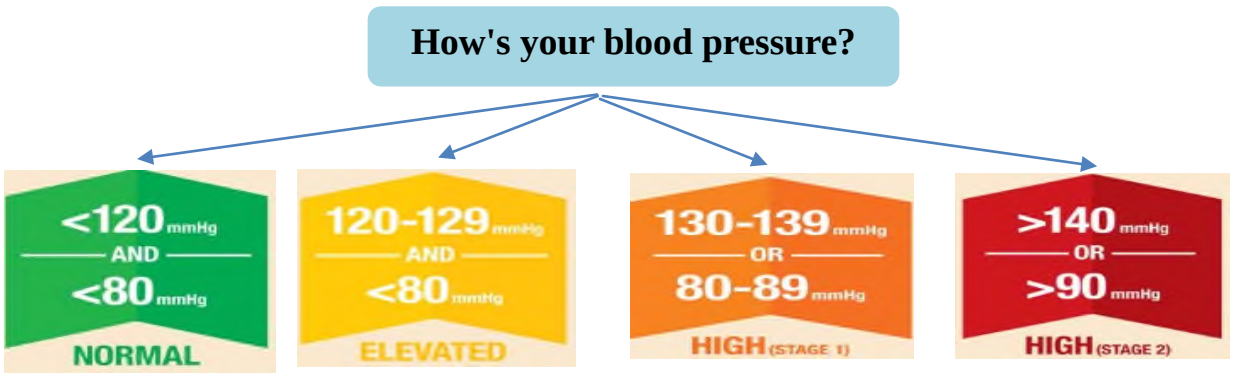


Figure 5: Chart of Blood Pressure (WHO)

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As per a statement of the World Health Organization (WHO), Hypertension is one of the silent killers in the 21st century and is one of the biggest global public health concerns. Hypertension is amajor contributor to cardiac complications, stroke, heart disease, kidney failure, blindness, premature death, and disabilities. Hypertension is curable and treatable, for which there is a need for involvement from individual entities, government and private sectors, health workers, and civil societies moreover, personal awareness is highly recommended.

As per the estimation of WHO, globally more than 1.13 billion people are affected with Hypertension among which less than 1 in each 5 is under control. Unhealthy diets, lack of physical activities, and consumption of alcohol & tobacco are the main contributing factors to Hypertension. In 2016, the Global Heart Initiative was launched by the World Health Organization and the US Centers for Disease Control and Prevention. Globally, Hypertension or High Blood Pressure leads7.5 million death cases which, share about 12.8% of all death cases recorded. Hypertension also accounts for about 57 million disabilities adjusted life years which is about 3.7% of total adjusted life years. In 2016, the Global Heart Initiative was launched by the World Health Organization and the US Centers for Disease Control and Prevention.

The Global prevalence of Hypertension in adults aged>25 was about 40% in 2008. From 1980 to 2008, there was a moderate prevalence. But, due to sharp growth in population, the aging population uncontrolled Hypertension reached 1 billion in 2008 from 600 million. The prevalence of Hypertension was highest in Africa (>40%) and lower in the Americas (35%) [22]

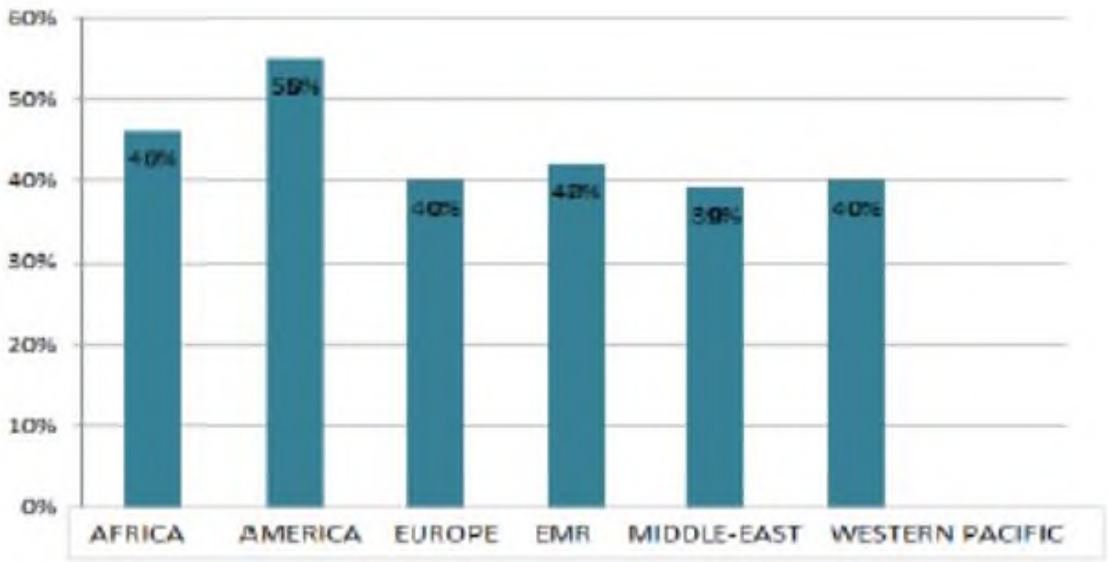


Figure 6: Hypertension Statistics World 2020 [22]

What is the risk factor?

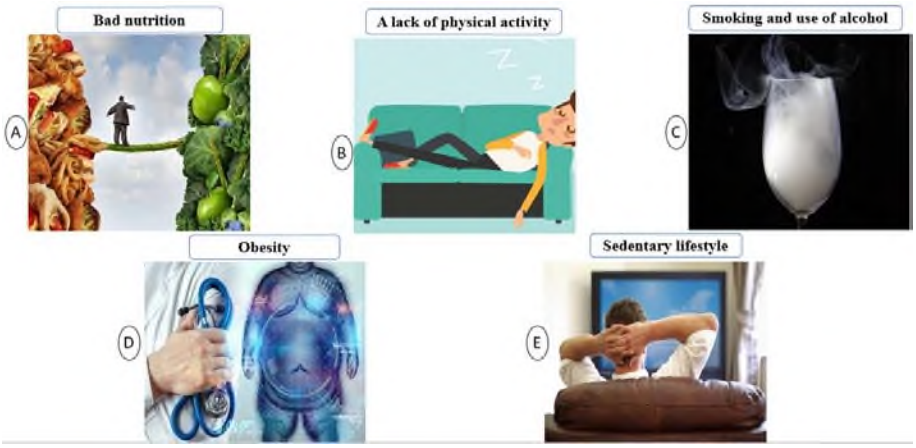


Figure 7: Factors of the Risk of Cardiovascular Disease and Hypertension (A&B [24], D [23] E [24], C [25])

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IV. Rosemary

Rosemary is a medicinal plant of the Lamiaceae family which is called in Arabic Azir and French Romarin, the scientific name of this plant is *Rosmarinus officinalis*. Rosemary, a plant Abundant in the wild all around the Mediterranean, is traditionally valued for its bioactive constituents, with antioxidant, anti-inflammatory, and anti-cancer, antifungal, antibacterial, insecticide, and hepaprotective properties, which can be removed by distillation under form aromatic oils or by extraction in solvents in the form of extract or oleoresin. Rosemary leaves contain macronutrients (Ca, K, P, Na) and micronutrients (trace elements) (Cu, Zn, Mn, Fe) [26]. They are a good source of vitamins A, B, and C [27].

Rosemary is similarly used in cosmetic formulations and in treating pathological and non-pathological conditions, such as cellulite, alopecia, ultraviolet damage, and aging [28].

a) Other Names

- Azir, barkella, haselban, Aklilljabal, klile (in North Africa) [29].
- Grass-aux-courounnes, sea dew, sea rose, rosemary of the troubadours, bouquet-de-la-vierge (France).
- Folia Anthos, Folia Rorismarini, Encensier, rosemary (Angle), Rosmarinblatter, Krankrautblatter, Kranzen-krautblatter, Rosmarein (Allemand).

Rosemary has long been known for its medicinal properties, especially by the Greeks and Romans. Later produced crowns, thus the Arabic name Iklilal-Jabal (mountain crowns), translated from Latin. In the Middle Ages, it enjoyed great prestige as a medicine for paralysis. The water of the Queen of Hungary, famous in the seventeenth century because Queen Isabella, tasty and paralytic, used it as water of youth, was nothing but rosemary alcoholate. It is also a grilling condiment. Rosemary supplies an important distillation industry in Maghreb [29]. Since Morocco has excellent potential in the field of aromatic and medicinal plants.

b) Botany

Branched, woody, 1 m tall, bushy, and aromatic shrub. The leaves are narrowly lanceolate and up to 3 cm long and 4 mm wide, causes, and friable; The edge is involuted downwards (top row). On their upper side, young leaves are pubescent, while the older ones are glabrous. They are wrinkled and streaked due to a sunken midrib, which is very prominent on the underside, and covered by a dense white pubescence. The January blooming of the flowers, pale blue or lilac, are grouped in axillary and terminal racemes in the upper part of the branches [29]. These spiciform inflorescences bear subsessile flowers in all seasons. The gamosepalous calyx, bilabiate bell-shaped, has three lobes. The gamopetalous corolla is long and tubular with a helmet-shaped upper lip with two lobes and a lower lip with three lobes. The two protruding stamens protrude well beyond the corolla; two others are reduced to square brackets. The fruit is brown achene [30]. This very polymorphic species has several varieties. But Many botanists prefer to use the chemical makeup of the essential oil to classify four chemotypes instead of this sporadic morphological differentiation., according to the dominant compound:

1. Rosemary in cineole,
2. Rosemary with verbenone,
3. Rosemary with camphor,

c) Chemical Composition

Essential Oils: 1 to 2.5% Rosemary flowering tops provide 10 to 25ml / kg of an essential oil whose main constituents are: camphor (15 to 25%), cineole (15 to 50%), alpha-pinene (10 to 25%) and borneol, free and esterified. Among other things, the essential oil's composition varies. Depending on the origin, the French pharmacopeia retains two types of products: the Morocco and Tunisia type and the Spain type. resulting from hydro-distilling natural populations, these essential oils differ slightly in their composition and physical constants [30].

Table 1: Chemical Composition of Rosemary EO [31]

Compound	Mroroccooil	Spain oil	Algerieoil
α-Pinene	12.51	24.7	5.2
1, 8-Cineole	47.44	18.9	52.4
Camphene	3.62	11.2	3.0
β-Myrcene	1.57	4.9	1.7
Borneol	2.97	4.5	3.4
Verbenene	-	-	-
Bornylacetate	0.23	1.0	1.1
Camphor	7.9	18.9	12.6
Verbenone	0.46	-	-
Verbenol	-	-	-
β-Pinene	7.2	3.4	5.7

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Linalool	0.7	1.0	1.1
β-Caryophyllene	3.31	2.2	4.2
3-Octanone	-	-	-
β -Phellanderene	-	-	-
Limonene	1.9	3.1	-
Sabinene	0.12	0.4	-

Other Chemical Compositions of Rosemary

- *Flavonoids:* luteolin, apigenin, quercetin, diosmin.
- *Diterpenes:*carnosic acid, rosmadial.
- Triterpenes and steroids: oleanolic acid, ursolic acid.
- Tannins.
- *Lipids:* n-alkanes, isolalkanes, alkenes.
- Rosmarin.
- Rosmarinic acid.

There are two ways to extract bioactive molecules from Rosemary.:

- *By conventional so-called conventional methods:* Hydrodistillation; Steam distillation; Aqueous Alcoholic Extraction by Fermentation; Maceration; Two-step extraction with organic solvents (hexane, ethanol); Extraction by pressurized liquid.
- *By new and innovative methods:* CO_2 extraction; Supercritical CO_2 extraction; Subcritical state extraction (H_2O); Extraction assisted by ultrasound; Microwave assisted extraction; Extraction by microwaves without solvents; extraction by hydrodiffusion by microwaves and gravity; Extraction by microwave vapor diffusion; Extraction by instantaneous controlled pressure drop; by Advanced Technology Phytonics (non-chlorinated fluorohydrocarbons); Extraction by High Voltage Pulse Fragmentation Technology…[32].

V. The Effect of Rosemary on Cardiovascular Diseases and Hypertension

Epidemiological and clinical studies on the influence of flavonoids on cardiovascular diseases are rare and inconsistent. The main issue outlines the evolutionary exposition of flavonoids through diet and developed mechanisms that reduce their bioavailability (transporters that reduce absorption and metabolism that increases excretion from the body). Although studies on the European population show consumption of flavonoids of more than 100 mg per day [33], the bioavailability of flavonoids is limited to up to 24 % as reported for quercetin. As the half-life of quercetin in plasma is 11 to 28 hours, it is regarded that slow elimination increases the accumulation of quercetin in the body [34]. Usually, concentrations up to 1 mol L-1 are reported for plasma [35]. According to research conducted by our team, flavonoids can have clinically significant interaction with the ristocetin and arachidonic acid-induced platelet aggregation assay. even at very low flavonoid concentrations (i.e., 60 nmol L–1) can influence platelet aggregation assays induced by arachidonic acid and ristocetin. These assays are used for the assessment of von Willebrand factor (vWF) function [36], and flavonoids can consequently cause misdiagnosis of blood clot disorders related to vWF. In a clinical study on healthy males, the influence of tomato (*Solanum lycopersicum L.*)[37] pomace extract was tested ex vivo [38]. It showed a reduction of platelet aggregation in the test induced by ADP (5 days after starting the treatment, three hours after ther last dose was consumed). While the polyphenol profile of tomato extract was analyzed by high-performance liquid chromatography coupled with mass spectrometry (HPLC-MS), no clear indication of component(s) responsible for the activity was provided. Polyphenols-enriched beverage (326 mg L^{-1} of gallic acid equivalent) reduced platelet aggregation in healthy athletes after stress (marathon)[35]. Anthocyanin consumption (320 mg per day) by healthy volunteers reduced ex vivo platelet aggregation by 29 % in platelet aggregation assay induced by ADP [38]. Nearly all epidemiological research on the impact of flavonoids on cardiovascular diseases is inconclusive and calls for more research [39]. However, one of the rare studies confirming the beneficial effects of flavonoids in cardiovascular diseases was one performed by Wang et al. [40]. Meta-analysis of published data from 1966 to 2013 shows that an increase in flavanol intake by 20 mg per day reduces the risk of stroke by 14 % in men. The same effect was not confirmed in females.

Rosmarinic acid (AR) is a purely natural compound derived from herbs belonging to the Lamiaceae family, such as Rosemary, sage, basil, and mint. These plants are widely and frequently utilized in recipes for food. Rosmarinic acid is an ester of caffeic acid and 3,4-dihydroxy phenyl lactic acid. The biological benefits of chronic RA use on cardiometabolic abnormalities have been revealed. Rosmarinic acid reduces blood pressure by inhibiting of angiotensin-converting enzyme (ACE). (ACE) [41], promotes nitric oxide production and regulates endothelin-1 (ET-1) production, and downregulates endothelin-1 (ET-1) production [42]. Chronic treatment with RA improves whole-body insulin sensitivity in fructose-fed hypertensive rats [43] and high-fat diet (HFD)-induced diabetic rats [44]. It also reversed streptozocin-induced decreases in skeletal muscle plasma membrane GLUT-

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4 content in diabetic rats [44]. However, the mechanisms through which RA increases glucose uptake need to be elucidated. Angiotensin II (ANG II) is a potent hypertensive agent. It is involved in the generation of reactive oxygen species (ROS) that activate p38 MAPK, reduce Akt phosphorylation, and decrease GLUT-4 translocation in skeletal muscles [45, 46]. The antioxidant properties of RA inhibit the production of ROS via c-Jun N-terminal kinase (JNK) and extracellular signal-regulated kinase (ERK) in a cell death model of cardiac muscle [47]. A previous study reported that ERK plays a crucial role in the therapeutic actions of RA in the hippocampus [48]. Moreover, exercise and 5-aminoimidazole-4-carboxamide-1-beta-d-riboside (AICAR) increase skeletal muscle glucose transport through the activation of ERK and adenosine monophosphate-activated protein kinase (AMPK) activities [49]. Together, RA might induce skeletal muscle glucose transport via the ERK pathway. In addition, RA could improve both cardiovascular and metabolic problems in hypertensive conditions. Therefore, this study aimed to evaluate the effects of acute and chronic RA administration on blood pressure and skeletal muscle glucose transport in rats treated with ANG II. Moreover, this study assessed the signaling pathways involved in skeletal muscle glucose transport.

VI. Treatment Regimen

The dosage of rosemary essential oil of 1 ml every eight hours has been indicated according to the German Commission E monograph on rosemary essential oil, as well as the safety profile derived from its use in its clinical and traditional use [50]. Rosemary essential oil samples were purchased by Meta pharmaceutical (Barcelona, Spain). The main components were 1,8-cineol (47.6%), camphor (13.8%), and α-pinene (11.7%), which corresponds to a Moroccan-type rosemary oil. The minor components were β-pinene, camphene, borneol, limonene, α-terpineol, p-cymene, β-myrcene, bornyl acetate, and verbenone. Rosemary essential oil and placebo were supplied in 30ml vials. made of topaz-colored glass with a dropper. Patients received the corresponding dose by dropping 1 ml on a sugar cube to avoid an unpleasant taste. Sugar cubes to avoid any unpleasant taste[51].This study is a part of many others works on the valorization of natural products started since 2006 [52-84].

VII. Conclusion

The antihypertensive and cardiovascular disease-stimulating effects of rosemary essential oil are shown in this work, along with the corresponding improvement in patients' quality of life. The study, which was conducted in a pharmacy, allowed for the evaluation of both the therapeutic efficacy and the significance of pharmaceutical care in patient compliance. The findings of this study can act as a roadmap for future investigations aimed at advancing scientific understanding of widely used plants.

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Electronic Structure of Chemical Elements Described by the Characteristic Graph of the Atom

By Ousmane Barry, Mamadou Saliou Diallo, Ousmane Wora Diallo, Mamadou Yaya Balde, Souleymane Balde & Aboubacar Safie Sylla

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Abstract- The characteristic graph of the atom makes it possible to explain the various concepts of atomics in a general way. Each concept is interpreted by specific graphics derived from the first. About the electronic structure, the particular graphs illustrating the order and the period of the subshells which obey the stability classify them in stacks according to the abscissas and the ordinates. The distribution of the electrons of the heaviest "118th" atom leads to new methods of illustrating classification called "condensed tables" with or without period and order subshells. The distribution of electrons according to the order of the energy level of the subshells denoted "OE," is based on a new criterion for the classification of chemical elements. This stems from arrays called condensed arrays to order. These condensed tables are summarized by numerical series whose informed reading makes it possible to explain the secrets of atomistic. The electronic structure can be worked out even by mental calculation. The counting from 1 to 7 or from 1 to 8 with the corresponding value series solves the development of the electronic structure. All the value series: 2, 8, 8, 18, 18, 32, 32, and 2, 2, 8, 8, 18, 18, 32, 30 made it possible to achieve this objective.

Keywords: *atomistic, electronic structure, subshells, order, period, affine equation.*

GJSFR-B Classification: *FOR Code: 0399*



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Electronic Structure of Chemical Elements Described by the Characteristic Graph of the Atom

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Abstract- The characteristic graph of the atom makes it possible to explain the various concepts of atomics in a general way. Each concept is interpreted by specific graphics derived from the first. About the electronic structure, the particular graphs illustrating the order and the period of the subshells which obey the stability classify them in stacks according to the abscissas and the ordinates. The distribution of the electrons of the heaviest "118th" atom leads to new methods of illustrating classification called "condensed tables" with or without period and order subshells. The distribution of electrons according to the order of the energy level of the subshells denoted "O_E," is based on a new criterion for the classification of chemical elements. This stems from arrays called condensed arrays to order. These condensed tables are summarized by numerical series whose informed reading makes it possible to explain the secrets of atomistic. The electronic structure can be worked out even by mental calculation. The counting from 1 to 7 or from 1 to 8 with the corresponding value series solves the development of the electronic structure. All the value series: 2, 8, 8, 18, 18, 32, 32, and 2, 2, 8, 8, 18, 18, 32, 30 made it possible to achieve this objective.

Keywords: atomistic, electronic structure, subshells, order, period, affine equation.

I. Presentation

Man has always tried to find an explanation for the complexity of the matter that surrounds him. It was first thought that the elements of matter consisted of water, earth, fire, and air. We realized over time, thanks to the improvement of experimental, chemical, and physical techniques, that matter was in reality more complex than it seemed. Nineteenth-century chemists then felt the need to order the new known elements. Mendeleev argued that in addition to periodic properties due to atomic weight, each element has its proper personality [1]. Several classifications were proposed before arriving at the current periodic table.

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The periodic table of elements, introduced by Mendeleev in 1869, was based on the following statement: "the chemical behavior of the elements is a periodic function of their atomic weight. "Currently, the periodic table is constructed as a result of the electronic structure of the elements [2]. The periodicity of electronic configurations finds its origin in the periodic values of the quantum numbers *n, l,*and*m*. Each new period starts with a new main layer [3].

To arrive at this elaborate version of the classification of the chemical elements showing the periodicity of the chemical properties, according to the atomic weight (mass), as planned by Mendeleïev, the scientists gradually penetrated the secrets of matter. By way of illustration, Joseph John Thomson will experimentally prove the existence of the electron in 1897; Ernest Rutherford discovered the atomic nucleus in 1911[3]. The discovery of the electron will prove the chemist's intuition right.

It is easy to see that the periodicity indeed corresponds to a "period" of filling of the electronic shells one after the other, conferring chemical properties more or less similar to the elements. This periodicity has made it possible to gradually and coherently build the painting, which now bears the name of its inventor. In 2010, it had 109 elements. In 2016, the International Union of Pure and Applied Chemistry (IUPAC) validated new "candidates," bringing to 118 the number of elements in the Mendeleev Table, which has become essential for anticipating the chemical properties and advancing knowledge [4].

It is important to remember that the electronic structure is the distribution of the electrons of an atom in the different sub-shells arranged in increasing order of their energy, respecting the electronic capacity of each sub-shell and especially the number of electrons (Z atomic number). This structure is done either by tables or in general order. This electronic structure poses three (3) simultaneous problems:

- The arrangement of the subshells in ascending order of their energy level;
- The electronic capacity of the sub-layers;
- The respect for the number of electrons to leave, gives rise to repetitive additions.

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The purpose of this work is to demonstrate how analytical geometry applied to the atomic model based on wave mechanics makes it possible to explain and construct the electronic structure of atoms.

In a previous article [3], it was recalled that the organization of the electronic cloud in energy levels (order) is the same for all atoms. The electronic cloud includes electronic shells subdivided into sub-shells having atomic orbitals (electronic cells). Each layer can be defined as the set of states (or electrons) characterized by the exact value of n; a subshell as the subset of states (or electrons) corresponding to the same value of n and the exact value of *l*. An atomic orbital is defined by the quantum triplet (*n, l, m*). However, the state of an electron is wholly determined by all four quantum numbers (n, l, m, m_s) [5]. Thus the distribution of electrons in the electronic cloud of a polyelectronic atom is how its Z electrons are distributed between shells, subshells, and atomic orbitals. The knowledge of Z gives few indications on the chemical behavior of an atom, but that of its electronic configuration allows, on the contrary, to explain or to predict this behavior to a large extent.It is an essential basis for understanding reactivity and the periodic table of elements.

The electronic configuration (or electronic structure) in polyelectronic atoms makes it possible to specify the distribution of electrons in the various layers and sub-layers of these atoms in the ground state according to the increasing order of the energy level. It obeys a certain number of principles which are: the principle of stability, the principle of Aufbau, the rule of Klechkowski, the principle of exclusion of Pauli,and the rule of Hund [6].

Thus the electronic structure makes it possible to divide the electrons of an atom into two groups: the "core electrons" and the "valence electrons". These represent the identity of the element. Indeed, a good reading of the valence electrons makes it possible to list all chemical elements and to develop the different concepts of chemical language, thusdescribing their periodic properties.

The characteristic graph of the atom [7], which correlates withall the literary diagrams, explains the composition of the electronic cloud. Among the various specific graphs of the electronic cloud, two are in agreement with the rule of stability; these are those which are specific to orders and periods. These classify the different subshells either in period or in order. In these charts, the sub-layers are arranged as a stack from one order to another or from one period to the next. Within a stack, the different sub-layers are classified according to the increasing order of "n" the period; this is the Aufbau principle. The different stacks follow each other as in the usual ordination of natural numbers; they are easily and logically memorized in each case.; thus expressing the highlights of atomistic. The exploiting of the characteristic graph of the atom and the Order and Period Tables presented in the previous works will allow easy assimilation of the atomistic concepts and their use for the electronic configuration of the atoms.

The objective of this work is to propose innovations simplified and concrete for the development of the electronic structure of atoms in the ground state. Its niche is to develop creative imaginations that will make it possible to work out this structure even mentally in the place of the rather abstract classical methods [8].

II. Methodology

a) *Description of the Electronic Structure*

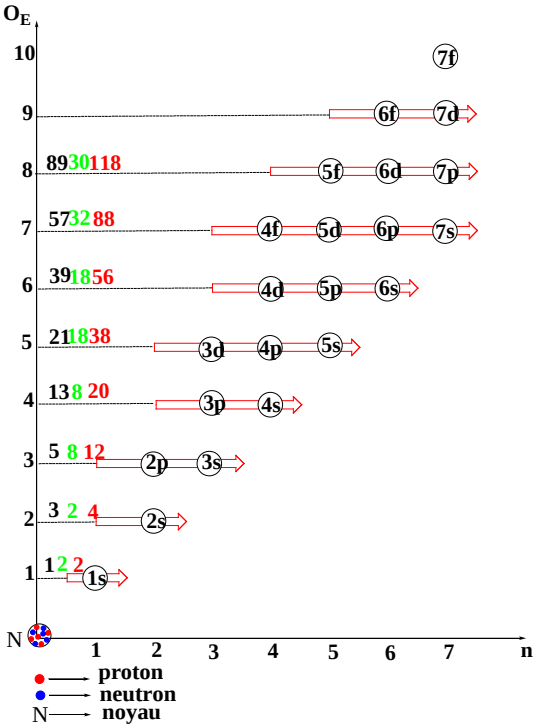
The electronic structure is the distribution of electrons on the shells and, or sub-shells of an atom under the principle of stability. The electrons saturate the sub-shells of the lowest energy levels and gradually move towards the sub-shells of the higher energy levels until the exhaustion of Z (atomic number). It is known that the process of saturation of the sub-layers is carried out under the rule of Klechkowski, that is to say, following the increasing order of O_E ($n+l$) and within the same order following "n" increasing. The different sub-layers of an order form a stack. The order of a stack is O_E, whatever the sub-layers compose it (*Aufbau principle*). Like the order, the period corresponds to a stack.

The layout of the subshells is described by graphs illustrating orders and periods [5]. From graphs 1 and 2, the distribution of the 118 electrons of the heaviest atom is carried out along the O_E axis (the order) and the n axis (the period).

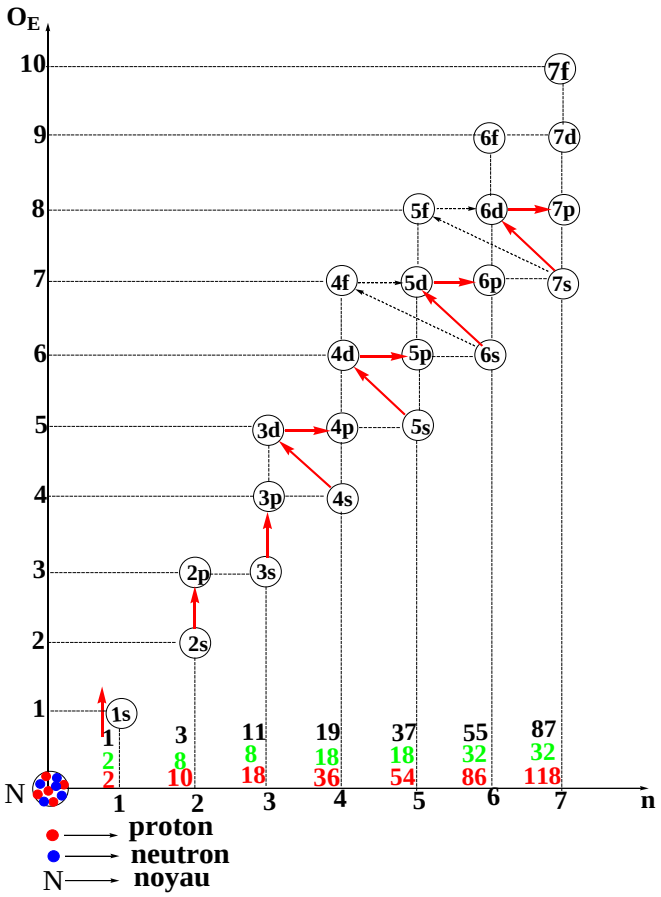
The known atoms are in a logical sequence of natural numbers ranging from 1 to 118. The distribution of the Z electrons of the element containing 118 protons makes it possible to fly over the electronic structure of all the others. The electrons of a polyelectronic atom are distributed in different populations by their energy levels whereas the nucleons exist at the same energy level. Thus from graph 1, it suffices to simply saturate the various sub-layers of each "OE" and "n" stack progressively, starting from 1 to 8 and 1 to 7, respectively. The distribution of the electrons is carried out,in this case, following a progression with variable steps at everyof two stage. We get graphs 1 and 2.

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b) Electronic Structure and Order and Period Charts



Graph 1: Electronic Structure of The Elements According to The Order



Graph 2: Electronic Structure of The Elements According to the Period

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According to graphs 1 and 2, the electrons are divided into populations characterized by their order of energy or their period. The number of electrons per energy level is a specific function of the electronic capacitance of the subshells that make up the indicated level (stacking); It also recalls the different types of known arrays, and it is then identical to the number of elements saturating each of the levels. Knowledge of these values (number of populations of electrons per level) is an inexhaustible source of the secrets of atomistic, of chemical language.

III. Results

a) Reading the two Graphs

Graphs 1 and 2 convey a set of parameters: the level orders, the periods and their subshells, the number of electrons, and the interval of the elements saturating each level. All these atomistic parameters are interdependent. From the coordinates ($O_E$and, or*n*) to the last sub-layer of the stack via the limits of each interval and their amplitudes (number of elements per stack), there is a perfect correlation. This is recorded in tables called condensed tables with order and period.

b) Condensed Arrays with Order and Period

The periods and, or(the orders) are formed by 1, 2, 3,or 4 sub-layers repeating 2 to 2 at each step change. They are: s(s) sp (ps) sdp (dps) sfdp (fdps). They are the different stacks. They are 2 to 2 at each the stepvariation of except the first in the case of periods. The electronic capacities per level or stack are: 2 8 18 32. These are also the various populations of electrons: 2(s) 8[sp/(ps)] 18[sdp/(dps)] 32[sfdp/(fdps)] satisfying the formula of the number of electrons per level.

In practice, all the subshells from 1s to 7p are divided into seven (7) periods and, or eight (8) orders. Each of its levels has some well-defined elements per interval. This number of chemical elements defines the electronic capacity of the level. All subshells are arranged according to the principle of stability, regardless of order or period. The electronic capacitance (amplitude) per period and/or order is given by the following formula: $N_{é/level} = \Sigma\ 2(2l+1)$; *l*: is the secondary quantum number at each of the sub-shells of the level (stack).

For example, if $O_E = 5$ and n = 5, What are the numbers of elements per level and their amplitudes?

For $O_E = 5$ the subshells are 3d 4p 5s, and the amplitude Né = $\Sigma\ 2(2l+1)$: 10 + 6 + 2 = 18é.

For n = 5 the subshells are 5s 4d 5p and the amplitude Né = $\Sigma\ 2(2l+1)$: 2 + 10 + 6 = 18é.

Please note that orders are not always similar to periods and vice versa.

For example, if $O_E = 4$, we obtain"3p 4s" and the fourth period is "4s 3d 4p".

Condensed tables are very captivating and even fascinating educational tools:

- *Condensed order table* (along the ordinate axis)

According to the order chart, the main information is summarized in this table:

Table 1: Condensed Order Table

Order	1	2	3	4	5	6	7	8
Subshells	*1s*	*2s*	*2p3s*	*3p4s*	*3d4p5s*	*4d5p6s*	*4f5d6p7s*	*5f6d7p…*
Z	1- 2	3- 4	5- 12	13- 20	21- 38	39- 56	57- 88	89-118…
Type tables	2	2	8	8	18	18	32	30……

- *Condensed period table* (along the abscissa axis)

According to the distribution of the electrons by the period, a table is obtained analogously to that of the order:

Table 2: Condensed Period Table

Periods	*1*	*2*	*3*	*4*	*5*	*6*	*7*
Subshells	*1s*	*2s2p*	*3s3p*	*4s3d4p*	*5s4d5p*	*6s4f5d6p*	*7s5f6d7p…*
Z	*1- 2*	*3- 10*	*11- 18*	*19- 36*	*37- 54*	*55- 86*	*87-118…*
Type tables	*2*	*8*	*8*	*18*	*18*	*32*	*32….*

- The first line for each of the tables corresponds to the usual ordination of the natural numbers "O_E" and "n," knowing that each number is linked to the coefficients of the different corresponding sub-shells (n, OE) are the coordinates of the sub-shells;
- The second line indicates the sequenced arrangement of the sub-shells according to the Aufbau principle and, or stability (KLECHKOWSKI rule); it gives the electronic structure to each of the elements;
- The third line gives the sequenced ordination of all the atomic numbers of the elements by interval in each level (this is a classification of the chemical elements).
- The last line of the tables has enough meanings, including among others the types of tables, the electronic capacity by stacking, the different

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populations of electrons by level, the amplitudes,thoseare to say the size (magnitude),etc…

These two very similar but different tables convey all the secrets of classification and electronic structure, which is why they are called condensed forms. They illustrate graphs 1 and 2.

Order and period condensed tables are new methods of illustrating the classification of elements. The condensed period form confirms all current periodic classifications of the chemical elements. The condensed period table summarizes all the classical period processes along the abscissa axis.

The condensed order table is also another classification process leading to entirely new forms of tables: Order tables of the elements obtained along the ordinate axis. The resulting tables are in the form of a staircase: they are also 8, 18,and 32 columns. They are carried out around a new classification criterion, namely the order "O_E" instead of " n" the period. Order tables or ordered tables of the elements already exist in the literature, but they continue to bear the name periodic [9]. They are different from periodic tables. The specific criteria for their formation are other from the order and from the period [2].

These new processes for illustrating classifications along the different "O_E and n" axes are summarized by series with period and order values, the main ones of which are, respectively:

- Series to order « O_E » : 2 2 8 8 18 18 32 30
- Series to period « n » : 2 8 8 18 18 32 32

IV. Discussions

By an informed reading, these two series with values (amplitudes) make it possible to interpret the various secrets of atomistic. It is possible to reconstitute each of the tables by their series of values or by the coordinates of their axes. These two very simple series, as simple as telephone numbers, are logically memorized. It is possible to work out the electronic structure of each and, or all the elements mentally. Knowing that the sub-shells of each level or stack are linked to the order numbers and, or period and to the amplitudes, i.e., the different populations of electrons, it is possible or even enjoyable to mask the sub-shells. Thus these condensed tables with order and period are summarized in numeral tables, that is to say, formed only of series with values.

Table 3: Condensed Order Table

Order	1	2	3	4	5	6	7	8
Z	2	4	12	20	38	56	88	118…
Type tables	2	2	8	8	18	18	32	30……

Condensed period table (along the abscissa axis)

According to the distribution of the electrons by the period, a table analogous to that of the order is obtained:

Table 4: Condensed Period Table

Period	*1*	*2*	*3*	*4*	*5*	*6*	*7*
Z	*2*	*10*	*18*	*36*	*54*	*86*	*118*
Type tables	*2*	*8*	*8*	*18*	*18*	*32*	*32….*

Interpreting the series with values resulting from these condensed tables makes it possible to bring out various secrets of atomics. These three series of each of the tables are strongly interdependent.

How to make the electronic structure of the elements?

These condensed tables of order and period numerals are also new ways of illustrating the classification of elements. The electronic structure of a chemical element is as follows:

$S_{elect} = E_{coeur} + E_{val}$, where E_{coeur} core electrons and E_{val} valence or exchangeable electrons.

In Tables 3 and 4, the lines of Z give the abbreviated numerical form of the electronic structure by the noble gases according to the period and by the alkaline earth metals according to the order. From one stack to another, the core electrons are the value of the previous one for the next ; to note,for the first stack,the core electrons are zero "0". The case is specific for the periodic classification ; for the ordered classifications one, hypothetically admits as core electrons the value of the preceding for the following for the need of the analogous calculation.

First Example

Table 3, either works out the electronic structure of Z=47. The chemical element Z= 47 is of the 5th period, whose subshells are: "5s 4d 5p". The electronic structure is then: Ecoeur = 36 (previous gas); Eval= 47-36=11é (11 electrons to leave for 5s 4d and 5p). Thus we get :$36//5s^2 4d^9$ $(5s^1 4d^{10})$.

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The same calculation is possible with the order table except that the hypothetical core electrons contain the two (2) electrons of the "s" sub-shell of the corresponding period. The electrons of hearts,if necessary, are redistributed for the periods preceding that of the elements to obtain the whole electronic structure (complete):$1s^2$; $2s^2\ 2p^6$; $3s^2\ 3p^6$; $4s^2\ 3d^{10}\ 4p^6$ // $5s^2\ 4d^9$ ($5s^1 4d^{10}$).

Second Example

By this method, making the electronic structure is carried out either by masking the different sub-layers or by making them appear :

a) Without highlighting the under layers (they are hidden)
For exampleZ=64

$$64 : 2 - 8 - 8 - 18 - 18 + 10\begin{cases}2\\7\\1\end{cases}$$

b) With subshells

$$Z=64 : 1s^2\ /2s^2 2p^6\ /3s^2 3p^6\ /4s^2 3d^{10} 4p^6\ /5s^2 4d^{10} 5p^6\ /6s^2 4f^7 5d^1.$$

Note that in the literature tables, the sublayers are placed by layer and by period.All forms of tables are interpreted by these small tables with values. Thus the arrangement of the subshells in a graph made it possible to simplify the electronic structure of the chemical elements by using either the period or the order.

V. Conclusion

This work made it possible to sequence the different sub-layers of the electronic cloud either in seven parts (periodically) or in eight, according to the order. These other parts are stacks whose characteristics (coefficients and amplitudes) are linked to the abscissas and, or ordinates of the graph. At each value of these, logically emerges the nature and the number of constituent sub-layers, as well as the amplitude of the corresponding stacking. This has led to new processes for illustrating the classification of elements, which are "condensed tables with period and order". It is by clinging to basic concepts and striving to reason logically that we will gradually realize that atomistic is only a matter of common sense.

A new classification criterion has emerged. All the classical classifications took place around period "n." The new classification criterion is, this time, the order of the energy level "O_E", which leads to a new classification; this is called the ordered classification by analogy to the periodic one. The interdependence of the quantities in these condensed tables even makes it possible to mask the different sub-layers of the electronic cloud, thus leading to condensednumerical tables. A careful reading of these describes all the secrets of atomistic and classification. Through these tables, it is now possible to mentally work out even the most formidable of atomistic,the electronic structure of one or more element(s) in one line.

In this paper, results of the simplification of the establishment of the electronic structure of the elements confirming the use of an affine equation have been provided. We hope to bring soon a rigorous proof of the terminology of atomistic using the equation of the straight line, such as the methods of illustrations of the different classifications of the chemical elements at order and period, the deduction of electrons valence,and electronic jump.

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The primary objective is to recognize the leaders in research and scientific fields of the current era with a global perspective and to create a channel between them and other researchers for better exposure and knowledge sharing. Members are most eminent scientists, engineers, and technologists from all across the world. Associate membership can later be promoted to Fellow Membership. Associates are elected for life through a peer review process on the basis of excellence in the respective domain. There is no limit on the number of new nominations made in any year. Each year, the Open Association of Research Society elect up to 12 new Associate Members.

To the institution

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Associate	Fellow	Research Group	Basic
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Certificate, LoR and Momento **2** discounted publishing/year **Gradation** of Research **10** research contacts/day **1 GB** Cloud Storage **GJ** Community Access	**Certificate,** LoR and Momento **Unlimited** discounted publishing/year **Gradation** of Research **Unlimited** research contacts/day **5 GB** Cloud Storage **Online Presense** Assistance **GJ** Community Access	**Certificates,** LoRs and Momentos **Unlimited** free publishing/year **Gradation** of Research **Unlimited** research contacts/day **Unlimited** Cloud Storage **Online Presense** Assistance **GJ** Community Access	**GJ** Community Access

Preferred Author Guidelines

We accept the manuscript submissions in any standard (generic) format.

We typeset manuscripts using advanced typesetting tools like Adobe In Design, CorelDraw, TeXnicCenter, and TeXStudio. We usually recommend authors submit their research using any standard format they are comfortable with, and let Global Journals do the rest.

Alternatively, you can download our basic template from https://globaljournals.org/Template.zip

Authors should submit their complete paper/article, including text illustrations, graphics, conclusions, artwork, and tables. Authors who are not able to submit manuscript using the form above can email the manuscript department at submit@globaljournals.org or get in touch with chiefeditor@globaljournals.org if they wish to send the abstract before submission.

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The corresponding author should mention the name and complete details of all co-authors during submission and in manuscript. We support addition, rearrangement, manipulation, and deletions in authors list till the early view publication of the journal. We expect that corresponding author will notify all co-authors of submission. We follow COPE guidelines for changes in authorship.

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Unless specified in the notification, the Editorial Board's decision on publication of the paper is final and cannot be appealed before making the major change in the manuscript.

Acknowledgments

Contributors to the research other than authors credited should be mentioned in Acknowledgments. The source of funding for the research can be included. Suppliers of resources may be mentioned along with their addresses.

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The following is the official style and template developed for publication of a research paper. Authors are not required to follow this style during the submission of the paper. It is just for reference purposes.

Manuscript Style Instruction (Optional)

- Microsoft Word Document Setting Instructions.
- Font type of all text should be Swis721 Lt BT.
- Page size: 8.27" x 11'", left margin: 0.65, right margin: 0.65, bottom margin: 0.75.
- Paper title should be in one column of font size 24.
- Author name in font size of 11 in one column.
- Abstract: font size 9 with the word "Abstract" in bold italics.
- Main text: font size 10 with two justified columns.
- Two columns with equal column width of 3.38 and spacing of 0.2.
- First character must be three lines drop-capped.
- The paragraph before spacing of 1 pt and after of 0 pt.
- Line spacing of 1 pt.
- Large images must be in one column.
- The names of first main headings (Heading 1) must be in Roman font, capital letters, and font size of 10.
- The names of second main headings (Heading 2) must not include numbers and must be in italics with a font size of 10.

Structure and Format of Manuscript

The recommended size of an original research paper is under 15,000 words and review papers under 7,000 words. Research articles should be less than 10,000 words. Research papers are usually longer than review papers. Review papers are reports of significant research (typically less than 7,000 words, including tables, figures, and references)

A research paper must include:

a) A title which should be relevant to the theme of the paper.
b) A summary, known as an abstract (less than 150 words), containing the major results and conclusions.
c) Up to 10 keywords that precisely identify the paper's subject, purpose, and focus.
d) An introduction, giving fundamental background objectives.
e) Resources and techniques with sufficient complete experimental details (wherever possible by reference) to permit repetition, sources of information must be given, and numerical methods must be specified by reference.
f) Results which should be presented concisely by well-designed tables and figures.
g) Suitable statistical data should also be given.
h) All data must have been gathered with attention to numerical detail in the planning stage.

Design has been recognized to be essential to experiments for a considerable time, and the editor has decided that any paper that appears not to have adequate numerical treatments of the data will be returned unrefereed.

i) Discussion should cover implications and consequences and not just recapitulate the results; conclusions should also be summarized.
j) There should be brief acknowledgments.
k) There ought to be references in the conventional format. Global Journals recommends APA format.

Authors should carefully consider the preparation of papers to ensure that they communicate effectively. Papers are much more likely to be accepted if they are carefully designed and laid out, contain few or no errors, are summarizing, and follow instructions. They will also be published with much fewer delays than those that require much technical and editorial correction.

The Editorial Board reserves the right to make literary corrections and suggestions to improve brevity.



Format Structure

It is necessary that authors take care in submitting a manuscript that is written in simple language and adheres to published guidelines.

All manuscripts submitted to Global Journals should include:

Title

The title page must carry an informative title that reflects the content, a running title (less than 45 characters together with spaces), names of the authors and co-authors, and the place(s) where the work was carried out.

Author details

The full postal address of any related author(s) must be specified.

Abstract

The abstract is the foundation of the research paper. It should be clear and concise and must contain the objective of the paper and inferences drawn. It is advised to not include big mathematical equations or complicated jargon.

Many researchers searching for information online will use search engines such as Google, Yahoo or others. By optimizing your paper for search engines, you will amplify the chance of someone finding it. In turn, this will make it more likely to be viewed and cited in further works. Global Journals has compiled these guidelines to facilitate you to maximize the web-friendliness of the most public part of your paper.

Keywords

A major lynchpin of research work for the writing of research papers is the keyword search, which one will employ to find both library and internet resources. Up to eleven keywords or very brief phrases have to be given to help data retrieval, mining, and indexing.

One must be persistent and creative in using keywords. An effective keyword search requires a strategy: planning of a list of possible keywords and phrases to try.

Choice of the main keywords is the first tool of writing a research paper. Research paper writing is an art. Keyword search should be as strategic as possible.

One should start brainstorming lists of potential keywords before even beginning searching. Think about the most important concepts related to research work. Ask, “What words would a source have to include to be truly valuable in a research paper?” Then consider synonyms for the important words.

It may take the discovery of only one important paper to steer in the right keyword direction because, in most databases, the keywords under which a research paper is abstracted are listed with the paper.

Numerical Methods

Numerical methods used should be transparent and, where appropriate, supported by references.

Abbreviations

Authors must list all the abbreviations used in the paper at the end of the paper or in a separate table before using them.

Formulas and equations

Authors are advised to submit any mathematical equation using either MathJax, KaTeX, or LaTeX, or in a very high-quality image.

Tables, Figures, and Figure Legends

Tables: Tables should be cautiously designed, uncrowned, and include only essential data. Each must have an Arabic number, e.g., Table 4, a self-explanatory caption, and be on a separate sheet. Authors must submit tables in an editable format and not as images. References to these tables (if any) must be mentioned accurately.

Figures

Figures are supposed to be submitted as separate files. Always include a citation in the text for each figure using Arabic numbers, e.g., Fig. 4. Artwork must be submitted online in vector electronic form or by emailing it.

Preparation of Eletronic Figures for Publication

Although low-quality images are sufficient for review purposes, print publication requires high-quality images to prevent the final product being blurred or fuzzy. Submit (possibly by e-mail) EPS (line art) or TIFF (halftone/ photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Avoid using pixel-oriented software. Scans (TIFF only) should have a resolution of at least 350 dpi (halftone) or 700 to 1100 dpi (line drawings). Please give the data for figures in black and white or submit a Color Work Agreement form. EPS files must be saved with fonts embedded (and with a TIFF preview, if possible).

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Tips for Writing a Good Quality Science Frontier Research Paper

Techniques for writing a good quality Science Frontier Research paper:

1. Choosing the topic: In most cases, the topic is selected by the interests of the author, but it can also be suggested by the guides. You can have several topics, and then judge which you are most comfortable with. This may be done by asking several questions of yourself, like "Will I be able to carry out a search in this area? Will I find all necessary resources to accomplish the search? Will I be able to find all information in this field area?" If the answer to this type of question is "yes," then you ought to choose that topic. In most cases, you may have to conduct surveys and visit several places. Also, you might have to do a lot of work to find all the rises and falls of the various data on that subject. Sometimes, detailed information plays a vital role, instead of short information. Evaluators are human: The first thing to remember is that evaluators are also human beings. They are not only meant for rejecting a paper. They are here to evaluate your paper. So present your best aspect.

2. Think like evaluators: If you are in confusion or getting demotivated because your paper may not be accepted by the evaluators, then think, and try to evaluate your paper like an evaluator. Try to understand what an evaluator wants in your research paper, and you will automatically have your answer. Make blueprints of paper: The outline is the plan or framework that will help you to arrange your thoughts. It will make your paper logical. But remember that all points of your outline must be related to the topic you have chosen.

3. Ask your guides: If you are having any difficulty with your research, then do not hesitate to share your difficulty with your guide (if you have one). They will surely help you out and resolve your doubts. If you can't clarify what exactly you require for your work, then ask your supervisor to help you with an alternative. He or she might also provide you with a list of essential readings.

4. Use of computer is recommended: As you are doing research in the field of science frontier then this point is quite obvious. Use right software: Always use good quality software packages. If you are not capable of judging good software, then you can lose the quality of your paper unknowingly. There are various programs available to help you which you can get through the internet.

5. Use the internet for help: An excellent start for your paper is using Google. It is a wondrous search engine, where you can have your doubts resolved. You may also read some answers for the frequent question of how to write your research paper or find a model research paper. You can download books from the internet. If you have all the required books, place importance on reading, selecting, and analyzing the specified information. Then sketch out your research paper. Use big pictures: You may use encyclopedias like Wikipedia to get pictures with the best resolution. At Global Journals, you should strictly follow here.



6. Bookmarks are useful: When you read any book or magazine, you generally use bookmarks, right? It is a good habit which helps to not lose your continuity. You should always use bookmarks while searching on the internet also, which will make your search easier.

7. Revise what you wrote: When you write anything, always read it, summarize it, and then finalize it.

8. Make every effort: Make every effort to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in the introduction—what is the need for a particular research paper. Polish your work with good writing skills and always give an evaluator what he wants. Make backups: When you are going to do any important thing like making a research paper, you should always have backup copies of it either on your computer or on paper. This protects you from losing any portion of your important data.

9. Produce good diagrams of your own: Always try to include good charts or diagrams in your paper to improve quality. Using several unnecessary diagrams will degrade the quality of your paper by creating a hodgepodge. So always try to include diagrams which were made by you to improve the readability of your paper. Use of direct quotes: When you do research relevant to literature, history, or current affairs, then use of quotes becomes essential, but if the study is relevant to science, use of quotes is not preferable.

10. Use proper verb tense: Use proper verb tenses in your paper. Use past tense to present those events that have happened. Use present tense to indicate events that are going on. Use future tense to indicate events that will happen in the future. Use of wrong tenses will confuse the evaluator. Avoid sentences that are incomplete.

11. Pick a good study spot: Always try to pick a spot for your research which is quiet. Not every spot is good for studying.

12. Know what you know: Always try to know what you know by making objectives, otherwise you will be confused and unable to achieve your target.

13. Use good grammar: Always use good grammar and words that will have a positive impact on the evaluator; use of good vocabulary does not mean using tough words which the evaluator has to find in a dictionary. Do not fragment sentences. Eliminate one-word sentences. Do not ever use a big word when a smaller one would suffice.

Verbs have to be in agreement with their subjects. In a research paper, do not start sentences with conjunctions or finish them with prepositions. When writing formally, it is advisable to never split an infinitive because someone will (wrongly) complain. Avoid clichés like a disease. Always shun irritating alliteration. Use language which is simple and straightforward. Put together a neat summary.

14. Arrangement of information: Each section of the main body should start with an opening sentence, and there should be a changeover at the end of the section. Give only valid and powerful arguments for your topic. You may also maintain your arguments with records.

15. Never start at the last minute: Always allow enough time for research work. Leaving everything to the last minute will degrade your paper and spoil your work.

16. Multitasking in research is not good: Doing several things at the same time is a bad habit in the case of research activity. Research is an area where everything has a particular time slot. Divide your research work into parts, and do a particular part in a particular time slot.

17. Never copy others' work: Never copy others' work and give it your name because if the evaluator has seen it anywhere, you will be in trouble. Take proper rest and food: No matter how many hours you spend on your research activity, if you are not taking care of your health, then all your efforts will have been in vain. For quality research, take proper rest and food.

18. Go to seminars: Attend seminars if the topic is relevant to your research area. Utilize all your resources.

19. Refresh your mind after intervals: Try to give your mind a rest by listening to soft music or sleeping in intervals. This will also improve your memory. Acquire colleagues: Always try to acquire colleagues. No matter how sharp you are, if you acquire colleagues, they can give you ideas which will be helpful to your research.

20. Think technically: Always think technically. If anything happens, search for its reasons, benefits, and demerits. Think and then print: When you go to print your paper, check that tables are not split, headings are not detached from their descriptions, and page sequence is maintained.

21. Adding unnecessary information: Do not add unnecessary information like "I have used MS Excel to draw graphs." Irrelevant and inappropriate material is superfluous. Foreign terminology and phrases are not apropos. One should never take a broad view. Analogy is like feathers on a snake. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Never oversimplify: When adding material to your research paper, never go for oversimplification; this will definitely irritate the evaluator. Be specific. Never use rhythmic redundancies. Contractions shouldn't be used in a research paper. Comparisons are as terrible as clichés. Give up ampersands, abbreviations, and so on. Remove commas that are not necessary. Parenthetical words should be between brackets or commas. Understatement is always the best way to put forward earth-shaking thoughts. Give a detailed literary review.

22. Report concluded results: Use concluded results. From raw data, filter the results, and then conclude your studies based on measurements and observations taken. An appropriate number of decimal places should be used. Parenthetical remarks are prohibited here. Proofread carefully at the final stage. At the end, give an outline to your arguments. Spot perspectives of further study of the subject. Justify your conclusion at the bottom sufficiently, which will probably include examples.

23. Upon conclusion: Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium though which your research is going to be in print for the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects of your research.

Informal Guidelines of Research Paper Writing

Key points to remember:

- Submit all work in its final form.
- Write your paper in the form which is presented in the guidelines using the template.
- Please note the criteria peer reviewers will use for grading the final paper.

Final points:

One purpose of organizing a research paper is to let people interpret your efforts selectively. The journal requires the following sections, submitted in the order listed, with each section starting on a new page:

The introduction: This will be compiled from reference matter and reflect the design processes or outline of basis that directed you to make a study. As you carry out the process of study, the method and process section will be constructed like that. The results segment will show related statistics in nearly sequential order and direct reviewers to similar intellectual paths throughout the data that you gathered to carry out your study.

The discussion section:

This will provide understanding of the data and projections as to the implications of the results. The use of good quality references throughout the paper will give the effort trustworthiness by representing an alertness to prior workings.

Writing a research paper is not an easy job, no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record-keeping are the only means to make straightforward progression.

General style:

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

To make a paper clear: Adhere to recommended page limits.



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- Submitting a manuscript with pages out of sequence.
- In every section of your document, use standard writing style, including articles ("a" and "the").
- Keep paying attention to the topic of the paper.
- Use paragraphs to split each significant point (excluding the abstract).
- Align the primary line of each section.
- Present your points in sound order.
- Use present tense to report well-accepted matters.
- Use past tense to describe specific results.
- Do not use familiar wording; don't address the reviewer directly. Don't use slang or superlatives.
- Avoid use of extra pictures—include only those figures essential to presenting results.

Title page:

Choose a revealing title. It should be short and include the name(s) and address(es) of all authors. It should not have acronyms or abbreviations or exceed two printed lines.

Abstract: This summary should be two hundred words or less. It should clearly and briefly explain the key findings reported in the manuscript and must have precise statistics. It should not have acronyms or abbreviations. It should be logical in itself. Do not cite references at this point.

An abstract is a brief, distinct paragraph summary of finished work or work in development. In a minute or less, a reviewer can be taught the foundation behind the study, common approaches to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Use comprehensive sentences, and do not sacrifice readability for brevity; you can maintain it succinctly by phrasing sentences so that they provide more than a lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study with the subsequent elements in any summary. Try to limit the initial two items to no more than one line each.

Reason for writing the article—theory, overall issue, purpose.

- Fundamental goal.
- To-the-point depiction of the research.
- Consequences, including definite statistics—if the consequences are quantitative in nature, account for this; results of any numerical analysis should be reported. Significant conclusions or questions that emerge from the research.

Approach:

- Single section and succinct.
- An outline of the job done is always written in past tense.
- Concentrate on shortening results—limit background information to a verdict or two.
- Exact spelling, clarity of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else.

Introduction:

The introduction should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable of comprehending and calculating the purpose of your study without having to refer to other works. The basis for the study should be offered. Give the most important references, but avoid making a comprehensive appraisal of the topic. Describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will give no attention to your results. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here.

The following approach can create a valuable beginning:

- Explain the value (significance) of the study.
- Defend the model—why did you employ this particular system or method? What is its compensation? Remark upon its appropriateness from an abstract point of view as well as pointing out sensible reasons for using it.
- Present a justification. State your particular theory(-ies) or aim(s), and describe the logic that led you to choose them.
- Briefly explain the study's tentative purpose and how it meets the declared objectives.

Approach:

Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done. Sort out your thoughts; manufacture one key point for every section. If you make the four points listed above, you will need at least four paragraphs. Present surrounding information only when it is necessary to support a situation. The reviewer does not desire to read everything you know about a topic. Shape the theory specifically—do not take a broad view.

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Procedures (methods and materials):

This part is supposed to be the easiest to carve if you have good skills. A soundly written procedures segment allows a capable scientist to replicate your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order, but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt to give the least amount of information that would permit another capable scientist to replicate your outcome, but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section.

When a technique is used that has been well-described in another section, mention the specific item describing the way, but draw the basic principle while stating the situation. The purpose is to show all particular resources and broad procedures so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step-by-step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

Materials may be reported in part of a section or else they may be recognized along with your measures.

Methods:

- Report the method and not the particulars of each process that engaged the same methodology.
- Describe the method entirely.
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures.
- Simplify—detail how procedures were completed, not how they were performed on a particular day.
- If well-known procedures were used, account for the procedure by name, possibly with a reference, and that's all.

Approach:

It is embarrassing to use vigorous voice when documenting methods without using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result, when writing up the methods, most authors use third person passive voice.

Use standard style in this and every other part of the paper—avoid familiar lists, and use full sentences.

What to keep away from:

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings—save it for the argument.
- Leave out information that is immaterial to a third party.



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Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part as entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Use statistics and tables, if suitable, to present consequences most efficiently.

You must clearly differentiate material which would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matters should not be submitted at all except if requested by the instructor.

Content:

- Sum up your conclusions in text and demonstrate them, if suitable, with figures and tables.
- In the manuscript, explain each of your consequences, and point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation of an exacting study.
- Explain results of control experiments and give remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or manuscript.

What to stay away from:

- Do not discuss or infer your outcome, report surrounding information, or try to explain anything.
- Do not include raw data or intermediate calculations in a research manuscript.
- Do not present similar data more than once.
- A manuscript should complement any figures or tables, not duplicate information.
- Never confuse figures with tables—there is a difference.

Approach:

As always, use past tense when you submit your results, and put the whole thing in a reasonable order.

Put figures and tables, appropriately numbered, in order at the end of the report.

If you desire, you may place your figures and tables properly within the text of your results section.

Figures and tables:

If you put figures and tables at the end of some details, make certain that they are visibly distinguished from any attached appendix materials, such as raw facts. Whatever the position, each table must be titled, numbered one after the other, and include a heading. All figures and tables must be divided from the text.

Discussion:

The discussion is expected to be the trickiest segment to write. A lot of papers submitted to the journal are discarded based on problems with the discussion. There is no rule for how long an argument should be.

Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implications of the study. The purpose here is to offer an understanding of your results and support all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of results should be fully described.

Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact, you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved the prospect, and let it drop at that. Make a decision as to whether each premise is supported or discarded or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."

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- You may propose future guidelines, such as how an experiment might be personalized to accomplish a new idea.
- Give details of all of your remarks as much as possible, focusing on mechanisms.
- Make a decision as to whether the tentative design sufficiently addressed the theory and whether or not it was correctly restricted. Try to present substitute explanations if they are sensible alternatives.
- One piece of research will not counter an overall question, so maintain the large picture in mind. Where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

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Describe generally acknowledged facts and main beliefs in present tense.

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Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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