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Highlights

Analysis of Chromium Soaps Digestion using Atomic Absorption

Nanocomposite Materials Based Synthesis and Biological Activities

Discovering Thoughts, Inventing Future

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By Kassa Belay
Adigrat University, Ethiopia

Abstract- Lead, Cadmium and Chromium concentration in spice samples collected from eastern Ethiopia were determined after digesting the samples by wet digestion methods using FAAS. Oven-dried 1 g spice samples were first Wet-digested in 8 ML of (69–70%) HNO3 and 2 ML of (30%) H2O2 for 3 hr at a temperature of 120 °C. Wet digestion method showed very fast, safer, simple and cleaner and also gives satisfactory recovery, detection limits and standard deviation relative to dry ashing method. Contents of investigated trace metal in spice samples of Lead in Fenugreek, Black cumin, garlic and ginger ranged from 0.0126 to 0.0155, 0.0205 to 0.0254, 0.0046 to 0.0066 and 0.0161 to 0.0178 mg/kg respectively. Cadmium is detected only in Fenugreek in the ranged of ND to 0.0175mg/kg where as Chromium in Fenugreek, Black cumin, Garlic and Ginger ranges from 0.0187 to 0.0219, 0.0134 to 0.0152, 0.0014 to 0.0016 and 0.0258 to 0.0346 mg/kg respectively.

Keywords: trace metals, wet digestion, fenugreek, black cumin, ginger, garlic, atomic absorption spectrometry.

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ANALYSISOFLEADPBCADMIUMCDANDCHROMIUMCRINETHIOPIANSPICESAFTERWETACIDDIGESTIONUSINGATOMICABSORPTIONSPECTROSCOPY

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Kassa Belay

Abstract- Lead, Cadmium and Chromium concentration in spice samples collected from eastern Ethiopia were determined after digesting the samples by wet digestion methods using FAAS. Oven-dried 1 g spice samples were first Wet-digested in 8 ML of (69–70%) HNO_3 and $_2$ ML of (30%) H_2O_2 for 3 hr at a temperature of 120 °C. Wet digestion method showed very fast, safer, simple and cleaner and also gives satisfactory recovery, detection limits and standard deviation relative to dry ashing method. Contents of investigated trace metal in spice samples of Lead in Fenugreek, Black cumin, garlic and ginger ranged from 0.0126 to 0.0155, 0.0205 to 0.0254, 0.0046 to 0.0066 and 0.0161 to 0.0178 mg/kg respectively. Cadmium is detected only in Fenugreek in the ranged of ND to 0.0175mg/kg where as Chromium in Fenugreek, Black cumin, Garlic and Ginger ranges from 0.0187 to 0.0219, 0.0134 to 0.0152, 0.0014 to 0.0016 and 0.0258 to 0.0346 mg/kg respectively.

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I. Introduction

Dry ashing is the standard method for the preparation of organic material for electrolyte analysis; hence this method has been used in most investigations of the electrolyte metabolism of soft tissues. The chief disadvantages of the method are [1] that it requires relatively expensive apparatus (platinum crucibles and muffle furnace) and [2] that there is danger of volatilization of significant amounts of some electrolytes when the digestion temperature is kept high enough to yield a white ash. And now a day it is replaced by wet digestion method.

Wet ashing of organic material requires less expensive apparatus and there is little danger of volatilization of electrolytes. The usual oxidizing agents employed are sulfuric, perchloric, and nitric acids, hydrogen peroxide, and their various combinations. The disadvantages which are responsible for the limited use of this method arise from the fact that the final solution is either very strongly acid, so that large amounts of base are required to neutralize the excess acid, or the digestion must be continued over a relatively hot flame to drive off the excess acid. Volatilization of sulfuric or perchloric acid over an open flame is a difficult procedure to carry out without occurrence of creeping or spattering resulting in partial loss of the sample, unless a relatively large and subsequently disadvantageous digestion tube is used. If only relatively volatile oxidizing substances, such as nitric acid [3] and hydrogen peroxide which can be removed by heating on a water bath, are used, a colored, incompletely oxidized, residue results which is not completely soluble and consequently difficult to work with.

Wet ashing is primarily used in the preparation of samples for subsequent analysis of specific minerals. It breaks down and removes the organic matrix surrounding the minerals so that they are left in an aqueous solution. A dried ground food sample is usually weighed into a flask containing strong acids and oxidizing agents (e.g, nitric, perchloric and/or sulfuric acids) and then heated. Heating is continued until the organic matter is completely digested, leaving only the mineral oxides in solution. The temperature and time used depends on the type of acids and oxidizing agents used. Typically, a digestion takes from 10 minutes to a few hours at temperatures of about 350oC. The advantage of this method is little loss of volatile minerals occurs because of the lower temperatures used, more rapid than dry ashing. The disadvantages of this method are, it is labor intensive, requires a special fume-cupboard if perchloric acid is used because of its hazardous nature, and low sample throughout [4].

Many analytical methods including Atomic Absorption Spectrometry for trace element determination in plant materials require the digestion of the sample [5]. Because of its sensitivity, specificity, simplicity and precision, Atomic Absorption Spectrometry (AAS) is the most widely recommended instrument utilized in analytical procedures for trace heavy metal analysis. In order to separate the analyte from the matrix and to avoid organic matter which may react with the metal ions or chemical reagents and interfere with the analyte in acid digestion methods are very important step. The most commonly used methods for the sample treatment of spices are dry ashing, wet ashing and microwave assisted treatment.

The aim of this article is to present the analytical method applied for the determination of metal content in Ethiopian spices and clearly some aspects, regarding

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the sample preparation and the validation of the method.

II. Materials and Methods

a) Apparatus

Buck scientific 210 VGP flame atomic absorption spectrometer was used in the experiments. A deuterium background corrector was used for background corrections. The operating parameters for the elements were set as recommended by the manufacturer (Table 1). For flame measurements, a 10-cm long slot-burner head, a lamp and an air-acetylene flame were used.

Heating digester (type DK 20) for wet ashing was used to digest the sample for the analysis of Heavy metals concentration. Sample volume, digestion times for the wet digestion, cleaning temperatures were optimized prior to analysis in order to obtain the maximum absorbance with minimum background.

Table1 : Working Conditions of Atomic Absorption Spectroscopy

Element	Wavelength nm	Slit Width nm	Lamp Current mA	IDL mg/L	MDL mg/L	Flame type
Cd	228.9	0.7	2	0.005	0.0062	Rich/yellow
Cr	357.9	0.7	2	0.05	0.051	Lean/blue
Pb	217.0	1.0	5	0.01	0.016	Lean/blue

b) Reagents

All reagents were of analytical reagent grade. Double distilled deionized water (Milli-Q Millipore 18.2 MΩ-cm resistivity) was used for all dilutions. HNO3 and H2O2 were of suprapure quality (E. Merck, Darmstadt). All plastic and glassware were cleaned by soaking in diluted HNO3 (10% v/v) and rinsed with distilled water prior to use. The element standard solutions used for calibration were prepared by diluting stock solutions of 1000 mg/L of each element.

c) Sampling

Total of four spice sample types were collected randomly from different traders and Samples were washed thoroughly with tap water followed by de-ionized water and dried in the oven at a temperature of 105oC for 24 hr for Fenugreek, Ginger, and Garlic. But black cumin was dried at a temperature of 105oC for 48 hr. The dried samples were ground in a stainless steel mill till obtaining fine particles that pass through a 0.5 mm mesh and kept dry in a polyethylene bag in desiccators until analysis.

d) Wet Ashing (Digestion) Procedure

Wet digestion of each spice sample was performed using an oxi-acidic mixture of HNO3/H2O2 (4:1) (10 ML for a 1.0 g sample) in a 100 ML beaker inside a hood. This mixture was heated up to 120˚C for 3 hr and brought to a volume of 25 ML with de-ionized water and blank digestion was also carried out in the same way [6].

e) Digestion Conditions

Different procedures for spices sample digestion were assessed based on varying reagent volume, digestion time and digestion temperature. For optimizing the procedure all spices were selected and it was digested with wet digestion methods. The selection for the respective optimum digestion methods was made based on the particular procedure which resulted to the following conditions: clear digestion solution, minimal reflux time/digestion time, minimal reagent volume consumption, absence of undigested spice samples [7]. The results are given in Table 2. The comparison of three digestion methods showed statistically significant differences in results. The recovery values were nearly (≥ 95%) for all digestion methods. ANOVA was used in this study ($p < 0.05$). The relative standard deviations were less than 10% for all elements. The approximate time required is 5hr.

f) Evaluation of Analytical Figures

i. *Accuracy and precision*

Accuracy and precision are probably the most often quoted terms to express the extent of errors in a given analytical results. Analytical results must be evaluated to decide on the best values to report and to attempt to establish the probable limits of errors of these values [8, 9]. The analyst will thus be concerned with the question of precision (repeatability of results), that is, the agreement between a set of results for the same quantity; and also with accuracy, that is the difference between the measured value and the true value of the quantity, which is determined [8]. In this study the precision of the results were evaluated by the standard deviation of the results of triplicate samples ($n = 3$), analyzed under the same condition. Standard deviation is a useful parameter in estimating and reporting the probable size of indeterminate errors. On the other hand, the accuracy and validity of the measurement were determined by analyzing spiked samples using BUCK SCIENTIFIC standard solutions.

The procedure of spiking was as follows: for the determination of the validity of the developed optimized procedures used for determination of metals in raw and roasted coffee bean samples, known concentration of standard solutions (that is 100 mg/L of Cr, Pb, and 10

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mg/L of Cd) were prepared. From these solutions based upon the amount that make the concentration the final solution 0.20 mg/L (Cr and Pb) and 0.02 mg/L (Cd); 0.15, 0.10 and 0.10 mL, respectively, were added to 0.50 g of spice samples. Then they were digested with the developed digestion Procedures. After diluting the spiked samples to the required volume with deionized water, they were analyzed with the same procedure followed for the analysis of coffee samples. Triplicate samples were prepared and triplicate readings were obtained.

ii. *Determination of detection limits*

Detection limit is the lowest concentration level that can be determined to be statistically Different from an analyte blank [9, 10] or the minimum concentration that can be detected by the analytical method with a given certainty [9]. For a measurement, detection limit can be properly estimated from the standard deviation of several blank determinations [11]. There are numerous ways of determining detection limits of a given measurement [12]. A general accepted definition of detection Limit is the concentration that gives a signal three times the standard deviation of the blank or background signal [14, 15]. In this study the detection limit of each element was calculated as three times the standard deviation of the blank (3σblank, n = 5).

iii. *Determination of limits of quantitation*

Limit of quantitation (or limit of determination) is the lowest concentration of the analyte that can be measured in the sample matrix at an acceptable level of precision and accuracy. An acceptable level of precision is typically 10 to 20 % of relative standard deviation depending upon the concentration level measured. However, in the absence of specified precision, the limit of quantification is the same as the concentration that gives a signal 10 times the standard deviation of the blank [12]. Limit of quantitation is the lowest limit for precise quantitative measurements [14]. The quantitation limit of each element was calculated as ten times the standard deviation of the blank (10σblank, n = 5).

g) Optimization of Working Procedure

The optimal procedure chosen on the basis of these criteria for wet ashing required 3 h at a temperature of 1200C for complete digestion of 1 g dried sample with 8 mL 70% HNO3 and 2 mL 30% H2O2 .

The optimized operating conditions were compared with literature report on similar study made by [13]. The work had improved for wet digestion methods employed there significantly as far as digestion time is concerned.st listed in table 2.

Table 2 : Optimum working conditions for the digestion methods used

Digestion Methods	Previous work optimum parameters [13]			Current work optimum Parameters			Difference		
Wet digestion	T (°C)	V(mL)	Time(hr)	T (°C)	V(mL)	Time(hr)	T(°C)	V(mL)	Time (hr)
	130	2:1(12)	4	120	4:1(10)	3	10	2	1

Note; T = temperature; V = volume WA= wet ashing

III. Results and Discussion

The method detection limit (MDL) is defined as the concentration corresponding to three times the standard deviation of blanks. Method detection limit values of the investigated elements for AAS were found to be 0.016 mg/L for Pb, 0.0062 mg/L for Cd and 0.051 mg/L for Cr.

Trace metal levels in the analyzed samples are given in Table 4. The metal contents in the samples studied depended on the specific species. Levels of the essential metals in the spice samples were found to be higher than those of the non-essential metals. The lowest and highest contents of Lead and chromium in fenugreek, Black Cumin, Garlic and Ginger were found 0.0140, 0.0224, 0.0058, 0.0172 and 0.0219, 0.0152, 0.0016, 0.0258 mg/kg and cadmium in fenugreek is 0.0175 mg/kg but in other spices ND.

a) Optimization of Working Procedure

The optimal procedure chosen on the basis of these criteria for wet ashing required 3 h at a temperature of 1200C for complete digestion of 1 g dried sample with 8 mL 70% HNO3 and 2 mL 30% H_2O_2.

b) Recovery Tests

The efficiency and accuracy of the optimized methods were evaluated by analyzing the digests of spiked samples. 0. 2, ppm of Pb, Cr and 0.02 ppm Cd respectively, were taken from stock solution of each metal and spiked in a 250 mL Erlenmeyer flask containing 1g spice sample. The recoveries of metals in the spiked spice samples were 95 to 103 %. The results are given in Table 3. Generally, good recoveries were obtained for all metals, (particularly in Garlic for metals like Cd, Pb and Cr). In Cd the percentage recovery for all samples except Fenugreek were not calculated since results obtained was not within the method detection limit. Each determination was carried out at least three times in order to ensure precision. The relative standard deviations were less than 10% for all measurements.

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Table 4 : Metal Concentration (mg/kg) in Spices from Dire Dawa Market (Mean± S.D)

Digestion Methods	Spices	Pb	Cr	Cd
WA	Fenugreek	0.0134±0.0003	0.0269±0.0016	0.0138± 0.0016
	Black cumin	0.0215±0 .0010	0.0150±0.0004	ND
	Garlic	0.0057±0.0009	0.0014±0.0002	ND
	Ginger	0.0168±0.0004	0.0270±0.0024	ND

Note; WA = wet ashing and ND = not detected at P≤0.05

IV. Conclusions

This study is focused on check the extraction efficiency of Microwave oven digestion methods in different spices including Garlic, Ginger, Black Cumin and Fenugreek for the determination of heavy metals like Cd, Pb and Cr by using Atomic Absorption spectrometry (AAS).

Spice samples were digested by using three digestion methods by using HNO3 and H2O2 used as digestion reagents. The concentrations of these reagents were optimized to minimize possible matrix interferences. Different temperature programs, reagent volume and digestion time were investigated to obtain maximum digestion efficiency with minimum digestion reagent consumption for all digestion methods. All methods gave almost similar results for the elements studied.

But dry ashing method is more time consuming and complicated than the wet digestion method in terms of digestion efficiency. The use of wet digestion system in spice samples provides very fast, safer, simple and cleaner method of sample preparation, increases analyte recoveries and useful volatile elements relative to dry ashing method. Effects of HNO3 and H2O2 concentrations on Pb (II), Cd (II) and Cr (VI) signals were investigated to see the effect of matrix matching on AAS and it was found that increasing concentrations of HNO3 has suppression effect on Pb and Cr concentration whereas the effect on Cd concentration were not detected by using neither direct calibration method or standard addition method by AAS.

The aim of this study was to prove the analytical performances of a quantitative method for metal determination in spice samples by FAAS method after their wet digestion. The method, previously optimized, was validated. The performance parameters obtained such as linearity, detection and determination limits, trueness, followed as recovery and, precision, evaluated as repeatability and intermediate precision of the spice samples investigated, respect the theoretical values provided by specialty literature permitting, in this way, validation of the method. This study confirms the suitability of the proposed methods for determination metal content in spice for routine and quality control laboratories.

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Spectroscopic Analysis of Chromium Soaps

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Keywords: chromium soaps, IR, x-ray diffraction and thermal measurement.

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SPECTROSCOPICANALYSISOFCHROMIUMSOAPS

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Spectroscopic Analysis of Chromium Soaps

Vasu Mitra [α], S. K. Upadhyay [σ] & R. K. Shukla [ρ]

Abstract- The spectroscopic characteristics of chromium soaps (butyrate and caprylate) in solid state were investigated by IR, X-ray diffraction and thermal measurements. The IR results reveal that the fatty acids exist in dimeric state through hydrogen bonding and soaps possess partial ionic character. The X-ray diffraction measurements were used to calculate the long spacings and the results confirm the double layer structure of chromium soaps. The decomposition reaction was found kinetically of zero order with energy of activation .275 and .180 kcal mol-1 for butyrate and caprylate, respectively.

Keywords: chromium soaps, IR, x-ray diffraction and thermal measurement.

I. Introduction

The survey of literature reveals that the physico-chemical characteristics of transition soaps have not yet been studied thoroughly in spite of the wide range of applications of these soaps in various industries and academic field [1-18]. The applications of the soaps depend largely on their physical state, stability and chemical reactivity together with their volalitity and solubility in common solvents. The present work deals with infrared, x-ray and thermal studies of chromium Soaps and have been used to determine structural information in solid state.

II. Experimental

All the chemicals used were of BDH /AR grade. Chromium soaps (butyrate and caprylate) were prepared by direct metathesis of corresponding potassium soap with slight excess of the solution of chromium nitrate at 50-55oC under vigorous stirring. The precipitated soap was filtered and washed with distilled water and acetone and recrystallized with a mixture of benzene and methanol and dried under reduced pressure.

The infrared absorption spectra of fatty acids and their corresponding chromium soaps were recorded with a Perkin-Elmer "577 model" grating spectrophotometer in the region of 4000-200 cm-1 using potassium bromide disc method.

The X-ray diffraction patterns were obtained with a Richseifert "2002 D" Isodebyeflex Diffractormeter using Cu-Kα radiations filtered by a nickel foil over the range of diffraction angle, 2θ = 30 to 650 where θ is Bragg's angle. The readings of the diffraction angle were made up to 0.001° and the wavelength of the radiation was taken as 1.542Å. The thermogravimetric Analyses of chromium soaps were carried out by Perkin-Elmer thermogravimetric analyzer TG-S-2 at a constant heating rate of 10o/min in nitrogen atmosphere and maintaining similar conditions throughout the investigations.

III. Results and Discussion

a) Infrared Spectra

The wave numbers of some important absorption bands in infrared absorption spectra of chromium (butyrate and caprylate) were assigned and compared with those of corresponding fatty acids (Table-1). The absorption bands observed at 2660-2580, 1700, 1430-1390, 930-910, 690 and 550 cm-^{1} in the spectra of fatty acids have indicated the presence of localized –COOH group in the form of dimeric structure and the existence of intermolecular hydrogen bonding between two molecules of the acid. The evenly spaced progressive bands at 1330-1140 cm-^{1} also observed which are characteristic of the hydrocarbon chain and remain unchanged during the preparation of the soap. The complete disappearance of, the carbonyl frequency at 1700 cm-^{1} in the spectra of chromium soaps indicates that there is a complete resonance between the two C=O bonds of the carboxylic groups of the soap molecule and the two bonds become identical with their force constant assuming an intermediate value between the normal double and single bonds. The appearance of two absorption bands corresponding to symmetric and antisymmetric stretching vibrations of carboxylate ion at 1470-1436 cm-^{1} and 1600-1550 cm-1 regions, respectively, in the spectra of chromium soaps place of one band of carbonyl frequency near 1700 cm-1 confirms the partial ionic nature of these soaps.

The results show that the fatty acids in the solid state exist with dimeric structure through hydrogen bonding between carboxyl group of the two acid molecules whereas metal-to-oxygen bonds in chromium soaps are not purely ionic, but somewhat covalent in character. The assigned frequencies are in agreement with the results of other workers [6-19].

b) X-ray Diffraction analysis

Since the metal soaps do not give large crystals for a detailed single crystal examination, so the X-ray powder diffraction patterns of chromium soaps have been investigated to characterize the structure of these soaps.The intensities of the diffracted X-ray as a function of the diffraction angle, 2θ for chromium soaps were observed and the interplanar spacings, d, have been calculated from the positions of the intense peaks

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using Bragg's relationship, $n\lambda = 2d \sin \theta$, where λ is the wavelength of the radiation. The calculated spacings together with the relative intensities with respect to the most intense peaks are recorded (Tables 2 and 3). The appearance of the diffractions up to the 5th order in chromium butyrate and up to 12th order in chromium caprylate confirms good crystallinity for these soaps. The average planar distance, i.e., long spacing of chromium caprylate and butyrate are 24.12 and 14.893 A respectively. The difference in the observed values of long spacings for chromium caprylate is 9.227 Å which corresponds to twice the length of the additional methylene ($-CH_2-$) groups in the fatty acid radical constituent of the soap molecules. The values of the long spacings for these soaps are approximately equal to double the length of the fatty acid radical of the soap molecules. It is therefore, suggested that the zig-zag chains of fatty acid radicals extended straightforward in these soap molecules.

Table 1 : Iinfrared Absorption Spectral Frequencies (Cm-1) of Acids and Their Soaps

S.	Assignments	Butyric Acid	Chromium Butyrate	Caprylic Acid	Chromium Caprylate
1	CH_3, C—H asymmetrical stretching	2960 W	2960 W	2950 W	2950 VS
2	CH_2, C—H asymmetrical stretching	2910 M	2930 S	2920 S	2920 S
3	CH_2, C—H symmetrical stretching	2855 S	2860 M	2850 S	2840 VS
4	OH, stretching	2660 S	—	2580 W	—
5	C = O, stretching	1700 S	—	1700 VS	—
6	COO–, C—O asymmetrical stretching	—	1550 S	—	1560 MS
7	CH_2, deformation	—	—	1470 M	1440 M
8	C—O Stretching + O—H inplane deformation	1390 M	—	1430 M	—
9	COO, C-O symmetrical stretching	—	1460 W	—	1400 S
10	CH_2 (adjacent to COOH group) deformation	1410 S	1440 S	1370 S	1400 S
11	CH_3, symmetrical deformation	1350 W	1340 W	1340 S	—
12	Progressive bands (CH_2 twisting and wagging)	1270-1220M	1330-1180W	1320-1140M	1180W
13	CH_3 rocking	1100 VS	1120 W	1110 VS	1100 S
14	OH, out-of-plane deformation	930 S	—	910 S	—
15	CH_2 rocking	720 S	722 M	720 M	720 VS
16	COOH bending mode	690 M	—	690 M	—
17	COOH wagging mode	550 M	—	550 M	—
18	Cr — O bond	—	425 M	—	420 M

Key to abbreviation: VW = Very weak
VS = Very sharpS = Sharp
M = Medium W = Weak

The observed values of the long spacing for caprylate (24.12 Å) and butyrate (4.893 Å) of chromium are smaller than the calculated dimension of caprylate (27.0 Å) and butyrate (17.0 Å) ions from Pauling's values of atomic radii and bond angles and this suggests that the molecular axes of these soaps are somewhat inclined to the basal planes. The metal ions fit into spaces between oxygen atoms of the ionized carboxyl group without a large strain of the bonds. A number of diffraction peaks in the intermediate range of the diffraction angles are also observed in the diffraction patterns of chromium soaps and these are attributed to the diffraction of X-ray by planes of atoms of much smaller separation than the basal planes. The calculated spacings from these peaks correspond to the shorter side spacings, i.e., the lateral distances between one soap molecule and the next in a layer. It is observed that the long spacing peaks are fairly intense while the short spacing peaks are relatively weak. On the basis of long and short spacings, it is proposed that the metal ions in chromium soaps are arranged in a parallel plane, i.e., a basal plane is equally spaced in the soap crystal with fully extended zig-zag chains of fatty acid radicals on both side of each basal plane and chromium soaps have double layer structure as proposed by Vold and Hattiangdi [20].

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Table 2 : X-Ray Diffraction Analysis of Chromium Butyrate

S.No.	2θ	θ	Sin θ	$\frac{\lambda}{2 \sin \theta}$	d	n	I/I_{max}
1.	5.94	2.97	0.0518	14.8649	14.865	1	1.00
2.	11.89	5.95	0.1037	7.4253	14.851	2	0.16
3.	17.89	8.95	0.1556	4.9486	14.846	3	0.06
4.	23.72	11.86	0.2055	3.7469	14.988	4	0.03
5.	29.79	14.90	0.2571	2.9949	14.975	5	0.05
6.	42.61	21.31	0.3634	2.1189	14.832	7	0.01

Average value of d = 14.893Å

Table 3 : X-Ray Diffraction Analysis of Chromium Caprylate

S.No.	2θ	θ	Sin θ	$\frac{\lambda}{2 \sin \theta}$	d	n	I/I_{max}
1.	7.31	3.66	0.0638	12.069	24.138	2	0.59
2.	11.03	5.51	0.0960	8.007	24.021	3	1.00
3.	14.69	7.35	0.1279	6.027	24.108	4	0.17
4.	18.34	9.17	0.1594	4.832	24.160	5	0.18
5.	22.23	11.12	0.1929	3.995	23.970	6	0.02
6.	25.61	12.80	0.2215	3.476	24.332	7	0.11
7.	29.90	14.95	0.2580	2.985	23.880	8	0.16
8.	33.36	16.68	0.2870	2.684	24.156	9	0.09
9.	41.69	20.85	0.3559	2.164	23.804	11	0.10
10.	44.56	22.28	0.3791	2.031	24.372	12	0.13
11.	64.99	32.50	0.5373	1.433	24.361	17	0.03

Average value of d = 24.12Å

c) Thermogravimetric Analysis

The results of thermogravimetric analysis of chromium soaps (caprylate and butyrate) show that the final residue is metal oxide and the weights of the residues are in agreement with the theoretically calculated weight of chromium oxide from the molecular formulae of the soaps. A white substance is found deposited at the cold part of the sample tube surrounding the sample and it is identified as caprylone (m.p. 39oC) and butanone (b.p. 79.6oC) in case of caprylate and butyrate, respectively.

The thermal decomposition of chromium soaps can be expressed as:

$$2(RCOO)_3Cr \rightarrow 3RCOR + 3CO_2 + Cr_2O_3$$

chromium Soap — Ketone

The thermogravimetric data have been used to calculate the energy of activation and to find the order of reaction for decomposition of chromium soaps using the equation of Freeman-Carroll [19] which may be written as:

$$\frac{[\log(dw/dt)]}{(\log W)} = \frac{-E}{2.303\,R} \cdot \frac{(1/T)}{(\log W)} + n$$

Where E = energy of activation, R = gas constant, n = order of decomposition reaction, T = temperature on absolute scale, W_r = difference between the total loss in weight and loss in weight at time, t, i.e., W0 – Wt, and dw/dt = value of rate of weight loss

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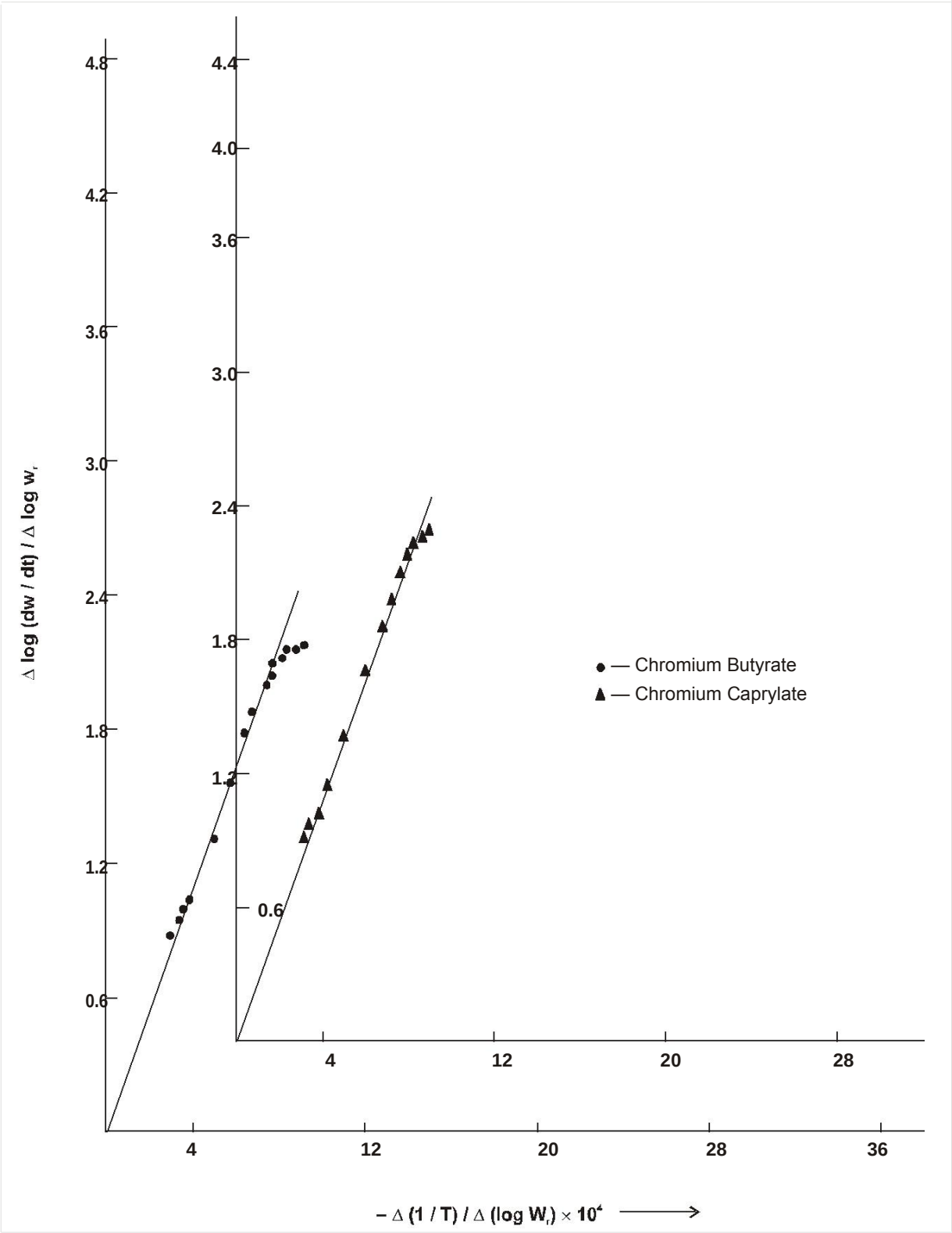
obtained from the loss of weight Vs time curves at appropriate times.

The plots of [log dw/dt)/log W_r] vs. [(1/T)/log W_r] have been found to be linear with the intercept equal to zero (Fig.1). It is, therefore, concluded that the order of reaction for the decomposition of chromium soaps is zero and the value of energy of activation from the slope (-E/2.303R) of the plots (Fig. 1) are .275 and .180 kcal.mol-1 for chromium (butyrate and caprylate) respectively.

Fig. 1 : Freeman – Carroll's Type Plots



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Synthesis and Biological Activities of Some Substituted 6H-Dibenzo [B,D] Pyran-6-One and 6,6-Dimethyl 6H-Dibenzo [B,D] Pyran Derivatives

By Jaya Pandey & Kanchan Hajela

Amity University, India

Abstract- A series of compounds have been synthesized and they were evaluated for anti - implantation, estrogenic, anti-estrogenic and anti-osteoporotic activities. The present paper describes synthesis, and the results of biological activities of accessed molecules.

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SYNTHESISANDBIOLOGICALACTIVITIESOFSOMESUBSTITUTEDGH-DIBENZOB,DPYRAN-G-ONEANDGG-DIMETHYLGH-DIBENZOB,DPYRANDERIVATIVES

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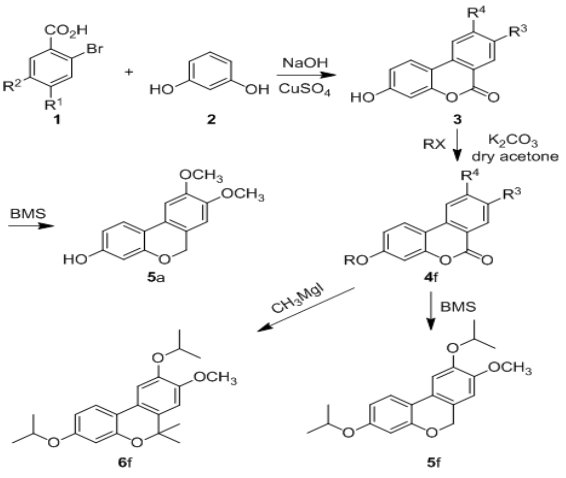
Jaya Pandey [α] & Kanchan Hajela [σ]

Abstract- A series of compounds have been synthesized and they were evaluated for anti-implantation, estrogenic, anti-estrogenic and anti-osteoporotic activities. The present paper describes synthesis, and the results of biological activities of accessed molecules.

I. Introduction

Fused carbocyclic and heterocyclic fused ring systems constitute an important class of natural products with immense pharmacological properties.[1,2] The biological importance of pyrans as an anticoagulant3, aflatoxins as mycotoxins4 and of coumestrol as an estrogen and a phytoalexin5 has led to a considerable amount of work in the field of fused ring systems. Recently, the dibenzopyranone/pyrans and naphthopyran nucleus have surfaced as common ring system of a group of antibiotics, antibacterials, antitumors and immunomodulators, etc. exemplified by alternariol6, ravidomycin7, shilajit8, ellagic acid9 etc. Several carbocyclic and heterocyclic compounds have recently been reported in literature such as KCA-09812, LY-35615613, and coumestrol analogue14 etc. which Selectively modulate the activity of estrogen receptor (ER) showing complete antagonist effect at breast and uterus yet retain the positive effect on central nervous, cardiovascular and skeletal systems. These compounds termed as 'selective estrogen receptor modulators (SERMs)' cause increase in bone mineral density (BMD), reduce serum cholesterol level and are completely antagonists to breast and uterus tissues, therefore, are being evolved as antiosteoporotic agents. Our continuing effort on the development of 2,3-diarylbenzopyrans as selective estrogen receptor modulators10,11 led us to synthesize some dibenzopyranone/pyran molecules as potential antiestrogenic agents. As a critical balance of estrogenic as well as antiestrogenic effect is required in a molecule, therefore, various structural modifications at C-3, 6, 8 and 9 positions were done in the molecule to study structure-activity relationship and evolve a novel selective estrogen receptor modulator. The present paper describes synthesis, estrogenic, anti-estrogenic, RBA, anti-implantation and anti-osteoporotic activities of the synthesized compounds.

The respective orthobromobenzoic acids (1a-h), were condensed with resorcinol in aqueous alkaline medium in presence of copper (II) sulfate as a catalyst



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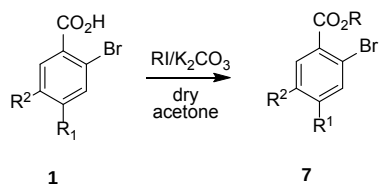
Compounds	a	b	c	d	e	f	g	h
1	R^1=H R^2=H	R^1=H R^2=OMe	R^1=OH R^2=H	R^1=OH R^2=OMe	R^1=OMe R^2=OMe	R^1=OBn R^2=OMe	R^1=$O(CH_2)_2$Py R^2=OMe	R^1=$O(CH_2)_2$Pi R^2=OMe
2	-	-	-	-	-	-	-	-
3	R^3=H R^4=H	R^3=OMe R^4=H	R^3=H R^4=OH	R^3=OMe R^4=OH	R^3=OMe R^4=OMe	R^3=OMe R^4=OBn	R^3=OMe R^4=$O(CH_2)_2$Py	R^3=OMe R^4=$O(CH_2)_2$Pi
4	R=O^{i}Pr R^3=H R^4=H	R=O^{i}Pr R^3=OMe R^4=H	R=O^{i}Pr R^3=OMe R^4=O^{i}Pr	R=O^{i}Pr R^3=OMe R^4=OH	R=O^{i}Pr R^3=OMe R^4=OMe	R=O^{i}Pr R^3=OMe R^4=O^{i}Pr	-	-
5	R=O^{i}Pr R^3=H R^4=H	R=O^{i}Pr R^3=OMe R^4=H	-	-	-	-	-	-
6	-	-	-	-	-	R=O^{i}Pr R^3=OMe R^4=O^{i}Pr	-	-

Py=pyrrolidine; Pi=piperidine

Scheme1 : Synthesis of 6H-dibenzopyran-6-one and dibenzopyran derivatives

to produce the corresponding dibenzopyranones (3a-h,) in good yields (Scheme 1).15a,b The various orthobromobenzoic acids, used in the Scheme 1 were in turn prepared by either known literature methods starting from simple starting materials16a, b, c or from bromovanillic acid esters 7 as shown in Scheme 2.



7a (R=Me, R^1= OH; R^2= OMe; **7b** (R=Me, R^1= OMe; R^2= OMe; **7c** (R=Bn, R^1= OBn; R^2= OMe; **7d** (R=Me, R^1=$O(CH_2)_2Py$, R^2= OMe); **7e** (R=Me, R^1=$O(CH_2)_2Pi$, R^2= OMe)

Scheme 2 : Synthesis of Substituted Orthobromobenzoic acid esters

The dibenzopyranones were alkylated with isopropyl bromide/methyl iodide, in dry acetone with anhydrous potassium carbonate as base to yield the respective alkylated products (4a-g, Scheme 1).

To obtain dibenzopyrans, the corresponding pyranones were reduced with borane methyl sulfide (BMS) (5a-d, Scheme I).

To study the structure-activity relationship various 6, 6-dimethyl dibenzopyran were also prepared. 9-Benzyloxy 8-methoxy 3-hydroxy dibenzopyranone (3f) was first protected with methoxy group (4g) and then reacted with methyl magnesium bromide to give 6, 6-dimethyl compound (6a). The 9-benzyloxy group was de-protected by hydrogenation with Raney Nickel in

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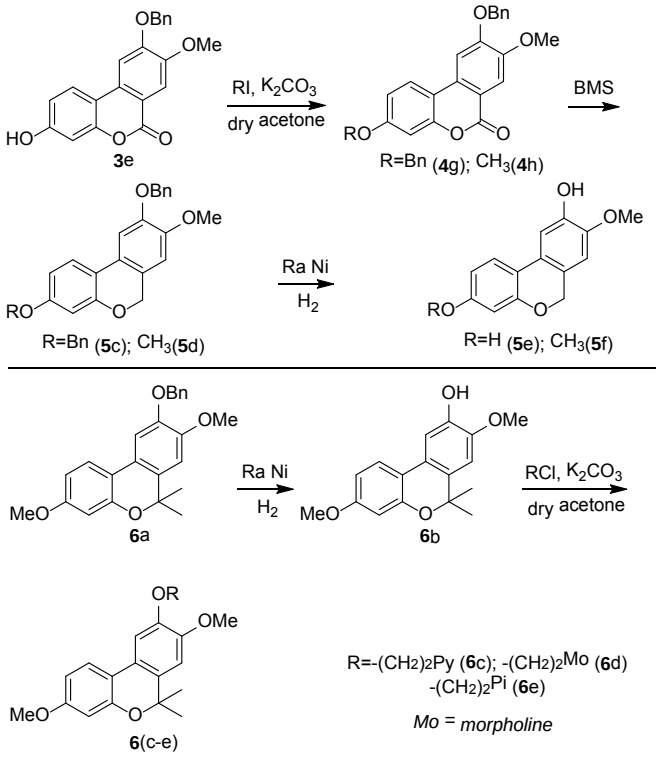
methanol, to give 6b which was alkylated with various alkyl/aminoalkoxy chains (e.g. 1-2-chloroethyl piperidine /pyrrolidine/morpholine hydrochlorides etc.) to yield the corresponding products (6c-e, Scheme 3).



Scheme 3 : Synthesis of Substituted Orthobromobenzoic acid esters

All the synthesized compounds were characterized by spectral data NMR, Mass and elemental analysis and evaluated for estrogenic, anti-estrogenic, RBA (receptor binding affinity), anti-implantation and anti-osteoporosis activities.

II. Result and Discussion

The results of biological activity reveal that the dibenzopyran-6-ones possess both anti-estrogenic as well as inherent estrogenic property in the basic molecule. Hydroxy group at C-3 position analogous to C-3 of estradiol (E_2) is primary requisite for binding to ER and substitution by methoxy or isopropyl group cause decrease in binding affinity and biological activities. Although these molecules show poor binding affinity in in vitro RBA assay experiment, the reason for showing strong estrogenic or anti-estrogenic effects by some molecule is that there may be some other mechanism operative in vivo, like conversion into active metabolites or the molecules may be acting via some other mechanism than receptor modulation like modulation of certain enzymes/ cofactors involved in manifestation of estrogenic action. The 6,6-dimethyl compound 6e has emerged as most potent anti-estrogenic molecule in this series (24.7%) showing anti-implantation activity of 60% at 10 μg/kg dose in 1-5 day schedule. The compounds are also moderately agonist at skeletal tissue. 5a being most active (7.78%). Reduction into 6H-dibenzopyran has drastically increased the estrogenic property of the molecule (compound 3h – 78.4%) though substitution by alkyl chain at C-3 again blocks the binding of the molecule to ER decreasing the activity.

a) Biological Activities

Some of the compounds listed in Table III, IV, V and VI (3c, 3d, 3g, 3h, 4a, 4b, 4c, 4d, 4f, 5a, 5b, 5e, 5f, 6c, 6d, 6e, 6f) were screened for anti-implantation, estrogenic, anti-estrogenic, receptor binding affinity and anti-osteoporotic activities. The biological methods are described below and the results presented in Table VII.

b) Anti-implantation activity

Implantation activity was evaluated by known biological method in Sprague-Dawley rats (21 day old, weighing 22-30 g) by the method given in literature17. The compounds were administered per oral dose at 10 mg/kg dose as aqueous gum acacia suspension on day 1-5 schedule. On the 11th day of the test, rats of both the control and treated groups were laparotomised and their uteri examined for implantation sites. The results were considered positive when implantation sites were totally absent in both the uterine horns.

c) Estrogenic activity

Different groups of animals were orally administered the test material in graded doses for 3

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consecutive days. Uterine weight and status of vaginal opening were noted at the time of autopsy, i.e. 24 h after the last treatment. The activity was assessed by uterine weight gain.

d) Anti-estrogenic activity

17β estradiol [E2] at 0.1μ g in olive oil was given by subcutaneous route along with graded doses (5-15 μg) of compounds for three consecutive days. Inhibition was expressed as percent inhibition of estradiol induced increase in uterine wet weight.

e) Receptor Binding Affinity (RBA)

The RBA values of test compounds were evaluated by method described in literature18. Briefly, 50μl aliquots of cytosol (1 uterine equivalent/ml) were incubated at 4°C for 18 hour with increasing concentrations of test compounds (10-8-10-4 M). In triplicate and fixed concentrations of 3H-E2 (SR 10-9 M) dissolved in 20μl of DMF-TEA buffer. For separation of free from bound 3H-E2, each incubate was treated with 10μl of charcoal-dextran slurry (2.5 and 0.25% v/v, respectively) in TEA buffer for 20 minutes. Radioactivity of 50μl aliquot of each incubate was measured in Packard tricarb liquid scintillation spectrometer. The binding affinities of compounds relative to reference ligand (E_2=100) were calculated.

f) Anti-osteoporotic activity (In vitro anti-resorptive assay)

The in vitro assay of anti-resorptive activity using ^{45}Ca prelabelled rat fetal bone was done according to known literature method19. Three month old Sprauge Dawley female rats were mated to males of proven fertility. 250 CI/30μl of $^{45}CaCl_2$ was administered to each rat on day μl of pregnancy and labelled humerus and radio-ulna bones were isolated 48 hours thereafter under sterile conditions. Bones were cultured in 300μl of the BGJB medium supplemented with antibiotic, antifungal and buffer (pH 7.3) for 24 hours. The bones were washed twice with PBS and transferred to BJGB medium containing PTH (0.4μM) and these cultured for 96 hours in the presence or absence of test compound (100μM) or the vehicle (0.1% ethanol/DMSO) in 300μ1 of BGJB, medium on termination of the culture, bones were transferred to 0.1 N HCl for 24 hours. Radioactivity due to ^{45}Ca in the spent medium collected at 48 and 96 hrs of culture and the HCl extracts was quantified by liquid scintillation spectrophotometer. Bone resorbing activity was expressed as percentage of released ^{45}Ca and the effect of test compounds as percent of control.

III. Conclusion

Compound 6e was found to inhibit estrogenic effect by 24.7% at 10 μg dose. Whereas compound 3g has shown estrogenic effect by 41.8%. RBA (ER) % of E2 for compound 3h was found to be the best (0.24%). Anti-implantation activity for compound 6e was recorded close to 60%. Compound 5a displayed anti-resorptive activity close to 7.78%. In essence, most of the compounds tested have shown good to moderate level of activities related to anti-estrogenic, receptor binding affinity and anti-osteoporotic activities.

IV. Acknowledgement

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Experimental Section

Procedure for one typical case for each step has been described. The melting points were determined in open capillaries Toshniwal melting point apparatus and are uncorrected. The 1H NMR were recorded on Brucker Avans DRX 300 (300 MHz, FT NMR) spectrometer using TMS as internal standard. The chemical shift values are reported in □ (ppm) scale and coupling constants in Hz. Electron impact (EIMS) mass spectra were run on a JEOL-JMSD 300 instruments fitted with a direct inlet system. Elemental analysis were performed on elemental analyser EA-1108 and were within +4% of theoretical values. The purity of the products was checked on precoated silica gel 60 F254 or aluminium oxide 60 F254 TLC plates and the spots were visualized by spraying with iodine vapors.

Methyl-2-bromo-5-methoxy 4-pyrrolidinoethoxy benzote-7c

To a solution of methyl 2-bromo 4-hydroxy 5-methoxy benzoate (261 mg; 1 mM) in dry acetone (60 ml) was added anhydrous potassium carbonate (276 mg; 2 mM) and 2-chloropyrrolidine hydrochloride (213 mg; 1.2 mM). The reaction mixture was stirred and refluxed (70-80oC) for 24 hrs. After the completion of reaction, the reaction flask was cooled and the contents filtered through G-3 sintered crucible. The residue was washed with acetone (5 ml x 3), the filtrate was concentrated and chromatographed over basic alumina column eluting with ethylacetate-hexane (2%). The product was obtained as yellow oil. Wt., 22.5 mg; Yield, 63%;

Methyl-2-bromo-5-methoxy-4-piperidinoethoxy benzoate 7d

It was prepared by similar procedure described for 3d. Ethyl acetate-hexane (2%) as yellow oil. Wt., 278 mg; Yield, 75%.

2-bromo-5-methoxy 4-pyrrolidino ethoxy benzoic acid hydrochloride 1f

To a solution of methyl 2-bromo 5-methoxy 4-pyrrolidinoethoxy benzoate (358 mg; 1 mM) in methanol (5 ml) was added sodium hydroxide solution (5N, 2 ml). The reaction mixture was stirred at room temperature for 48 hrs. After the completion of reaction, the excess methanol was evaporated off and the reaction flask was cooled in ice and neutralised with hydrochloric acid solution (6N, 3 ml). The precipitated product was filtered off and crystallized in methanol (3 ml) - water (6 drop) to yield pale yellow crystals of the product. Wt., 342 mg; Yield, 92%,m.p., 218°C.

2-bromo 5-methoxy 4-piperidino ethoxy benzoic acid hydrochloride 1g

It was prepared by similar procedure as described for 1g, Wt., 352mg; Yield, 90%; m.p., 216 °C.

3, 9-dihydroxy 8-methoxy dibenzo [b,d] pyran-6-one 3d

To a solution of methyl 2-bromovanillic acid (598 mg; 2.42 mM) in aqueous sodium hydroxide solution (8%, 2.5 ml) was added resorcinol (550 mg, 5 mM). The reaction mixture was refluxed (120oC) in an oil bath for 45 min, then aqueous copper sulfate solution (5%, 1 ml) was added into it and the reaction mixture was refluxed for another 15 min. The reaction mixture was cooled and the precipitated product was filtered off and washed with water (1 ml x 3), dried and crystallized in acetic acid (2 ml) - methanol (5 ml) to yield brown amorphous solid. Wt.,157 mg; Yield, 61%, m.p., 288oC.

It was prepared by similar procedure described for 3d from 4,s-dimethoxy z-bromobenzoic acid (568.98, 2.18 mM) and resorcinol (550 mg; 5 mM), Weight: 142 mg, Yield: 52% m.p. >280oC.

9-Benzyloxy 3-hydroxy 8-methoxy dibenzo [b,d] pyran-6-one 3f

It was synthesized by similar method starting from 4-benzyloxy 3-hydroxy 8-methoxy 2-bromobenzoic acid (630 mg; 2.18 mM) and resorcinol (550 mg; 5 mM). Wt., 141 mg; Yield, 52 %.

3-Hydroxy 8-methoxy 9-pyrrolidinoethoxy dibenzo [b,d] pyran-6-one 3g

It was prepared by similar procedure as above from 2-bromo 5-methoxy 4-pyrrolidinoethoxy benzoic acid (826 mg; 2.12 mM) and resorcinol (550 mg; 5 mM). The product was crystallized in methanol (2 ml)-water (0.5 ml) as white crystalline solid. Wt., 170 mg; Yield, 47.8%; m.p., 196°C.

3-hydroxy 8-methoxy 9-piperidinoethoxy dibenzo [b,d] pyran-6-one 3h

It was prepared by similar procedure described for 3d from 1g (830 mg; 2.18 mM) and resorcinol (550 mg, 5 mM) and crystallized in methanol (2 ml) - water (0.5 ml) to yield white crystalline solid.Wt., 184 mg; Yield, 49.8%; m.p., 205°C.

3-isopropyloxy 8-methoxy dibenzo [b,d] pyran-6-one 4b

To a solution of 3-hydroxy 8-methoxy dibenzo [b,d] pyran-6-one (242 mg; 1 mM) in dry acetone (60 ml) was added anhydrous potassium carbonate (276 mg; 2 mM) and isopropyl bromide (0.19 ml; 2mM). The reaction mixture was stirred and refluxed (70°C) for 24

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hr. After the completion of reaction, the reaction flask was cooled and the contents filtered through G-3 sintered crucible and the residue washed with acetone (5 ml x 3). The filtrate was concentrated and chromatographed over silica gel column eluting the product with ethyl acetate-hexane (2%). Wt., 260 mg; Yield, 91.5%; m. p., 96°C.

It was prepared by similar procedure described for 4b from 3-hydroxy dibenzopyranone (212; 1 MM) and isopropyl bromide. Wt. 170 mg; Yield: 70.24%, m. p. 78°C.

3, 9-Diisopropyloxy dibenzo [b,d] pyran-6-one 4c

It was synthesized by similar method from 3, 9-dihydroxy dibenzopyranone (228 mg; 1 mM). Wt.,130 mg; Yield, 42%; m. p., 78°C.

3, 8-Diisopropyloxy dibenzo [b, d] pyran-6-one 4d

It was synthesized by similar method described for 4a from 3, 8-dihydroxy dibenzo [b, d] pyran-6-one (228 mg; 1 MM) and isopropyl bromide (0.19 ml; 2 MM) in dry acetone using potassium carbonate as base. Wt., 295mg; Yield, 94.5%; m .p., 98°C.

3, 9-Diisopropyloxy 8-methoxy dibenzo [b,d] pyran-6-one 4e

It was synthesized by similar method described for 4a from 3-hydroxy 8, 9-dimethoxy dibenzopyranone (272 mg; 1 MM). Wt., 240 mg; Yield, 88.23%; m. p., 218°

3-Isopropyloxy 8, 9-dimethoxy dibenzopyranone 4f

It was synthesized by similar method from 3, 9-dihydroxy 8-methoxy dibenzopyranone (258 mg; 1mM).Wt., 148 mg; Yield, 43.2%; m.p., 196°C.

3, 9-dibenzyloxy 8-methoxy dibenzo [b,d] pyran-6-one 4g

It was prepared by similar method described for 4b from 3-hydroxy 9-benzyloxy 8-methoxy dibenzo [b,d] pyran-6-one (348 mg; 1 mM) and benzyl bromide (0.239 ml; 2 mM) in dry acetone (60 ml) using anhydrous K2CO3 as base. The product crystallized in ethylacetate (3 ml) hexane (1 ml) as white crystalline solid. Weight 368 mg; Yield: 90.05%; m .p. 186°C.

9-Benzyloxy 3, 8-dimethoxy dibenzo [b, d] pyran-6-one 4h

It was prepared by similar procedure described for 4g from methyl iodide (0.124 ml; 2 MM) Weight: 326 mg; Yield: 90.05%; m.p. 192°C.

3, 9-Diisopropyloxy 3-methoxy dibenzo [b, d] pyran 5a

The mixture of 3, 9-diisopropyloxy 8-methoxy dibenzopyranone (342 mg; 1 MM) in borane methyl sulfide complex (0.5 ml; 2M solution in THF) was left overnight in dry RB flask (50 ml) with guard tube. After the completion of reaction, the reaction flask was cooled in ice-bath and quenched with cold saturated solution of ammonium chloride (5 ml) with stirring. The reaction mixture was refluxed on water bath with ethanol (5 ml) for 10 minutes. The ethanol was distilled of and the product extracted with dichloromethane (10 ml). The organic layer was washed with water (3 ml x 3), dried Na_2SO_4 and concentrated. The product was recrystallized in ethyl acetate (5 ml) - hexane (2 ml) to yield the product s pure white crystals. Wt., 260 mg; Yield, 79.2%; m. p., 96°C.

9-benzyloxy 3, 8-dimethoxy 6, 6-dimethyldibenzo[b, d] pyran 6a

A solution of 9-benzyloxy 3,8-dimethoxy dibenzo pyranone 13c (372 mg; 1 mM) in dry THF (20 ml) was added dropwise over 15 minutes to a solution of methyl magnesium iodide, prepared from Mg-turnings (360 mg; 15 mM) and methyl iodide (0.74 ml; 15 mM) in dry ether (5 ml). The reaction mixture was heated under reflux for 8 hours. The reaction mixture was cooled and poured into a mixture of conc. H2SO4 (0.2 ml) and ice (4 gm) with vigorous stirring. The product was extracted in benzene (20 ml), the organic layer was washed with water (3 ml x 3), dried (Na2SO4), concentrated in vacuo. The concentrate was chromatographed over silica gel column. The product was crystallized in benzene (2 ml)-hexane (0.5 ml) to give white crystalline solid. Wt., 280 mg; Yield, 74.4%; m.p.152oC.

Preparation of 9-hydroxy 3, 8-dimethoxy 6,6-dimethyl dibenzo [b,d] pyran 6b

To a solution of 3, 8-dimethoxy 9-benzyloxy 6, 6-dimethyl dibenzopyran (376 mg; 1 MM) in methanol (10 ml) was added Raney-nickel (10 mg). The reaction mixture was hydrogenated (60 psi) and shaken for 4 hr. After the completion of reaction, the reaction mixture was filtered through G-3 sintered crucible with cellite bed (2 cm) and washed with methanol (5 ml x 3). The filtrate was concentrated and the product crystallized in methanol (2 ml) to yield the product as white crystalline solid. Wt.,230 mg; Yield, 80.4%.

3, 8-dimethoxy 9-piperidinoethoxy 6, 6-dimethyl dibenzo [b,d] pyran 6c

It was prepared by same procedure as describe above for 4a from 3,8-dimethoxy 9-hydroxy 6,6-dimethyl dibenzopyran (286 mg; 1 mM) and 1-(2-chloroethyl piperidine hydrochloride) (222 mg; 1.2 mM) in dry acetone (60 ml) using anhydrous K_2CO_3as base. The product was crystallized in benzene (0.5 ml)-hexane (4 drops) as white crystalline solid. Wt., 320 mg; Yield, 80.6%; m.p., 118°C.

3, 8-Dimethoxy 9-morpholinoethoxy 6,6-dimethyl dibenzo [b,d] pyran 6d

It was prepared by similar procedure as described above for 4a from 3,8-dimethoxy 9-hydroxy 6,6-dimethyldibenzo pyran (286 mg; 1 mM) and 1-(2-chloroethyl) morpholine hydrochloride. The product was

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crystallized in ethyl acetate (3ml). Wt., 248 mg;Yield, 62%; m.p., 126°C.

3, 8-Dimethoxy 9-pyrrolidinoethoxy 6,6-dimethyl dibenzopyran 6e

It was prepared by same procedure as described above for 4a from 3,8-dimethoxy 9-hydroxy 6,6-dimethyl dibenzopyran (286 mg; 1 mM) and 1-(2-chloroethyl) pyrrolidine hydrochloride (204 mg; 1.2 mM) in dry acetone (60 ml) using anhydrous K_2CO_3 as base. The product was crystallized in ethyl acetate (3ml). Wt., 237 mg; Yield, 61.8%; m.p., 124°C.

3,9-Diisoprophyloxy-8-methoxy 6,6-dimethyl dibenzo [b, d] pyran 6f

It was prepared by similar procedure as described above from 3,9-diisopropyloxy 8-methoxy 6,6-dimethyl dibenzo [b, d] pyran (356 mg; 1 MM). The product was crystallized in ethyl acetate (3ml). Wt., 206 mg; Yield, 58%; m.p., 82°C.

3, 9-Dibenzyloxy 8-methoxy dibenzo [b,d] pyran-6- one 5c

It was prepared by similar method described for 5a from 3, 9-dibenzyloxy 8-methoxy dibenzo [b,d]pyran-6-one (438 mg; 1 mM) and BMS (0.5 ml). The product crystallized in benzene (5 ml) hexane (2 ml) as white crystalline solid. Wt. 324 mg; Yield: 77.2% m.p. 122°C.

3, 9-Dihydroxy 8-Methoxy dibenzo [b,d] pyran 5e

It was prepared by similar method described for 5a from 3,9-dibenzyloxy 8-methoxy dibenzo [b,d] pyran (424 mg; 1 mM) and Raney Nickel (50 mg) in methanol (10 ml). The product was crystallized in ethyl acetate (4 ml), Wt. 152 mg; Yield: 62.29%; m.p. 194°C.

9-Benzyloxy 3, 8-dimethoxy dibenzo [b,d] pyran 5d

It was prepared by similar method described for 5a from 9-benzyloxy 3, 8-dimethoxydibenzopyran-6-one (362 mg; 1 mM) and BMS (0.5 ml). The product was crystallised in ethyl acetate (2 ml) hexane (0.5 ml) as white crystalline solid. Wt. 294 mg; Yield: 84.4%; m.p. 124°C.

9-Hydroxy 3, 8-dimethoxy dibeno [b,d] pyran 5f

It was prepared by similar method described for 6b from 9-benzyloxy 3, 8-dimethoxy dibenzo [b,d] pyran (348 mg; 1 mM) and Raney nickel in methanol (5 ml). The product was crystallised as white solid. Wt. 182 mg; Yield: 70.8%; m.p. 188°C.

Table I : Physical data and characterization of orthobromobenzoic acid (1d-1h)

Compd. No.	Mol. Formula	Mass: m/z	m.p. °C	^{1}H NMR ($CDCl_3$, δ ppm)
1e	$C_9H_9O_4Br$	261(M^+), 263 (M^++2), 246, 200, 160	178	3.91 (s, 3H, OCH_3), 3.94 (s, 3H, OCH_3), 7.13 (s, 1H, H^3), 7.58 (s, 1H, H^6)
1f	$C_{11}H_{13}O_4Br$	426 (M^+), 181, 105, 91	160	3.9 (s, 3H, OCH_3), 5.2 (s, 2H, $COCH_2$), 5.4 (s, 2H, CH_2), 7.3-7.6 (m, 12H, Ar-H), 7.59 (s, 1H, H^6)
1g	$C_{14}H_{18}O_4BrN$	344 (M^+), 246, 98, 84	218	2.0-2.1 (m, 4H, $(CH_2)_2$ of pyrrolidine ring), 2.8-2.9 (m, 4H, $N(CH_2)_2$ of pyrrolidine ring), 2.8-2.9 (m, 4H, $N(CH_2)_2$), 3.56-3.61 (t, 2H, NCH_2), 3.8 (s, 3H, OCH_3), 4.28-4.33 (t, 2H, OCH_2), 7.23 (s, 1H, H^3), 7.42 (s, 1H, H^6)
1h	$C_{15}H_{20}O_4BrN$	357 (M^+), 237, 137, 120, 112, 98	210	1.5-1.8 (m, 6H, $(CH_2)_3$ of piperidine ring), 2.4-2.48 (m, 4H, $(CH_2)_2$ of piperidine ring), 2.50 (t, 2H, NCH_2), 3.81 (s, 3H, OCH_3), 4.46-4.51 (t, 2H, OCH_2), 7.30 (s, 1H, H^3), 7.40 (s, 1H, H^6)

Table II : Physical data and characterization of substituted orthobromobenzoates (7a-d)

Compd. No.	Mol. Formula	Mass: m/z	m.p. °C	^{1}H NMR ($CDCl_3$, δ ppm)
3d	$C_{14}H_{10}O_5$	258 (M^+) 243, 219, 215, 187, 131, 91, 69	>250	3.91 (s, 3H, OCH_3), 6.72-6.73 (d, 1H, H^4), 6.79-6.85 (dd, 1H, H^2), 7.40 (s, 1H, H^{10}), 7.50 (s, 1H, H^7), 7.88-7.92 (d, 1H, H^1)
3e	$C_{15}H_{12}O_5$	272 (M^+), 258, 228, 214, 158, 121	>280	3.85 (s, 3H, 8-OCH_3), 3.98 (s, 3H, 9-OCH_3), 6.70-6.71 (d, 1H, H^4), 6.78-6.82 (dd, 1H, H^2), 7.5 (s, 1H, H^{10}), 7.6 (s, 1H, H^7), 8.13-8.18 (d, 1H, H^1)

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3f	$C_{20}H_{16}O_5$	272 (M^+), 258, 228, 214, 158, 121	198	3.91 (s, 3H, OCH_3), 5.49 (s, 2H, OCH_2), 6.86-6.87 (d, 1H, H^4), 6.94-6.98 (dd, 1H, H^2), 7.49-7.69 (m, 7H, H^7, H^{10} and Ar-H), 7.93 (s, 1H, H^7), 8.26-8.30 (d, 1H, H^1)
3g	$C_{20}H_{21}O_5N$	355 (M^+), 307, 289, 242, 154, 136	196	1.70-1.80 (m, 4H, $(CH_2)_2$ of pyrrolidine), 2.50-2.57 (m, 4H, $N(CH_2)_2$ of pyrrolidine), 2.88-3.08 (t, 2H, NCH_2), 3.98 (s, 1H, OCH_3), 4.30-4.36 (t, 2H, OCH_2), 6.73-6.79 (d, 1H, H^4), 6.84-6.86 (dd, 1H, H^2), 7.53 (s, 1H, H^7), 7.64 (s, 1H, H^{10}), 8.18-8.22 (s, 1H, H^1), 9.13 (s, 1H, OH)
3h	$C_{21}H_{23}O_5N$	369 (M^+), 149, 121, 91, 55	205	1.4-1.5 (m, 6, $(CH_2)_3$ of piperidine rign), 2.4-2.7 (m, 4H, $N(CH_2)_2$ of piperidine ring), 2.71-2.76 (t, 2H, NCH_2), 3.77 (s, 3H, OCH_3), 4.30-4.36 (t, 2H, OCH_2), 6.72-6.74 (d, 1H, H^4), 6.79-6.85 (dd, 1H, H^2), 7.5 (s, 1H, H^7), 7.71 (s, 1H, H^{10}), 8.18-8.23 (dd, 1H, H^2)

Table III : Physical data and characterization of 6H-dibenzo [b,d] pyron-6-ones (3d-3h)

Compd. No.	Mol. Formula	Mass: m/z	m.p. °C	^1H NMR ($CDCl_3$, δ ppm)
3d	$C_{14}H_{10}O_5$	258 (M^+) 243, 219, 215, 187, 131, 91, 69	>250	3.91 (s, 3H, OCH_3), 6.72-6.73 (d, 1H, H^4), 6.79-6.85 (dd, 1H, H^2), 7.40 (s, 1H, H^{10}), 7.50 (s, 1H, H^7), 7.88-7.92 (d, 1H, H^1)
3e	$C_{15}H_{12}O_5$	272 (M^+), 258, 228, 214, 158, 121	>280	3.85 (s, 3H, 8-OCH_3), 3.98 (s, 3H, 9-OCH_3), 6.70-6.71 (d, 1H, H^4), 6.78-6.82 (dd, 1H, H^2), 7.5 (s, 1H, H^{10}), 7.6 (s, 1H, H^7), 8.13-8.18 (d, 1H, H^1)
3f	$C_{20}H_{16}O_5$	272 (M^+), 258, 228, 214, 158, 121	198	3.91 (s, 3H, OCH_3), 5.49 (s, 2H, OCH_2), 6.86-6.87 (d, 1H, H^4), 6.94-6.98 (dd, 1H, H^2), 7.49-7.69 (m, 7H, H^7, H^{10} and Ar-H), 7.93 (s, 1H, H^7), 8.26-8.30 (d, 1H, H^1)
3g	$C_{20}H_{21}O_5N$	355 (M^+), 307, 289, 242, 154, 136	196	1.70-1.80 (m, 4H, $(CH_2)_2$ of pyrrolidine), 2.50-2.57 (m, 4H, $N(CH_2)_2$ of pyrrolidine), 2.88-3.08 (t, 2H, NCH_2), 3.98 (s, 1H, OCH_3), 4.30-4.36 (t, 2H, OCH_2), 6.73-6.79 (d, 1H, H^4), 6.84-6.86 (dd, 1H, H^2), 7.53 (s, 1H, H^7), 7.64 (s, 1H, H^{10}), 8.18-8.22 (s, 1H, H^1), 9.13 (s, 1H, OH)
3h	$C_{21}H_{23}O_5N$	369 (M^+), 149, 121, 91, 55	205	1.4-1.5 (m, 6, $(CH_2)_3$ of piperidine rign), 2.4-2.7 (m, 4H, $N(CH_2)_2$ of piperidine ring), 2.71-2.76 (t, 2H, NCH_2), 3.77 (s, 3H, OCH_3), 4.30-4.36 (t, 2H, OCH_2), 6.72-6.74 (d, 1H, H^4), 6.79-6.85 (dd, 1H, H^2), 7.5 (s, 1H, H^7), 7.71 (s, 1H, H^{10}), 8.18-8.23 (dd, 1H, H^2)

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Table IV : Physical data and characterization of substituted 6H-dibenzo [b,d] pyron-6-ones (4a-h)

Compd. No.	Mol. Formula	Mass: m/z	m.p. °C	^{1}H NMR ($CDCl_3$, δ ppm)
4a	$C_{16}H_{14}O_3$	254 (M^+), 212, 184, 128, 83	78	1.37 x 4.00 (s, 6H, $(CH_3)_2$ of isopropyl gp), 4.59-4.68 (q, 1H, –CH– of isopropyl gp), 7.4-7.7 (m, 2H, H^2 & H^4), 7.5-7.6 (t, 1H, H^8), 7.78-7.81 (t, 1H, H^9), 7.90-7.97 (m, 2H, H^7 and H^{10}), 8.33-8.37 (d, 1H, H^1)
4b	$C_{17}H_{16}O_4$	284 (M^+), 282, 241, 226, 212, 170, 105, 68	96	1.37-1.40 (m, 6H, $(CH_3)_2$ of isopropyl gp), 3.92 (s, 3H, OCH_3), 4.51-4.69 (m, 1H, –CH– of isopropyl gp.), 6.75-6.95 (m, 2H, H^4 & H^2), 7.34-7.39 (dd, 1H, H^9), 7.76-7.77 (d, 1H, H^7), 7.83-8.12 (m, 2H, H^{10} & H^1).
4c	$C_{19}H_{20}O_4$	312 (M^+), 270, 228, 200, 170, 83, 57	78	1.25-1.44 (m, 12H, $(CH_3)_2$ of isopropyl group x 2), 4.55-4.67 (m, 1H, –CH–), 4.71-4.83 (m, 1H, –CH–), 6.83-6.88 (m, 2H, H^8 and H^4) 6.97-7.02 (dd, 1H, H^2), 7.14-7.16 (d, 1H, H^{10}), 7.82-7.86 (d, 1H, H^1), 8.25-8.29 (d, 1H, H^7)
4d	$C_{19}H_{20}O_4$	312 (M^+), 228, 171, 149, 115, 91	98	1.36-1.39 (m, 12H, $(CH_3)_2$ x 2 of isopropyl gp), 4.54-4.64 (m, 1H, –CH–), 4.66-4.76 (m, 1H, –CH–), (m, 1H, –CH– of isopropyl gp.), 6.84-6.85 (s, 1H, H^4), 6.88-6.89 (d, 1H, H^2), 7.30-7.34 (dd, 1H, H^{10}), 7.75-7.76 (d, 1H, H^7), 7.82-8.19 (m, 2H, H^9 & H^1)
4e	$C_{18}H_{18}O_5$	314 (M^+), 272, 257, 229, 159, 146	218	1.37-1.40 (d, 6H, $(CH_3)_2$), 3.99 (s, 3H, 8-OCH_3), 4.07 (s, 3H, 9-OCH_3), 4.35-4.67 (s, 1H, CH-isopropyl gp.), 6.85-6.86 (d, 1H, H^4), 6.90-6.95 (d, 1H, H^2), 7.32 (s, 1H, H^7), 7.77 (s, 1H, H^{10}), 7.79-7.84 (d, 1H, H^1)
4f	$C_{18}H_{18}O_5$	342 (M^+), 300, 258, 243	196	1.37-1.59 (m, 12H, $(CH_3)_2$ x 2 of isopropyl gps), 4.0 (s, 3H,OCH_3), 4.54-4.66 (m, 1H, –CH–), 4.76-4.92 (m, 1H, –CH–), 6.75 (s, 1H, H^4), 6.84-6.89 (dd, 1H, H^2), 7.39 (s, 1H, H^7), 7.72 (s, 1H, H^{10}), 7.81-8.12 (d, 1H, H^1)
4g	$C_{28}H_{22}O_5$	438	186	3.88 (s, 3H, OCH_3), 5.02 (s, 2H, CH_2 benzylic) 5.12 (s, 2H, –CH_2- benzylic), 6.60-6.61 (d, 1H, H^4), 6.62-6.66 (m, 2H, H^7 and H^2), 7.35-7.56 (m, 14H, ArH benzylic, H^{10} and H^{11})
4h	$C_{22}H_{18}O_5$	362	192	3.80 (s, 3H, OCH_3), 3.96 (s, 3H, OCH_3), 5.44 (s, 2H, OCH_2), 6.83 (s, 1H, H^4), 6.85-6.89 (d, 1H, H^2), 7.34-7.52 (m, 7H, Ar-H, H^7 and H^{10}), 7.72-7.74 (d, 1H, H^1)

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Table V : Physical data and characterization of substituted 6H-dibenzo [b,d] pyrans (5a-f)

Compd.	Mol. Formula	Mass: m/z	m.p. °C	^{1}H NMR ($CDCl_3$, δ ppm)
5a	$C_{20}H_{24}O_4$	328 (M^+), 286, 244, 228, 215, 201, 155	96	1.33-1.40 (m, 12H, $(CH_3)_2$x2 of isopropyl groups), 3.86 (s, 3H, OCH_3), 4.47-4.57 (m, 2H, –CH– x 2 of isopropyl groups), 5.04 (s, 2H, –CH_2– of pyran ring), 6.51-6.52 (d, 1H, H^4), 6.55-6.61 (m, 2H, H^2 & H^7), 7.15 (s, 1H, H^{10}), 7.35-7.50 (d, 1H, H^1)
5b	$C_{15}H_{14}O_4$		224	
5c	$C_{28}H_{24}O_4$	424 (M^+), 334, 306, 242, 91		3.90 (s, 3H, OCH_3), 5.04 (s, 2H, –CH_2 benzylic), 5.06 (s, 2H, –CH_2– of pyran ring), 6.59-6.60 (d, 1H, H^2), 6.62-6.66 (m, 2H, H^7 and H^2), 7.35-7.56 (m, 14H, Ar-H benzylic, H^{10} and H^1)
5d	$C_{22}H_{20}O_4$	348 (M^+), 257, 229, 201, 141, 115, 91		3.80 (s, 3H, OCH_3), 4.10 (s, 3H, OCH_3), 5.04 (s, 2H, –CH_2 of pyran ring), 5.20 (s, 2H, –OCH_3–), 6.51-6.53 (d, 1H, H^4), 6.55-6.66 (dd, 1H, H^2), 6.76 (s, 1H, H^7), 7.14 (s, 1H, H^{10}), 7.30-7.49 (m, 6H, Ar-H and H^1)
5e	$C_{14}H_{12}O_4$	244 (M^+), 229, 201, 115, 44	194	3.78 (s, 3H, OCH_3), 4.96 (s, 2H, CH_2 of pyran ring), 6.32-6.33 (d, 1H, H^4), 6.43-6.49 (dd, 1H, H^2), 6.82 (s, 1H, H^7), 7.05 (s, 1H, H^{10}), 7.40-7.44 (d, 1H, H^1), 9.03 (s, 1H, 3-OH), 9.58 (s, 1H, 9-OH)
5f	$C_{15}H_{14}O_4$	257 (M^+), 242, 214, 114, 82		3.80 (s, 3H, OCH_3), 4.10 (s, 3H, OCH_3), 5.13 (s, 2H, –CH_2– of pyran ring), 5.60 (s, 1H, OH), 6.52-6.53 (d, 1H, H^4), 6.58-6.72 (m, 2H, H^2 and H^7), 7.15 (s, 1H, H^{10}), 7.41-7.57 (m, 1H, H^1)

Table VI : Physical data and characterization of substituted 6, 6-dimethyl dibenzo [b,d] pyrans (6a-d)

Compd. No.	Mol. Formula	Mass: m/z	m.p. °C	^{1}H NMR ($CDCl_3$, δ ppm)
6a	$C_{24}H_{24}O_4$	376 (M^+), 361, 269, 147, 91	152	1.56 (s, 3H, CH_3 of pyran ring), 1.60 (s, 3H, CH_3 of pyran ring), 3.79 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 5.20 (s, 2H, CH_2 of benzyloxy group), 6.59-6.60 (d, 1H, H^4), 6.70-6.76 (dd, 1H, H^2), 7.03 (s, 1H, H^7), 7.47-7.65 (m, 6H, Ar-H & H^{10}), 7.80-7.85 (dd, 1H, H^1)
6b	$C_{17}H_{18}O_4$	286 (M^+), 271, 256, 135, 105		1.56 & 1.60 (s, s, 6H, CH_3 x 2 of pyran ring), 3.80 (s, 3H, 8.0 CH_3), 3.92 (s, 3H, 3-OCH_3), 5.58 (s, 1H, OH), 6.39 (s, 1H, H^4), 6.49-6.54 (dd, 1H, H^2), 6.68 (s, 1H, H^7), 7.36 (s, 1H, H^{10}), 7.50-7.45 (dd, 1H, H^2), 6.68 (s, 1H, H^7), 7.36 (s, 1H, H^{10}), 7.50-7.45 (dd, 1H, H^1)
6c	$C_{24}H_{31}O_4N$	397 (M^+), 366, 269, 121, 112, 98	64	1.60-1.70 (m, 12H, $(CH_2)_3$ of piperidine ring and CH_3 x 2 of pyran ring), 2.51-2.56 (m, 4H, $N(CH_2)_2$), 2.81-2.87 (t, 2H, $N(CH_2)_2$), 3.80 (s, 3H, 8.0 CH_3), 3.98 (s, 3H, 3-OCH_3), 4.18-4.25 (t, 2H, OCH_2), 6.49-6.50 (d, 1H, H^4), 6.55-6.60 (d, 1H, H^2), 6.70-7.06 (s, 1H, H^7 and H^{10}), 7.47-7.51 (d, 1H, H^1)

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6d	$C_{23}H_{29}O_5N$	399 (M^+), 296, 270, 196, 152, 113, 99	126	1.60-1.70 (s, s, 6H, CH_3x2 of pyran ring), 2.50-2.54 (m, 4H, $N(CH_2)_2$ of morpholine ring), 3.68-3.80 (m, 4H, $(CH_2)_2O$ of morpholine ring), 3.90 (s, 3H, OCH_3), 3.98-4.22 (t, 2H, OCH_2), 6.49-6.51 (d, 1H, H^4), 6.55-6.60 (dd, 1H, H^2), 6.71 (s, 1H, H^7), 7.16 (s, 1H, H^{10}), 7.46-7.51 (d, 1H, H^1)
6e	$C_{23}H_{29}O_4N$	383 (M^+), 219, 149, 131, 99, 86	124	1.49 & 1.50 (s, s, 3H x 2, CH_3 x 2 of pyran ring), 1.79-1.86 (m, 4H, $(CH_2)_2$ of pyrrolidine ring), 2.64-2.67 (m, 4H, $N(CH_2)_2$), 2.95-3.02 (t, 2H, NCH_2), 3.80 (s, 3H, 3-OCH_3), 3.99 (s, 3H, 8-OCH_3), 4.19-4.26 (t, 2H, OCH_2), 6.49-6.50 (d, 1H, H^4), 6.55-6.59 (dd, 1H, H^2), 6.60 (s, 1H, H^7), 7.16 (s, 1H, H^{10}), 7.46-7.51 (d, 1H, H^1).
6f	$C_{22}H_{28}O_4$	356 (M^+), 341, 299, 257, 141, 83, 47	82	1.31-1.39 (m, 12H, $(CH_3)_2$ x 2 isopropyl groups), 1.55 (s, 3H, CH_3), 1.60 (s, 3H, CH_3), 3.87 (s, 3H, OCH_3), 4.50-4.61 (m, 2H, –CH– x 2 of isopropyl groups), 6.48-6.49 (d, 1H, H^4), 6.52-6.56 (dd, 1H, H^1), 6.57 (s, 1H, H^7), 7.15 (s, 1H, H^{10}), 7.44-7.48 (d, 1H, H^1)

Table VII : Results of Biological Activity

Compd. No.	Antiestrogenic effect (% inhibition at 10 μg dose)	Estrogenic effect (% uterine wt. gain)	RBA (ER) % of E_2	Anti-implantation activity (%) 10 μg dose (1-5 day schedule)	Antiresorptive activity % inhibition in PTH induced resorption
3c	0.61	12.2	0.01	6	1.1
3d	8.6	10.8	0.11	11	NIL
3g	9.8	41.8	0.8	22	5.6
3h	4.3	78.4	0.24	17	6.2
4a	12.7	6.1	0.02	18	2.8
4b	14.9	12.4	0.09	12	1.9
4c	10.2	7.2	0.04	20	2.1
4d	16.8	8.9	0.02	24	3.4
4e	9.4	13.4	0.11	12	2.64
4f	0.62	16.2	0.10	10	1.2
5a	9.9	6.9	0.18	33	7.78
5b	4.8	20.8	0.07	35	3.1
5e	7.2	11.2	0.12	51	1.4
5f	10.1	12.8	0.16	30	2.1
6c	18.6	10.9	0.18	26	3.1
6d	20.2	20.1	0.14	32	0.8
6e	24.7	20.4	0.10	60	4.2
6f	12.8	15.8	0.12	35	

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A Review on Controlled Release Advanced Glassy Fertilizer

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Abstract- Large scale applications of fertilizer nitrogen (N) have also shown deleterious effects on groundwater quality, especially its nitrate content, which is harmful to health. Furthermore, gaseous losses of N as NH_3 and NO_x resulting from N fertilization have adverse effects on the environment. Therefore, the goal of all agriculture has to be to "increase food-grain production with the minimum and efficient use of chemical fertilizers". This calls for a sincere effort on the part of agricultural scientists including extension workers to increase the efficiency of fertilizers applied in the farm fields. Glass fertilizers are new type of advanced and controlled released fertilizer and made of glass matrixes with macro elements (K, P, Mg, S, Ca) most useful for plants and also incorporated with microelements (B, Fe, Mo, Cu, Zn, Mn) which are important to the growth and development of corps or plants. The quantity of the microelements incorporated in the glass as oxide in the range 1-5%. The use of glass fertilizers offers lot of advantages: due to low or controlled solubility it avoid underground water pollution; the soil pH can be regulate by the pH of the glass matrix; do not release acid anions (Cl-, SO2-) which are harmful for plants so there is no risk of soil burning when they are incorrectly dosed; in a single type of fertilizer can be embedded almost all useful elements for plants; the controlled rate of solubility in water can be adjust easily by changing the composition of glass matrix.

Keywords: glass fertilizer; controlled release; plant nutrients; phosphate glass; advanced fertilizer; vitreous fertilizer; eco-friendly fertilizer.

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AREVIEWONCONTROLLEDRELEASEADVANCEDGLASSYFERTILIZER

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A Review on Controlled Release Advanced Glassy Fertilizer

G. Hazra [α] & T. Das [σ]

Abstract- Large scale applications of fertilizer nitrogen (N) have also shown deleterious effects on groundwater quality, especially its nitrate content, which is harmful to health. Furthermore, gaseous losses of N as NH_3 and NO_x resulting from N fertilization have adverse effects on the environment. Therefore, the goal of all agriculture has to be to "increase food-grain production with the minimum and efficient use of chemical fertilizers". This calls for a sincere effort on the part of agricultural scientists including extension workers to increase the efficiency of fertilizers applied in the farm fields. Glass fertilizers are new type of advanced and controlled released fertilizer and made of glass matrixes with macro elements (K, P, Mg, S, Ca) most useful for plants and also incorporated with microelements (B, Fe, Mo, Cu, Zn, Mn) which are important to the growth and development of corps or plants. The quantity of the microelements incorporated in the glass as oxide in the range 1-5%. The use of glass fertilizers offers lot of advantages: due to low or controlled solubility it avoid underground water pollution; the soil pH can be regulate by the pH of the glass matrix; do not release acid anions (Cl-, SO2-) which are harmful for plants so there is no risk of soil burning when they are incorrectly dosed; in a single type of fertilizer can be embedded almost all useful elements for plants; the controlled rate of solubility in water can be adjust easily by changing the composition of glass matrix.

Keywords: glass fertilizer; controlled release; plant nutrients; phosphate glass; advanced fertilizer; vitreous fertilizer; eco-friendly fertilizer.

I. Introduction

The worldwide per capita land base for agricultural production has declined dramatically over the past few decades and is expected to continue to decrease. For example, it's estimated that by the year 2025 the land in production per person will be 56 percent less than it was in 1965. The world population in 25 years is expected to be about 8 billion…2 billion more than the current 6 billion so the world average arable land per capita (ha) gradually decreases in every year which is shown in Fig.1. This trend will require that crop yields per unit of land continue to increase. The revolution which has resulted in a phenomenal increase in crop output per unit of land and has so remarkably scaled down the dimensions of the food crisis, has its roots in two main sources – the evolution of innumerable new varieties of crops with high yield potentials and the ready availability of fertilizers which form the life line for the meeting their increased nutritional demands i.e. these yield increases will in turn require greater nutrient inputs. The essential plant nutrients and their forms and typical concentration in plants is given in the Table-1. The effect of pH on nutrient availability is shown in Table-2.

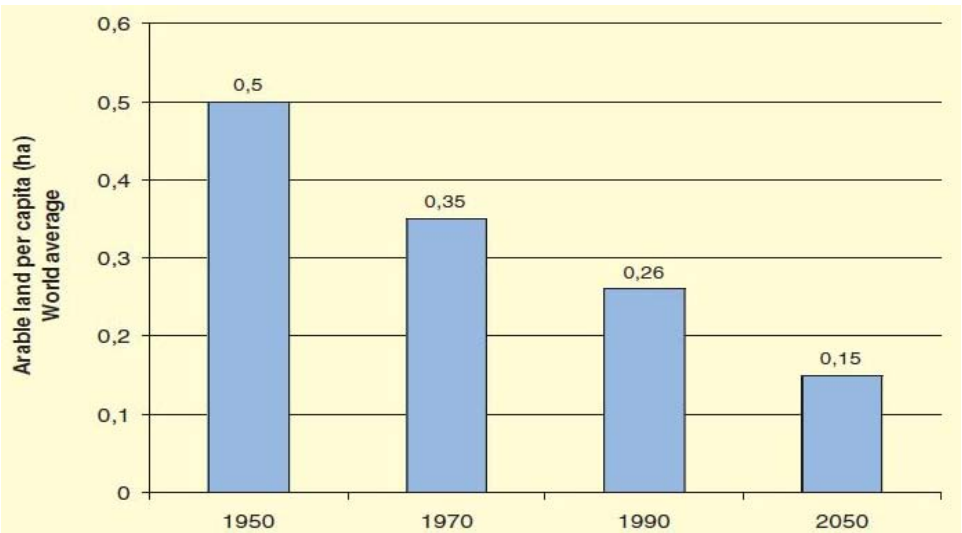

Figure 1 : Globally, arable land per capita is diminishing as population increases: while arable land remains constant, improved yield is required to meet the growing world food demand; [From: Phosphate Newsletter 23 (2005)]

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Concomitant with frequent addition of high doses of fertilizers, is the magnification of the environmental hazards the soil and water. Accumulation of anions like Cl^- and SO_4^-, soil salinisation and acidification, leaching of NH_4^+, NO_3^-, etc., and subsequent contamination of ground water are some of the damages caused by long term fertilizer application. It's also reasonable to assume that the impact of agriculture on the environment will be increasingly scrutinized since the public's influence over production is growing [1].

The main reason for this is the near total dependence on the use of water soluble salts as macro- and micro-nutrient fertilizers. The high solubility of fertilizers is not only the factor for the leaching and contamination of ground water but is also, for same reason, an economically wasteful proportion. Thus quite often, even up to 80% of urea added to soil, may be lost by leaching and volatilisation and only a small fraction micronutrients that are used as foliar sprays, is available for plants.

Glass fertilizers are new type of advanced and controlled released fertilizer and made of glass matrixes with macro elements (K, P, Mg, S, Ca) most useful for plants and also incorporated with microelements (B, Fe, Mo, Cu, Zn, Mn) which are important to the growth and development of corps or plants. The quantity of the microelements incorporated in the glass as oxide in the range 1-5%. The use of glass fertilizers offers lot of advantages: due to low or controlled solubility it avoid underground water pollution; the soil pH can be regulate by the pH of the glass matrix; do not release acid anions (Cl-, SO2-) which are harmful for plants so there is no risk of soil burning when they are incorrectly dosed; in a single type of fertilizer can be embedded almost all useful elements for plants; the controlled rate of solubility in water can be adjust easily by changing the composition of glass matrix. With the growing need for efficient utilisation of resources, such glass fertilizers (CRF) are most deplorable and call for a radical changes in the inorganic fertilizers.

Table 1: Essential plant nutrients, forms taken up and their typical concentration in plants [2]

Nutrient(symbol)	Essentiality established by	Forms absorbed	Typical concentration in plant dry matter
		Macronutrients	
Nitrogen (N)	De Saussure (1804)	NH_4+, NO_3^-	1.5%
Phosphorus (P, P2O5)	Sprengel (1839)	$H_2PO_4^-$, HPO_4^{2-}	0.1–0.4%
Potassium (K, K2O)	Sprengel (1839)	K^+	1–5%
Sulphur (S)	Salm-Horstmann (1851)	SO_4^{2-}	0.1–0.4%
Calcium (Ca)	Sprengel (1839)	Ca^{2+}	0.2–1.0%
Magnesium (Mg)	Sprengel (1839)	Mg^{2+}	0.1–0.4%
		Micronutrients	
Boron (B)	Warington (1923)	H_3BO_3, $H_2BO_3^-$	6–60 μg/g (ppm)
Iron (Fe)	Gris (1943)	Fe^{2+}	50–250.μg/g (ppm)
Manganese (Mn)	McHargue (1922)	Mn^{2+}	20–500.μg/g (ppm)
Copper (Cu)	Sommer, Lipman (1931)	Cu^+, Cu^{2+}	5–20.μg/g (ppm)
Zinc (Zn)	Sommer, Lipman (1931)	Zn^{2+}	21–150.μg/g (ppm)
Molybdenum (Mo)	Arnon& Stout (1939)	MoO_4^{2-}	below 1.μg/g (ppm)
Chlorine (Cl)	Broyer et al., (1954)	Cl^-	0.2–2 percent

Table 2 : Effect of pH on nutrient availability [2]

Nutrient availability	Very low pH (lessthan 5.0)	Low pH (5.0–5.5) Optimum pH	Optimum pH(5.6–6.2)	High pH (6.5–7.0)
Soluble—available to plant roots		Manganese, iron,	Copper and zinc	Boron
Insoluble—not available to plant root	Magnesium, calcium	Molybdenum, Calcium, Magnesium, sulfur		Phosphorous, iron,manganese, copper, zinc, boron
Highly soluble—toxic levels	Ammonium, manganese, iron,copper, zinc, boron			

II. Different Kind of Controlled Release Fertilizers

Only compounds from which plant roots can extract ions by exchange reactions, and compounds which undergo hydrolysis and solubilisation at optimum rate to fulfil the requirements of the plants, are suitable as fertilizers. The controlled release fertilizers must be, therefore, either 'slow-releasing' or must contain nutrients in exchange sites. Slow-releasing or controlled-

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releasing fertilizers are the latest concept in fertilizer technology. A real controlled-releasing fertilizer can only be formulated at the molecular level. In recent use there have different types of slow or controlled release fertilizers [3] some of them are as follow:

- Sulphur Coated Urea (SCU)
- Sulphur Coated Compound Fertilizer
- Resin Coated Fertilizer
- Urea formaldehyde
- Urea and Nitrification inhibitors
- Tower Melt Spraying Granulation Compound Fertilizer
- Urea Melt Spraying Granulation Compound Fertilizer
- Chemically Modified Biomass Coating Urea for Controlled Released
- Bulk Blend Fertilizer and
- Glass fertilizer

a) Glass Fertilizers

Projected growth of population approximately 1% a year over the next 20 years will take the world population from its current level of 6 billion to 7.5 billion by 2020. Due to economic growth as people become wealthier, they consume more and higher-quality food; the International Food Policy Research Institute (IFPRI) forecasts a 40% increase in demand for grain by 2020.The arable land is scarce in many parts of the world and under pressure from urbanization and industrial uses; accordingly, there is continual pressure to increase the productivity of available land resources. Without increases in productivity, more land will have to be brought under cultivation, with potentially severe adverse impact on the environment. The projected food grain production in relation to nutrient (N-P2O5-K2O) consumption, removal and gap are clear from the Fig.2. The innovations provide new benefits and new opportunities in crop production, e.g. precision agriculture, use of environment friendly glassy fertilizers…etc.[4].Phosphate nutrient is part of the fertilizer package that remains the driving force for the growth of crop yields and crop production that is necessary to meet the global food demand

Glass is an amorphous (non-crystalline) solid material. Most of the glasses are typically brittle, optically transparent, as a substance, plays an essential role in science and industry. The chemical, physical, and in particular optical properties make them suitable for applications such as flat glass, container glass, optics and optoelectronics material, laboratory equipment, thermal insulator (glass wool), reinforcement materials (glass-reinforced plastic, glass fiber reinforced concrete), glass art (art glass, studio glass) and recently as glass fertilizers for plants nutrients (macro & micro).

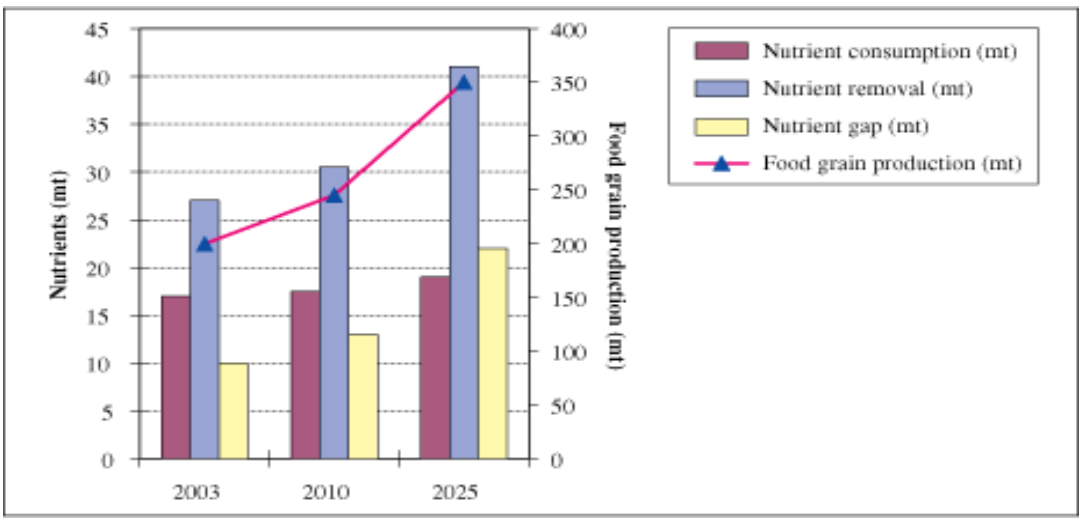

Figure 2 : Projected food grain production in relation to nutrient (N-P_2O_5-K_2O) consumption, removal and gap [5].

i. *Glass ingredients*

Quartz sand (silica) and P2O5 are the main raw material in commercial glass production. While fused quartz (primarily composed of SiO2) is used for some special glass applications but pure silica or quartz are not very common used due to its high glass transition temperature of over 2300°C. Normally, other substances are added to simplify processing i.e. to minimise the melting temperature. One of them is sodium carbonate (Na2CO3), which lowers the glass transition to about 1500°C. However, calcium oxide (CaO), generally obtained from limestone, magnesium oxide (MgO) and aluminium oxide (Al2O3) are added to provide for a better chemical durability [6]. The resulting glass contains about 70% - 74% silica by weight is called a soda-lime glass. Soda-lime glasses are comparatively more water soluble and account for about 90% of manufactured glass. The oxide components added into a glass batch may be sub-divided as (1) glass formers, (2) intermediates and modifiers. These are grouped on the basis of functions that they performed with in the glass.

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Glass formers and network formers include oxides such as SiO_2, B_2O_3, GeO_2, P_2O_5, V_2O_5 and As_2O_3 which are indispensible in the formation of glass since they form the basis of the random three dimensional networks of glasses. For the glasses which are used as fertilizers for plants nutrients P_2O_5 or phosphate salts of alkali metals or alkaline earth metals are used as glass former which have low melting point as well as serve as phosphate nutrients for the plants nutrients.

Intermediates include Al_2O_3, Sb_2O_3, ZrO_2, TiO_2, PbO, BeO and ZnO. These oxides are added in high proportions for linking up with the basic glass network to retain structural continuity [7].Modifiers include MgO, Li_2O, BaO, CaO, SrO, Na_2O and K_2O. These oxides are added to modify the properties of glass. The other additions in glass are the fluxes which lower the fusion temperature of the glass batch and render the molten glass workable at reasonable temperature. But, fluxes may reduce the resistance of glass to chemical attack render it water- soluble or make it subject to partial or complete devitrification, or what is called crystallisation, upon cooling. Devitrified glass is un desirable since the crystalliareas are externally weak and brittle. Stabilizers are therefore added to the glass batch to overcome these problems. Most common glass has other ingredients added to change its properties. The common silicate glass network structure and glass network with modifiers is shown in Fig.3.

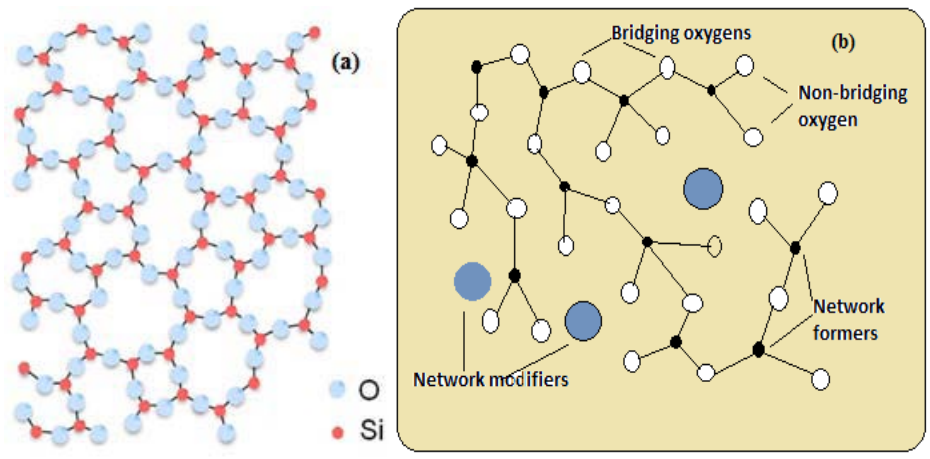

Figure 3 : (a) Silicate network structure, (b) Glass network with modifier

The interesting characteristic of phosphates which are used as former for glass fertilizers makes them so suitable for the production of polymeric fertilizers is that the ortho-phosphate ion, i.e., PO_4^{3-} , polymerises on hating with formation of linear chains of P-O-P bonds, In final stages of condensation, branches chain polymers may also be formed [8]. Thus, in a metaphosphate containing linear phosphate chain the negativity charged oxygen atoms may be neutralised by K^+, Mg^{2+}, Ca^{2+} or NH_4^+ ions (corps nutrients). Since these ions are held in exchangeable positions on an anionic polymer chain, they possess the dual property of being almost insoluble in water but being readily solubilised by complexants and by cation exchange. Moreover, slow hydrolysis of the P-O-P group occurs [9] causing solubilisation of the cations. It is noteworthy that polyphosphates of all the macro- and micro- nutrient ions may be prepared; additionally, their solubility can be varied to desire to levels by controlling the degree of polymerisation of chain. The model Network structure of the glass fertilizers with different corps nutrients is drawn in Fig.4.

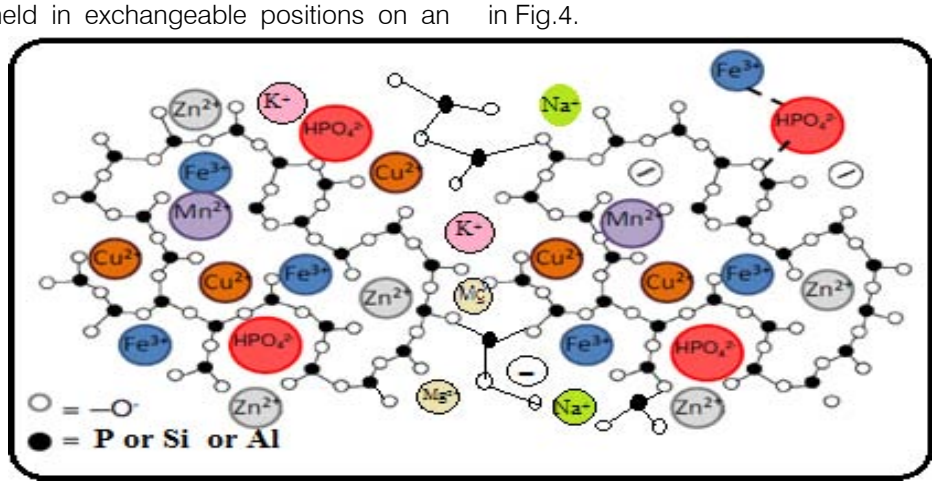

Figure 4 : Network structure of the glass fertilizers with different corps nutrients

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ii. *Composition of different glass fertilizers with various types of nutrients*

The composition of different glass fertilizers with various types of nutrients for different corps is presented in the following tables (Table-3a to Table-3e).

Table 3a : Oxide composition of some vitreous fertilizer used in field crops[10].

Code	P_2O_5mol%	MgOmol%	K_2O mol%	B_2O_3mol%	Fe_2O3mol%	ZnOmol%
AG2	41.84	22.45	35.71	-	-	-
AG2.1	32.08	16.98	26.42	24.52	-	-
AG2.2	40	21.05	32.63	-	6.32	-
AG2.3	38	20	32	-	-	10

Table 3b : Oxide composition of some Van Dien FMP fertilizer

P_2O_5 (%)	MgO (%)	Ca0 (%)	SiO_2 (%)	Microquantities
a15 - 18	≥15	≥28	≥ 24	Fe, B, Mn, Z Zn, Co, Cu,Mo

Table 3c : Oxide composition of some nitrate sulphate phosphate glass fertilizer used in field crops

Sample No.	Chemical composition (wt.%)					
	P_2O_5	KNO_3	$NaNO_3$	$KHSO_4$	Admixture	Temp.(350°-400°)C
1	61.5	28.5	10.0	-	-	Fused
2	45·3	36·5	18·2	-	-	Fused
3	45·3	27·4	27·3	-	-	Fused
4	29·2	27·5	9·6	33·8	-	Fused
5	36.5	28.5	9.9	25.0	-	Fused
6	45·0	28·8	10·0	16·2	-	Fused
7	49·0	28·7	10·0	12·4	-	Fused
8	56·7	28·5	9·9	4·9	-	Fused
9	44·8	28·8	3·6	17·5	5·3	Fused
10	42·3	27·2	3·4	16·5	10·5	Fused

Types of admixtures: $Mg(NO_3)_2$, $Zn(NO_3)_2$, $Cu(NO_3)_2$, $Fe(NO_3)_3$, B_2O_3, NH_4VO_3, $NH_4Mo_2O_7$

Table 3d : Oxide composition of glasses for spring and autumn crops, in weight % [11]

Oxide→ ↓Sample	P2O5	MgO	K2O	B2O3	Fe2O3	ZnO	MoO2	Total
AG2	58.76	8.74	32.5	-	-	-	-	100
AG2.1	47.96	7.13	26.52	18.39	-	-	-	100
AG2.2	53.42	7.94	29.54	-	9.1	-	-	100
AG2.3	54.05	8.04	29.9	-	-	8.01	-	100
AG2.4	53.18	7.92	29.41	-	-	-	9.5	100

Table 3e : Oxide composition of glasses for wine-grape, in weight % [11]

Oxide→ ↓Sample	P_2O_5	MgO	K_2O	CaO	B_2O_3	Fe_2O_3	ZnO	MoO_2	MnO_2	Total
AG3	43.47	18.48	32.61	5.44						100
AG3.1	39.64	16.85	29.74	4.96	8.81					100
AG3.2	40.57	17.25	30.43	5.08		6.67				100
AG3.3	42.41	18.03	31.82	5.31			2.43			100
AG3.4	42.15	17.91	31.61	5.27					3.06	100
AG3.5	42.21	17.94	31.66	5.28				2.91		100

iii. *Structure and some properties of some glassy fertilizers*

In theFig.5 the different types of magnesium phosphate and their network-structure is shown which is formed in the magnesium containing phosphate glass fertilizers. The Typical structure of a new slow-releasing iron fertilizer is shown in the Fig.6.

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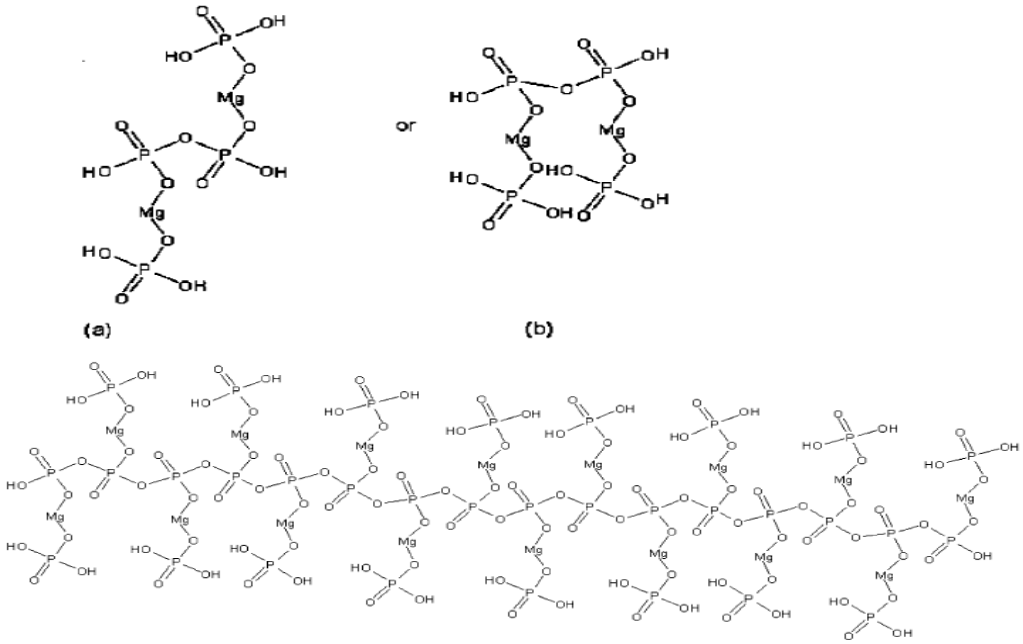

Figure 5 : Polymerization steps showing metal tetraphosphate dimer of less-stable (b) and more-stable (a) forms, plus the stable form of a multidimensional polymer (brickwall-like structure). Magnesium is shown as an example of a metal [12]

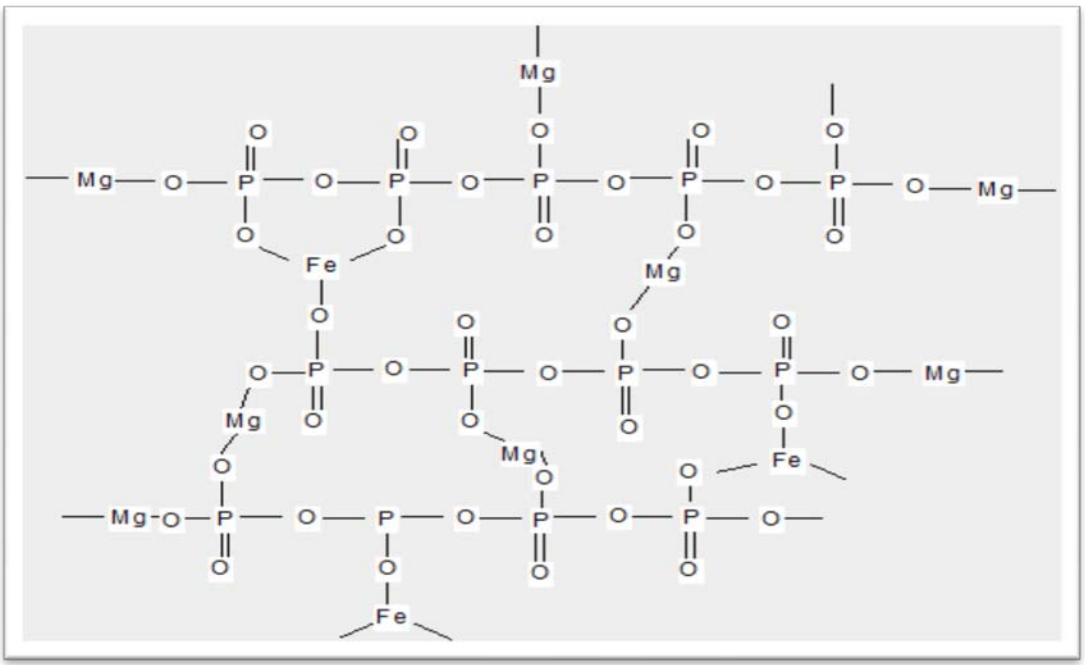

Figure 6 : Typical structure of a new slow-releasing iron fertilizer
(From: Chem. Eng. Journal, 2009)

The FTIR spectra and Raman spectra of various types of glass fertilizers with different compositions, shown in the Table-3d and Table-3e are shown in the Fig.7 and Fig.8 respectively.

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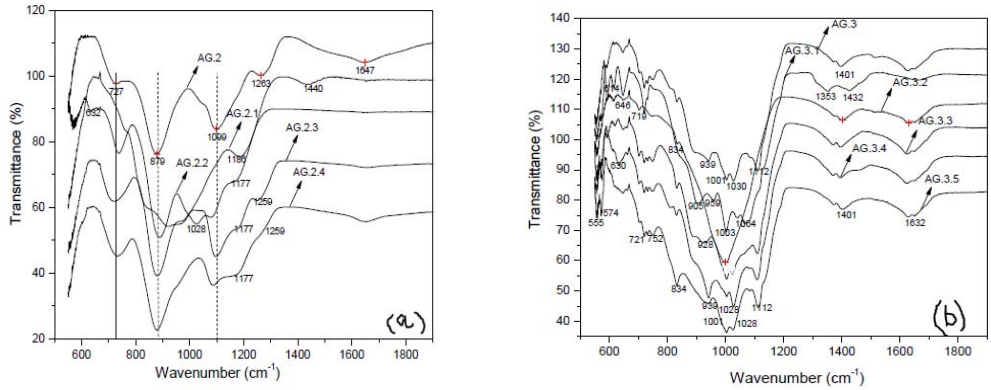

Figure 7 : Transmission FTIR spectra for potassium magnesium-phosphate glass samples, (a) AG2 type (Table-3d) and (b) AG3 type (Table-3e) [11].

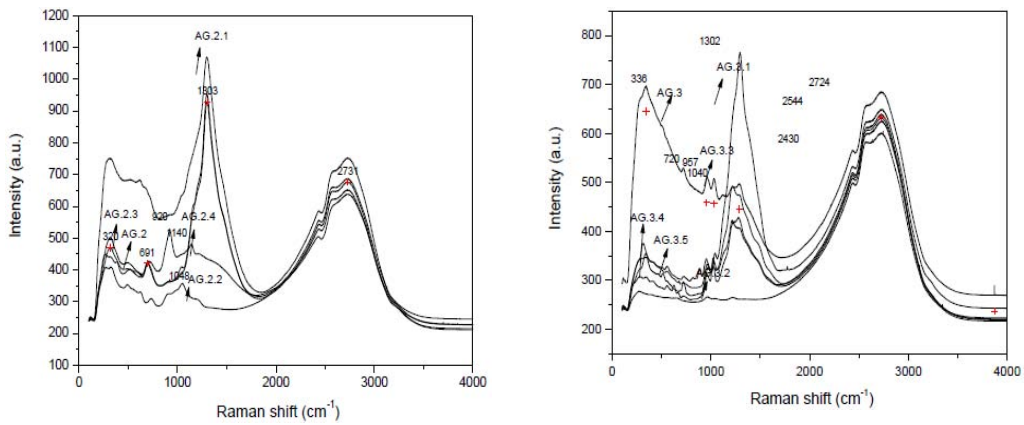

Figure 8 : Raman spectra of the glass samples from AG2 series (Table-3d) and AG3 series, (Tablle-3e) recorded in the 100-4000 cm-1 domain [11].

III. Functional Activity (With Structures)

The schematic binding procedure of 'glass fertilizers nutrients' with the soil component and plant's root showing its' network structure is presented in the Fig.9.

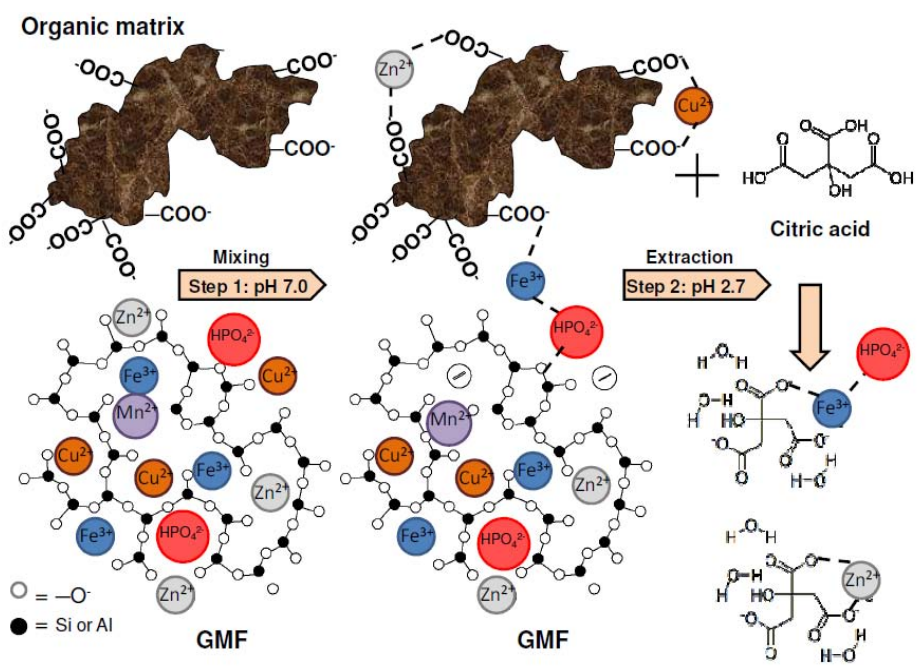

Figure 9 : Schematic binding procedure of glass fertilizers' nutrients with soil showing its' network structure [13].

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IV. Leaching Studies

a) Destruction of Glass Surfaces

Just like metal rusts, glass is undergoes to a corrosion process caused by reactions between the glass surface and gases in the atmosphere or different (chemical) solutions which come in contact. Glass is hydrophilic i.e. it attracts and holds moisture. All glass has a molecular layer of moisture on the surface as shown in the Fig.10.

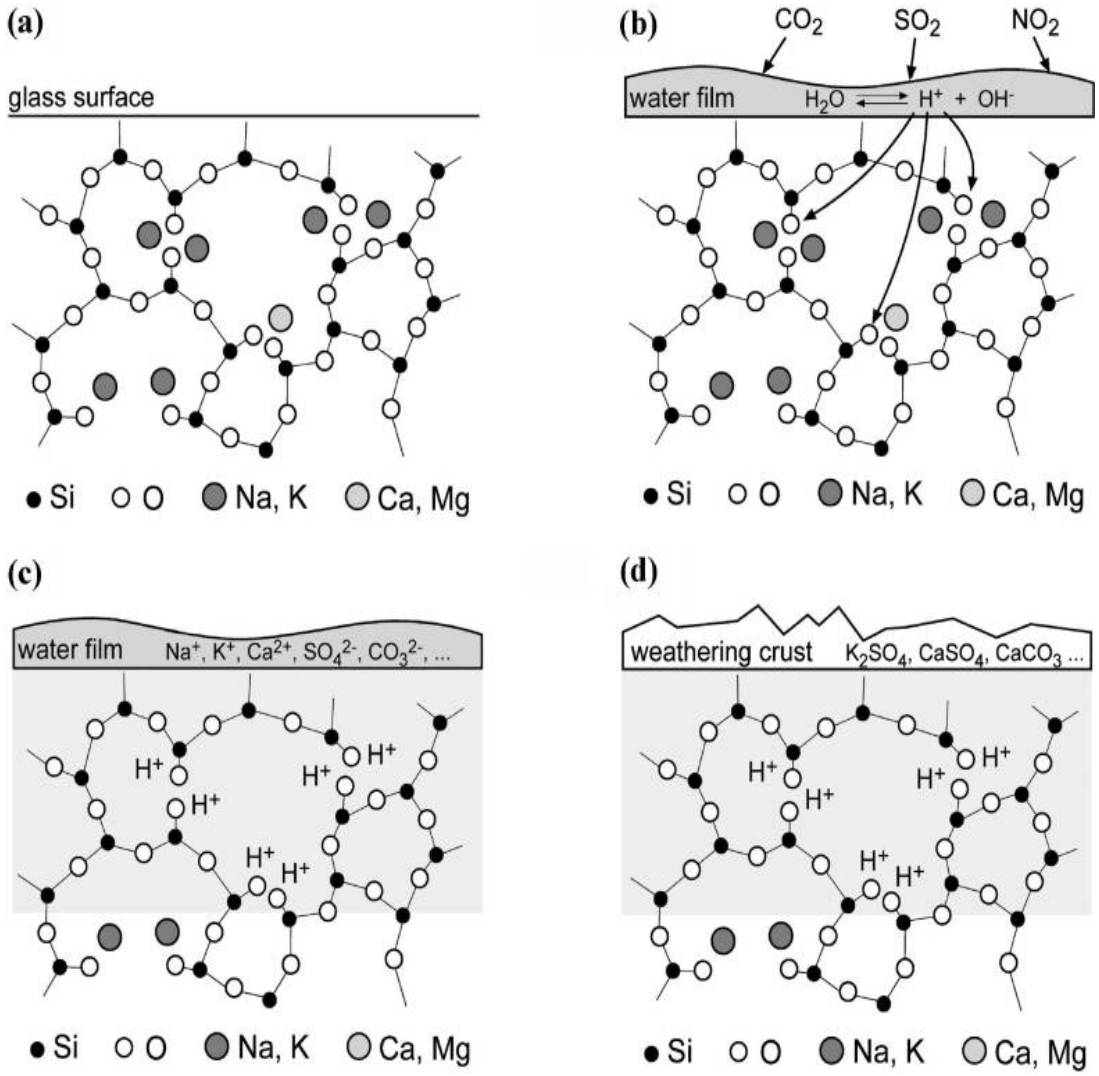

Figure 10 : (a) Glass weathering process starting from a clean surface, (b) a formation of a water film, (c) a leached layer containingHydrogen,(d) crystalline weathering products on the glass surface.

When this layer increases because of humidity or rainfall, it participates greatly to the destruction of the surface of the glass which is shown in Fig.10. There are two distinct stages to the corrosion process, occurring together or separately. One of them is aqueous corrosion, caused by moisture and is referred to as ion exchange or alkali extraction (leaching). An ion exchange occurs between alkali ions (K^+,Na^+) from the glass and hydrogen ions from the corrosion solution. The remaining components of the glass are not altered, but the effective surface area in contact with the solution is increased. This increase in surface area leads to extraction or leaching of the metal ions as nutrients from the glass fertilizers. As former (SiO_2 / P_2O_5) concentration in the glass goes down, surface area increases through dissolution of the glass surface. The pH of the solution in contact with the glass surfaces will greatly affect the corrosion process. The rapid increase in pH will cause a rapid breakdown of the glass surface. There are two types of aqueous corrosion, static and dynamic. Static aqueous corrosion is caused by an entrapment of moisture on the surface of the glass. In dynamic aqueous corrosion, the corrosion solution is replenished due to condensation run-off. In the mass transfer controlled leaching process, the fluids are always in motion e.g. batch processes with continuous mixing thus means that the fluid flows in a turbulent state past a solid surface, however, because the fluid

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velocity is zero at the surface of the particles, there must be a film of fluid adjacent to the surface. Using the idea that a thin film is responsible for the resistance of transfer, one can write the equation for mass transfer as [14].

$$\frac{dM}{dt} = \frac{k' A(C_S - C)}{b} \quad (1)$$

Where,

A is the area of solid -liquid interface,

b is the effective thickness of the liquid film surrounding the particles,

C is the concentration of the solute in the solution bulk at time t,

C_S is the concentration of the saturated solution in contact with the particles.

M is the mass of solute transferred in time t, and

k' is the diffusion coefficient.

$$ln\frac{C_S - C_o}{C_S - C} = \frac{k' A}{Vb} t \quad (2)$$

For pure solvent $C_o = 0$, therefore

$$C = C_S(1 - e^{\frac{k' A}{Vb} t}) \quad (3)$$

To made the suitable glass fertilizer, physical, chemical and dissolution properties were investigated according to variation of the composition in both phosphate and silicate glass systems. Among them phosphate system is more suitable one because phosphate component act as a former as well as macronutrient for the glass fertilizers and plant respectively. In glass forming region, K_2O-CaO-SiO_2-P_2O_5 and K_2O-MgO-SiO_2-P_2O_5 glass systems were used as most of the glass fertilizers. The glass transition temperature (Tg) and softening temperature (Ts) were gradually shifted to the higher temperature range according to increase of SiO_2 contents. The K_2O and Na_2O contents, which could cause the structure change from network structure to polymeric chain structure, have direct proportion with the thermal expansion coefficient and inverse proportion with Tg and Ts[15].

For the application of environment friendly glass-fertilizer, K_2O-CaO-P_2O_5 glasses were chooses and the dissolution properties of these glasses were investigated using pH meter and ICP analyzer by H.K. Lee et al in 2005.[Hoi Kwan Lee et al., 2005, Materials Science Forum, 486-487, 407].The results shown that pH values depended on the glass compositions, and the ICP analysis confirmed that the dissolution rate was inversely proportional to the change of the K_2O/P_2O_5 ratio, which was a main factor in controlling chemical durability of the glass fertilizer, and which could be controlled by mother glass matrix composition. Therefore, the phosphate glasses are expected to provide the slow-releasing nutrient fertilizers that are easy to produce, environmentally safe, and widely applicable [16].

Pure vitreous P_2O_5 consists in a continuous random network (polymeric structure) of quasi-tetrahedral PO_4 units wherein phosphorous is four coordinated and only three of the oxygen atoms of each unit bridge to neighbouring units, while the forth is doubly bonded to the central phosphorous atom. The presence of the modifier like alkali and alkaline earth species decreases the number of bridging oxygens (P-O-P bridge) in PO_4 units, while its negative charge increases. Two PO_4tetrahedra sharing an oxygen, that can be represented as $(PO_3)^{2-}$ –O–$(PO_3)^{2-}$, form the $(P_2O_7)^{4-}$ pyrophosphate anions. Both in the melt and during the quenching process will occur an equilibrium between the pyrophosphate anions and their products as follows [17] $(PO_3)^{2-}$ –O– $(PO_3)^{2-}$ ↔ $(PO_4)^{2-}$ + –O–(PO_2). The dissolution resistance has to be also related to the presence of Na2O in the glass matrix, having in view that the alkali ions diminish the network consistency [18].

The initial stages of the aqueous reactions always results in the leeching of alkali and alkaline earth species from the surface of the glass to create a P2O5-rich surface layer. It is generally believed that in the initial stages of the leaching reaction, the contact of liquid water or vapour water with the glass surface leads to an exchange of alkali and alkaline earth ions in the glass with hydrogenated ions in the aqueous environment. (i.e. ion exchange or interdiffusionmechanism).

Another mechanism proposed is based on the diffusion of molecular water into the glass and its chemisorption at the non-bridging oxygen sites where alkali and alkaline earth species reside in the glass [19].

b) Advantages of Glassy Fertilizers

- Each element (former and modifier) in glass fertilizer has an effect to give a very high increase in the fertility of the soil and they are not water-soluble yet easily soluble in weak acidic content in the soil or generated by plant roots. Solubility of each substance in 2 %citric acid is same as shown in the Table 4.

Table 4 : Solubility of some oxides in the 2 % citric acid.

P_2O_5 :	98 – 99%
CaO, MgO :	over 98%
SiO_2:	over 95%
Fe_2O_3 :	approx. 90%

If the glass fertilizers are water-soluble, then P_2O_5 shall combine with minerals (i.e. iron, aluminium) presence in the soil to form precipitants which are hardly absorbed by plants thereby reducing considerably the effect to increase the fertility of the soil. The residual of the common fertilizer shall dissolve in water and is washed out after few hours of its application. Glass fertilizer does not have that weak point, thus it is not washed

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out easily, not disintegrated in the soil and can supply the nutrients for a long time with effect of increasing fertility of the soil.

- The glass fertilizer can neutralize toxic acids and can bind the heavy metals in the soil and from other fertilizers. The effect of glass fertilizer is characterized by acidity, within pH 8.0 – pH8.5.
- Controlled release glass fertilizer is very convenient for use and can be preserved for a long time because it absorb less moisture, does not disintegrate even in damp weather or (below 500°C).
- Glass fertilizer does not contain toxics substances, since it does not have an acidic sulphate or chloricradical, glass fertilizer does not cause acidity to the soil, toxic gas or hydro sulphuric acid that can destroy plant roots on rice-fields [20]. Normally, the soil is poor in phosphate (P2O5), therefore, P_2O_5 is necessarily to be added. P2O5 is the important constituents of plant root cells which assist the roots in growing strongly thus further improving the yield. The glass fertilizer is not easily water-soluble, it lies within the soil and continues providing necessary nutrients for the plants on the other hands common fertilizers are easily soluble in water, for example, super phosphate, and ammoniac sulphate can have immediate effects but are easily held by aluminium in the soil thus rapidly washed out. Plant roots still continue to dissolve P_2O_5 via immediate contact with glass fertilizer in the soil. This effect is very important to the type of soil originating from volcano ashes, wild soil and exhausted fields poor in P_2O_5 [20].
- The glass fertilizer not only helps increase the fertility of the soil, suitable for many kinds of plant but also help prevent lack of magnesium and some other nutrients in the soil that support the plants' growth [3]. Mg is very necessary for creating Chlorophyll in plant leaves, the main constituent of the plants. Mg plays an essential role in the production of protein and fat in plants. Mg improves the effect of phosphate, helping plants absorb the nutrients lying inside the soil and also participate in transporting P_2O_5 that has been absorbed in the tree-trunk. Fused Magnesium Phosphate (FMP) fertilizer i.e. one kind of glass fertilizer can be seen as the most suitable one in tropical and subtropical zones poor in P_2O_5. In such zones, many kinds of nutrient of plants are in the process of washing out; this situation can be improved by using controlled released glass fertilizer continuously, on the other hand it assists the soil in maintaining the nutrients in an efficient manner [11,20].
- Unlike classical common fertilizers, which are used only 35-40% by plants, glass fertilizers are totally absorbed, which protects the soil from pollution. On the other side, glass fertilizers used quantities was at least two times smaller than in the case of classical ones, which implies decreasing of production costs and very significant reduction of pollution. At the same time, the soil pressing grade is significantly reduced. The use of vitreous fertilizers showed it efficiency, together with the classical ones, but also at using them without the classical ones [11,21].

V. Conclusion

Since the inception of Green Revolution there has been a race for increasing food grain (mainly cereals) production using chemical fertilizers in India. However, cereal production in the country increased only five fold, while fertilizer consumption increased 322 times during the 1950–51 to 2007–08 period, implying a very low fertilizer use efficiency [22]. The Controlled Release Fertilizers delivers up to 10 weeks of healthy plant growth and colour, so you can make fewer applications in a season. Less product breakage means less quick release, less surge growth and longer residual feeding. Fewer products are lost to leaching and volatilization, reducing environmental impact. Slow release fertilisers are less nitrogen "lock-off" that means we get the nitrogen we're paying for in the expected time frame. The CRF can trace elements that can be fitted into slightly soluble glasses for slow release in soil. The experiments have shown a 25-50% increase in the crop production with use of these micro nutrient glass fertilizers and the benefits can be seen for over 20 years of each addition. Micro Nutrient Glass Fertilizers release micronutrient trace chemicals in soil for balanced plant growth, over a 10-20 year period, and are not easily washed away [3,20].

If a mixture of phosphate rock and olivine or serpentine (magnesium silicate) is fused in an electric furnace [11]. The molten product is quenched with water and used in a finely divided state as a fertilizer. The product, a calcium magnesium phosphate (CMP) glass, contains about 20% P_2O_5 and 15% MgO. Over 90% of the product is soluble in citric acid. The minerals are variable in compositions; iron, nickel, and sometimes manganese may substitute for magnesium.

The change of the K_2O/P_2O_5 ratio is the main key factor to control water solubility, physical properties such as density and hardness and chemical durability. In the abnormal glass properties such as fast dissolution in aqueous solution, it was presented that the glass can be a good candidate for agriculture fertilizer [15,23].

It can be concluded that the glass composition and structure can be designed in order to control the solubility in water and to obtain valuable vitreous fertilizer with special application in plant production.

Most important of all, water and soil pollution hazards are minimised and the economics of fertilizer use is significantly improved. All this can be achieved with just cheap and readily available raw materials and

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using processes that are both technical simple and fairly low energy consuming. It would appear that in the long run polyphosphates are indeed the answer to the problem of choosing the right fertilizers for the needs of the future [1].

A higher effectiveness of lead ions elimination from the examined chloride solutions in relation to cadmium ions has been observed [24]. The presence of citric acid solution simulating natural soils environment has an inhibiting effect on the process of bonding lead and cadmium into the form of insoluble phosphates.

For maximizing health and growth of crops, plants need to ingest certain elements, such as borax, cobalt, iron, manganese and nickel in trace quantities. Use of the common salts of these chemicals do not help very much, not only because excess quantities may actually be harmful, but these salts are usually soluble in water, and are washed away with the first rain, and so, are not only wasted, but contaminate the soil nearby with excess micronutrients. Micronutrient glass fertilizers, on the other hand, contain these micronutrients in the form of slightly soluble glassy granules, which cannot be washed away easily, and dissolve into the soil slowly, so that 200gms per sq. metre of micronutrient glass fertilizer provides the required nutrients over a period of 20-30 years, for the fertilized area before replenishments are required. So this is holistic approach to the environment.

VI. Acknowledgements

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Antibacterial and Antifungal Activities of Essential Oils from Satureia biflora D. Don, Benth, Speng (Chepsagitiet). Lippia javanica Burm.F. (Labotuet) and Toddalia asiatica (L) Lam. Rutaceae (Chepindoruet)

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Keywords: *nosocomial, opportunistic, infections, essential oils phytomedicine, and drugs.*

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Antibacterial and Antifungal Activities of Essential Oils from Satureia biflora D. Don, Benth, Speng (Chepsagitiet). Lippia javanica Burm.F. (Labotuet) and Toddalia asiatica (L) Lam. Rutaceae (Chepindoruet)

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Abstract- Three plants, parts used, by the Ogiek community as medicines were collected from Marioshoni in the Mau Forest Complex in the Rift Valley of Kenya, idenfied at the National Museums in Nairobi; *S. biflora* Voucher No.AO/08/12/12/2006 *L. javanica* O/015/NMK/06/12/2007 *T. asiatica* Voucher No. AO/017/NMK/04/07/2007. Each plant species was steam distilled individually. All yielded oils that were separately assayed against some known pathogens that inflict maladies in humans. The two species showed some markable antimicrobial activities for instance, *S. biflora* was effective against *S. aureus*, *K. pneumoniae* and *E. coli* including all their drug resistant strains. The essential oils from all the three plant species possessed activities against all the strains of *S. aureus,* different zones of inhibitions *L. javanica* is amongst other medicinal essential oils that have been studied elsewhere and proved useful medically. However, *S. biflora* which has varied activities when screened in the laboratory *in vitro* has not been studied exhaustively. The plant oil extracts gave promising results against both Gram-ve and Gram +ve bacteria. *T. asiatica* showed good activities against the bacteria and some known pathogenic fungi Further studies on wet bench chemistry and GC showed known classes of compounds which are of known medicinal values. It was recommended that further studies be done to ascertain if they can be used as phytomedicine or as components of current allopathic drugs.

Keywords: nosocomial, opportunistic, infections, essential oils phytomedicine, and drugs.

I. Introduction

Plants have been used by human against bacterial and fungus there is and other related conditions *in vogue* –"time immemorial". Currently this is influx in nosocomial and opportunistic infections, therefore an urgent need for new antibiotics to tackle the epidermic. *Lippia javanica,* a Verbenaceae, are used to treat fresh wounds. The same species has been used as a mosquito repellent by populations in East Africa up to South Africa for a long time (Lukwa, 1996). Previous studies in Kenya have shown that oils from *L. javanica* have very strong and lasting repellent activity against Bruchids (Tarus, 2006). Other studies showed that extracts of *L. javanica* were not active against *E. coli* and other bacteria (McGraw *et al.*, 2000).Long before mankind discovered the existence of microbes, the idea that certain plants possess healing potential and indeed, that they contained what we could currently identify as antimicrobial principles, was well-accepted (*Rosa damscena* mill. L. *Camiphora* spp). The plant species used in the study are those that are used by the Ogiek community of Kenya who are forest dwellers as hunters and forest product users.

The three plants that are traditionally used by the community and were analysed are: 1. *Satureia biflora* D. Don. Benth. Lamiaceae. (Labiatae) (Chepsagitiet) (Voucher No. AO/08/2/12/2006) Plant description; an erect woody herb to 50 cm, hairless to hairy with elliptic to circular entire leaves 12 (-20) x 8 mm; inflorescence bracts leaf like flowers 2–20 in dense auxiliary clusters, pink, 8 mm long; sepal teeth all equal in lowland individuals but flower up to 2 x as long upper ones in alpine forms. Common in wastelands and roadsides.

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Satureia biflora (See arrow) Plate left, regenerating plant and right full grown plant Mariashoni (Dec, 2006).

Usage and Dosage of the Medicine

The preparations from the plant are used in the following manner: About 200 gm of the roots crushed and boiled in one litre of water, decanted and 200 ml of decoction drunk daily for 4 days against stomachache, gastroenteritis and pneumonia.

a) *Lippia javanicca* (Burm.F.) Spreng. Verbenaceae. (Mwokyot) (Voucher No. AO/015/NMK/06/12/2007)

i. *Plant Description*

Shrub 0.5-3 m leaves opposite (rarely in threes), aromatic ovate or elliptic, base cuneate,apex acute margin crenate, 2-8 by 0.6-3 cm, sandpapery abovepubscent beneath. Flowers white or cream with yellow throat, in short-peduncles (rarely long-stalked) crowded spikes 0.5-1cm long; corolla tube about 2 mm long. Locally abundant in secondary bushland or grassland; found abundantly in Mau wastelands.

Lippia javanicca Plants, Marioshoni, Dec, 2007

ii. *Usage and Dosage of the medicine*

About 50 grams of fresh leaves a bunch carefully wrapped around fresh wound to enhance healing.

b) *Toddalia asiatica* (L) Lam. Rutaceae (Chepindoruet) (Voucher No. AO/017/NMK/04/07/2007)

i. *Plant Description*

Semi-climber, spiny, stem green when young turning yellowish-brown, leaves green, palmately compound; leaves found in clusters. Flowers greenish yellow orange fruits when ripe in axillary and terminal panicles; petals 2-3 mm long. Commonly distributed in forest margin or secondary regrowth and grassland thickets, 450 m coast and 1200-3000 m has profile growth at high altitudes.

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Toddalia asiatica: left, Thicket, right, fruiting branch; Kaptuiget Forest (July, 2007)

ii. *Usage and Dosage of the Medicine*

The roots chewed to cure various body ailments ranging from malaria, stomachache and sore throat. A handful of the roots boiled in 1 litre of water and the decoction taken as tonic against harsh environmental conditions.

II. Materials and Methods

The three plant species that were used are part of the flora of Mau Forest complex and were collected from Mariashoni area in the Rift Valley of Kenya, identified at the National Museums in Nairobi and assigned the following: *S. biflora* Voucher No.AO/08/12/12/2006, *L. javanica* Voucher No. AO/015/NMK/06/12/2007 and *T. asiatica* Voucher No. AO/017/NMK/04/07/2007. Several studies were carried out as given in the text:

a) Collection and Extraction

Fresh leaves of *L javanica* whole plant of *S. biflora* 2 kg of each of the plants were separately steam distilled through hydrodistillation using Cleveland apparatus until no more of the oil was recoverable from the samples. The oily substances obtained from each were put into separate vials, tightly corked, sealed with parafilm and frozen at 4^{0}C in a dark refrigerator for future use. The same process was done fresh leaves and ripening fruits of *T. asiatica* separately. The tests were then individually carried out.

b) Screening for Antimicrobial Activity

Antimicrobial efficacies were tested using the filter paper disc diffusion method (Elgayyar *et al.*, 2000). 0.1ml of the extracted oils was individually loaded onto 6mm diameter paper disc. The Muller-Hinton and Potato Dextrose Agar (PDA) were used in the culture of bacteria and fungi, respectively. Each medium was prepared by weighing the quantities recommended by the manufacturer and dissolving in recommended quantities of distilled water. The media was then sterilized using an autoclave set at 121°C for 15 min and poured onto sterile Petri dishes then allowed to cool on a clean bench. Each plate was seeded with 0.1 ml of bacterial and yeast culture directly from the 24 hr broth culture diluted to match 0.5 and 1.0 McFarland's standard, respectively (10^8 Colony Forming Units (CFU)/ ml) and fungi diluted to match 1.0 McFarland's standard (10^8 spores/ml). The discs loaded with the extracts were then placed onto the seeded plates. The bacterial and fungal cultures were incubated at 37°C for 24 to 48 hrs, while fungi were incubated at 25°C for 5 days. After the incubation period the zones of inhibition were measured and recorded in mm as described by (Elgayyar *et al.*, 2000). Negative control plates had discs with sterile methanol. The plants which were found to posses bioactivities were further subjected to screening for purposes of obtaining their inhibition abilities at different concentrations in milligram. *Trichophyton mentagrophytes* and *Microsporum gypseum* were incubated at 25°C for a period of five days to ascertain their antimicrobial sensitivity and resistance were confirmed by use of standard discs containing ampicillin (10μg), chloramphenicol (30μg), erythromycin (15μg), gentamycin (10μg), ciprofloxacin (10μg), tetracycline (30μg), amikacin (30μg) and an additional oxycacilin (1μg) for *S. aureus* (oxoid, London). The standards for fungi were discs containing fluconazole.

c) Disc Diffusion and MIC ratings of the Extracts

Disc diffusion and MIC of the seam extracts were carried out. The negative controls of the disc diffusion testing was done by use of methanol did not show any inhibition, The negative control was the paper disc dipped in methanol and showed no growth, while positive control was done by use of Standard antibiotic discs (Oxoid). The average zone of inhibition was calculated for the 3 replicates. A clearing zone of 9 mm for Gram-positive and Gram-negative bacteria and 10 mm for fungi or greater was used as the criterion for designating significant antibacterial and antifungal

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activity (Faizi *et al.*, 2003). The *in vitro* MIC results were classified as per Pessini *et al*. (2003). The extracts that displayed MIC lower than 100 µg/ml, the antimicrobial activity was considered very high; from 100-500 µg/ml, high; 500-1000 µg/ml, moderate; 1000-4000 µg/ml, low and anything above this, the extracts were considered inactive for both bacteria and fungi solvent had the least activity from the root bark extracts.

d) Gas Chromatography (GC)/mass Spectrophotometer (MS)

GC and MS co-injection were performed on capillary gas chromatograph Hewlett Packard (HP) 5890 A series II, equipped with a split- less capillary injector system, cross-linked Hewlett Packard ultra 1-methyl silicone (50m length, 0.22 m internal diameter, 0.33µm carbowax film thickness) capillary column and a flame ionization detector coupled to Hewlett Packard 3396 series II integrator. Hydrogen gas was used as source of fuel, while nitrogen gas flowing at a speed of 0.8ml/min was used as carrier gas. GC-MS analysis was carried out on a Hewlett Packard 5790A series II Gas Chromatograph coupled to a VG Analytical Organic Mass Spectrophotometer (Micromass, United Kingdom) formally known as VG Biotech. The mass spectrophotometer (MS) was operated in the electron ionization (EI) mode at 70 electron volts (eV) and an emission current of 200 micro amperes (µA). The temperature of the source was held at 180 °C and a multiplier voltage of 300 volts. The pressure of the ion source and MS detector were held at 9.4 x10^{-6} and 1.4 x 10^{-5} milli-bar, respectively. The MS had a scan cycle of 1.5 seconds (scan duration of 1s and inter-scan delay of 0.5 s). The mass and scan ranges were set at mass charge ration (m/z) of 1-1400 and 38-650 respectively. The instrument was calibrated using heptacosafluoro-tributyl amine, $[CF_3\text{-}(CF_2)_3]_3N$. The column used for GC-MS was the same as the one described for GC above except that the film thickness was 0.5µm. The temperature programme was similar to the ones earlier described for GC. Helium was used as a carrier gas. In both GC and GC-MS, High Performance Liquid Chromatography (HPLC) grade dichloromethane (DCM) was used as a dilution solvent.

III. Results

The plants used posses' oils that have medicinal value. They were: *T. asiatica, S. biflora,* and *L. javanica.* Oils extracted from the leaves and fruits of *T. asiatica* showed high activities against both human pathogenic fungi bacteria. *S. biflora* whole plant distillate showed high activities against pathogens. This supports and justifies their continued use as sources of medicines by the community.

Table 1 : Screening for Activity

Name of plants	*S.a.*	*P.a.*	*E.f.*	*K.p.*	*E.c.*	*S.t.*	*C.a.*	*C.b.*	*C.n.*	*T.m.*	*M.g.*
T. asiatica	+++	-	-	-	-	-	+++	+++	+++	++	++
L. javanica	++	-	+	-	-	-	+	+	+	-	-
S. biflora	+++	+	+	-	+++	-	+++	+++	-	++	+

Legend: *Staphylloccocus aureus (S.a.) Pseudomonas aeruginosa(P.a.) Klebsiella pneumoniae (K.p.) Escherichia coli (E.c.) Salmonella typhi (S.t.) Candida albicans (C.a.) Candida brusei C.b. Cryptococcus neoformas(C.n) Trichophyton mentagrophyte (T.m.) Microsporum gypseum(M.g.)*> 16 mm. high activity, 9-15 mm. moderate activity, 8-9 mm slight activity and <6mm no activity.

Extracts from *S. biflora*, *were* effective against most of the bacteria and *C. albicans*, *C. neoformas* and *T. mentagrophyte. S. biflora* were found to be slightly inhibiting, *S. aureus,* and *P. aeruginosa* and high inhibition of *E. coli.*. *S. biflora* was found to be effective on *K. pneumoniae, S. aureus P. aeruginosa* and *E. coli.*

Table 2 : MFC of fungi to various stem distillate of *T. asiatica* (µl/ml)

Plant part used	Fungi/Conc. (µl/ml)				
	C.a	*C.b*	*Cr.n*	*T.m*	*M.g*
Fruits	-	-	4.1	1.2	1.0
Leaves	-	-	-	4.5.	-

Distillate of the fruits of *T. asiatica* showed broad antifungal activities as compared to the leaves which had only an activity against *T. mentagrophyte* (MFC in µg/ml) of (*C.a*) *C. albicans* (clinical isolates), (*C.b*) *C. brusei*, (*Cr.n*) *C. neoformas,* (*T.m*) *T mentagrophytes* (clinical isolates) and (*M.g*) *M. gypseum* (clinical isolates) showed activities Essential oils *S. biflora* and *L. javannica* had similar activities (Table 3)

Table 3 : Zones of inhibition (mm) by *S. biflora* essential oils activity (100 µg/ml)

Organism	Zones of inhibition (6 mm)
S. aureus (ORSA)	19
S. aureus	10
K. pneumoniae	10
K. pneumoniae (MDRS)	11
E. coli	17
Control	6

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Table 4 : Zones of inhibition (mm) by: *L. javanica* essential oils against *S. aureus* (100 µg/ml)

Organism	Zones of inhibition(mm)
S. aureus (ORSA)	13
S. aureus	13
Control	6

There were no activities against the organism by the extracts of *Satureia biflora* and *Toddalia asiatica* extracts (Table 4).

Table 5 : MIC/MBC of Various plants extracts in µg/ml or µl/ml

Plant	Test Organism	MIC	MBC
S. biflora	*Pseudomonas spp*	1000	2000
T. asiatica	*E .coli* its strains	20	No reaction
	S. aureus and (ORSA)	400	No reaction

There were also activities although not shown in Table 5 of the oil extracts (MFC in µg/ml (MFC) results in (µml) against: (*C.a*) *C. albicans* (clinical Isolates), (*C.b*) *C. brusei*, (*Cr.n*) *C. neoformas*, (*T.m*) *T. mentagrophytes* (clinical isolates) and (*M.g*) *M. gypseum* (clinical isolates).

Table 6 : Essential oils from *T. asiatica, S. biflora* and *L. javanica* (GC)

T. asiatica	*S. biflora*	*L. javanica*
β-phellandrene	Mycene	Limonene
1,6-Octadien-3-ol	Linalool	β-Caryrophylene
3,7-dimethyl-trans-sabinenehydrate	β- Caryophylene	Mycerene
2-Cyclohexene-1-ol	Sabinene	Camphor
Methyl-4-(1-methyl)-1-octene,	Trans- sabinene	
6-methyl-2-Cyclohexen-1-ol,	Humulene	
Bicyclo[3.1.1]hept-2-ene-2-methanol	Dihydro tagotene	
(S)-(+)-6-methyl-1-octenol	3,9-Epoxy- p-menthal	
Caryophylene oxide	Cyclohexyl-1-pentyne	

The GC indicate the classes of compounds found (Table 6)

IV. Discussion

Satureia biflora oils had varied activities in that it was effective against both Gram negative and Gram positive bacteria. Its oil possessed activities against *S. aureus, K. pneumoniae* and *E. coli* at 12 µl/ml and its strains. Such activities may be attributed to different chemical composition of the oil as compared to *L. javanica* and *T. asiatica.* The activities confirm the use of the leaves and roots by the Ogiek community to combat upper respiratory tract ailments like colds, pneumonia, coughs and stomachache. This could be attributed to the fact that the drugs are able to penetrate the cell wall of the organisms. The inability may be, due to the fact that fungi being plants, though from the lower classes may have similar compounds as the extracts.

The mode of antimicrobial action of the oil may be due to the inhibition of respiration and disrupting the permeability of the cell wall structures. The other speculation on the enhanced efficacy of the oil is due to differential permeability as a result of molecular actions which have been prompted by adhesive activities of the oil molecules (Lukwa, 1994). Ultimately, even some Gram negative bacteria which possess complex cell wall structures that more often resist foreign molecules penetration will succumb to the oil.

Members of the Lamiaceae have been used in Asian medicine and involve several species in the family. Besides steroidal compounds which are found in the family, essential oils are of universal occurrence and have been used in medication in Asia and elsewhere successfully (Rajan *et al.*, 2002). The presence of these oils, steroidal compounds confirm the ability of *S. biflora* to combat pneumonia and stomachache. Furthermore, the plant is also used in cooking and warm drinks by the local community of the Ogiek. It is also often boiled and the decoction taken as a hot cup due to its strong aroma from the Artimesinin, terpenes and other aromatics. It is believed that the plant's strong aroma is able to alleviate common cold. The essential oils are found in the leaves which are freshly harvested. Some of these include *O. basilcum* (L), *O. sanctum, M. piperita* all of which yield eugenol and caryophyllene (Mehrotra and Rastogi, 1995). The same inferences conform to the other medicinal uses of *Dracocephalum moldavica* whose volatile oils are used in culinary, astringent and tonic. There are many plants like *Ocimum basilcum, Rosmarinus officinalis* and *Mentha piperita* whose extracts are used in perfumery and in the treatments of various antibacterial ailments (Willis, 1995) and are known to contain monoterpenes (Viyotch *et al.*, 2006).

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Traditionally, amongst the Ogiek the infusion of the plant is used to treat fresh wounds, colds and stomach complaints. The variable antimicrobial activity might be due to chemotypes (Vijoen *et al.*, 2005). The oils found in freshly harvested and steam distilled leaves yielded limonene, camphor, *ß*- caryophyllene, and myrcene which give the same results as the leaf when tested against the test organisms. It has been observed that geographical location and time of plant collection has a lot of influence on the type and quantities of the oil collected through stream distillation of the plant leaves (Samie *et al.*, 2005).

From the same genus in the same Verbenaceae, it has been reported that essential oil and selected terpenoid components of *Lippia scaberrima* posses fungastatic activities against *Bartyosphaeria parva* and Collectotricum gloespoiodes that are known to cause Mango spoilage (Sadeghi and Azish, 2013). However, during this study methanol extract and essential oils of this plant inhibited *S. aureus* at MIC of 140 μl/ml and 11μl/ml. traditionally, this plant is used by the community as mosquito repellant and fresh leaves rapped onto fresh wounds to assist in healing. Its action might be directed at the fresh wounds and thus combats the organism directly by preventing it from penetrating the host. Furthermore, sequential extractions of various portions of the plant had significant activities against selected human pathogenic fungi. When various extracts were set against the organisms with fluconazole as control, the ANOVA for all the extracts from both parts of the plant showed df=2 with leading results in the petroleum ether followed by methanol. Although ethyl acetate was not active against most of the test organisms, it was significantly active against *Microsporum gypseum* with a test of df=2 when compared to other extracts. Outstanding activities across the board were also reached when dealing with the extracts from the root bark. The phenomena are that most of the metabolites are transported from the sites of synthesis and ultimately stored in the roots in majority of the higher plants. This is perhaps why the activity is higher in root extracts.

Rutaceae are known to possess strong antimicrobial agents which have been tested (Quiroga, 2004). The genera *Pseudomonas, Enteroccocus* and *Salmonella* were all resistant to various levels of treatments of the root bark extracts *T. asiatica* root bark extracts of the plant had significant activity, $P < 0.05$ with a pooled StDev=0.725 and all the tested strains of *S. aureus* and *E. coli* with growth being inhibited significantly. Its inhibition increased proportionally with the increase in the drug concentration without any or much variation. Sensitivity of the organism to the plant extract justifies the fact that the community uses the plant against respiratory tract ailments such as cough, cold, stomachache and other chest related conditions. The plant was also mentioned to be used as tonic, possibly as an immune booster whereby, the roots are boiled decanted and the decoction taken by the Ogiek. However, members of the Studies of the plant extracts indicate that it can be used in abortion in India due to the presence of berberine, toddaline and toddanaline as selected chemical constituents (Mehrotra, 1993). The families are also known to contain triterpenoids as limonoids which are important in pharmaceutical chemistry due to their use in traditional medicine (Rajan *et al.,* 2002). The essential oils, like limnoides, were contained both in the leaves and in the fruits but with higher concentration in the fruits than in the leaves (Table: 6).

V. Conclusions

This study set out with the objectives to: document the ethnomedicine of the Ogiek people, document the plant medicines used by the Ogiek. The community has empirical and vast knowledge in the use of the forests and forest products. Observations revealed that there are wanton and excessive environmental degradations which have led to drastic reduction in the biodiversity in the study area. Hence ascertain plant species are rare to find with the Mau forest complex and elsewhere. Such species are thus endangered.

VI. Recommendations

The study has demonstrated that further studies can be carried concerning the medicinal plants used by Ogiek Community and the following recommendations came up. Documentation using other methods, other than ethnomedical approach should be employed to close the data gaps. Both *in situ* and *ex situ* conservation efforts are *introduced* in the area to counter the encroachment of fragile habitats by the fast increasing population. An exclusive reserve is created since it will allow natural regeneration of the ecosystem to take place thus the restoration of degraded areas. Failure to do so will lead to drastic reduction in the floral diversity which would otherwise be used in future studies. A comprehensive natural resource inventory, type on the plant species used by the people and the methods employed in their exploitation be made so that a holistic approach be employed for sustenance of the diversity.The plants which did not show any bio-activities against the test pathogens be further studied to ascertain their efficacies and the plants that showed bioactivities be studied *in vivo* using small laboratory animals to validate their efficacies. An interdisciplinary approach is employed in studying the community's ethnomedicine involving the pharmaceutical industry for purposes of new drug discovery and development. There should be a proper epidemiological record

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keeping so as establish the efficacy of the drugs used in traditional systems on endemic diseases and the newly emerging and reemerging ones.

The toxicity of plant medicines to human and livestock be carried out by laboratory cytological evaluations using small animal models. More organisms should be covered in future studies so as to leave no doubt on the validation of the efficacy of such plants. For purposes of scientific progress, phytochemists should be carried out comprehensive elucidation of the active principles. Thereafter, pharmacists may work on the several combinations and cocktails which could be effective and used in the manufacture of new drugs to combat the maladies currently afflicting humankind. Further assay these sequential extracts against some common human pathogenic fungi and profile the classes found in the biologically active plants. Several plants that were found to contain essential oils of reasonable efficacies against known human and animal pathogens, further researches be carried on their possible incorporation into toiletries, and other oral medicines for purposes of pharmaceutical uses. Post-harvest loses of perishable commodities like fruits are common in horticultural produce; it would be advisable to explore the possibility of incorporating extracts from botanicals in their spoilage reduction strategies as opposed synthetic pesticides which are often environmentally unfriendly.

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1. Choosing the topic: In most cases, the topic is searched by the interest of author but it can be also suggested by the guides. You can have several topics and then you can judge that in which topic or subject you are finding yourself most comfortable. This can be done by asking several questions to yourself, like Will I be able to carry our search in this area? Will I find all necessary recourses to accomplish the search? Will I be able to find all information in this field area? If the answer of these types of questions will be "Yes" then you can choose that topic. In most of the cases, you may have to conduct the surveys and have to visit several places because this field is related to Computer Science and Information Technology. Also, you may have to do a lot of work to find all rise and falls regarding the various data of that subject. Sometimes, detailed information plays a vital role, instead of short information.

2. Evaluators are human: First thing to remember that evaluators are also human being. They are not only meant for rejecting a paper. They are here to evaluate your paper. So, present your Best.

3. Think Like Evaluators: If you are in a confusion or getting demotivated that your paper will be accepted by evaluators or not, then think and try to evaluate your paper like an Evaluator. Try to understand that what an evaluator wants in your research paper and automatically you will have your answer.

4. 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.

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

6. Use of computer is recommended: As you are doing research in the field of Computer Science, then this point is quite obvious.

7. Use right software: Always use good quality software packages. If you are not capable to judge good software then you can lose quality of your paper unknowingly. There are various software programs available to help you, which you can get through Internet.

8. Use the Internet for help: An excellent start for your paper can be by using the Google. It is an excellent search engine, where you can have your doubts resolved. You may also read some answers for the frequent question how to write my research paper or find model research paper. From the internet library you can download books. If you have all required books make important reading selecting and analyzing the specified information. Then put together research paper sketch out.

9. Use and get big pictures: Always use encyclopedias, Wikipedia to get pictures so that you can go into the depth.

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

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

12. Make all efforts: Make all efforts to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in introduction, that what is the need of a particular research paper. Polish your work by good skill of writing and always give an evaluator, what he wants.

13. Have backups: When you are going to do any important thing like making research paper, you should always have backup copies of it either in your computer or in paper. This will help you to not to lose any of your important.

14. Produce good diagrams of your own: Always try to include good charts or diagrams in your paper to improve quality. Using several and unnecessary diagrams will degrade the quality of your paper by creating "hotchpotch." So always, try to make and include those diagrams, which are made by your own to improve readability and understandability of your paper.

15. Use of direct quotes: When you do research relevant to literature, history or current affairs then use of quotes become essential but if study is relevant to science then use of quotes is not preferable.

16. Use proper verb tense: Use proper verb tenses in your paper. Use past tense, to present those events that happened. Use present tense to indicate events that are going on. Use future tense to indicate future happening events. Use of improper and wrong tenses will confuse the evaluator. Avoid the sentences that are incomplete.

17. Never use online paper: If you are getting any paper on Internet, then never use it as your research paper because it might be possible that evaluator has already seen it or maybe it is outdated version.

18. Pick a good study spot: To do your research studies always try to pick a spot, which is quiet. Every spot is not for studies. Spot that suits you choose it and proceed further.

19. Know what you know: Always try to know, what you know by making objectives. Else, you will be confused and cannot achieve your target.

20. Use good quality grammar: Always use a good quality grammar and use words that will throw positive impact on evaluator. Use of good quality grammar does not mean to use tough words, that for each word the evaluator has to go through dictionary. Do not start sentence with a conjunction. Do not fragment sentences. Eliminate one-word sentences. Ignore passive voice. Do not ever use a big word when a diminutive one would suffice. Verbs have to be in agreement with their subjects. Prepositions are not expressions to finish sentences with. It is incorrect to ever divide an infinitive. Avoid clichés like the disease. Also, always shun irritating alliteration. Use language that is simple and straight forward. put together a neat summary.

21. 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 to your topic. You may also maintain your arguments with records.

22. Never start in last minute: Always start at right time and give enough time to research work. Leaving everything to the last minute will degrade your paper and spoil your work.

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

24. Never copy others' work: Never copy others' work and give it your name because if evaluator has seen it anywhere you will be in trouble.

25. Take proper rest and food: No matter how many hours you spend for your research activity, if you are not taking care of your health then all your efforts will be in vain. For a quality research, study is must, and this can be done by taking proper rest and food.

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

27. Refresh your mind after intervals: Try to give rest to your mind by listening to soft music or by sleeping in intervals. This will also improve your memory.

28. Make colleagues: Always try to make colleagues. No matter how sharper or intelligent you are, if you make colleagues you can have several ideas, which will be helpful for your research.

29. Think technically: Always think technically. If anything happens, then search its reasons, its benefits, and demerits.

30. Think and then print: When you will go to print your paper, notice that tables are not be split, headings are not detached from their descriptions, and page sequence is maintained.

31. Adding unnecessary information: Do not add unnecessary information, like, I have used MS Excel to draw graph. Do not add irrelevant and inappropriate material. These all will create superfluous. Foreign terminology and phrases are not apropos. One should NEVER take a broad view. Analogy in script is like feathers on a snake. Not at all use a large word when a very small one would be sufficient. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Amplification is a billion times of inferior quality than sarcasm.

32. Never oversimplify everything: To add material in your research paper, never go for oversimplification. This will definitely irritate the evaluator. Be more or less specific. Also too, by no means, ever use rhythmic redundancies. Contractions aren't essential and shouldn't be there used. Comparisons are as terrible as clichés. Give up ampersands and abbreviations, and so on. Remove commas, that are, not necessary. Parenthetical words however should be together with this in commas. Understatement is all the time the complete best way to put onward earth-shaking thoughts. Give a detailed literary review.

33. Report concluded results: Use concluded results. From raw data, filter the results and then conclude your studies based on measurements and observations taken. Significant figures and appropriate number of decimal places should be used. Parenthetical remarks are prohibitive. Proofread carefully at final stage. In the end give outline to your arguments. Spot out perspectives of further study of this subject. Justify your conclusion by at the bottom of them with sufficient justifications and examples.

34. After 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 to 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 in 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 criterion for grading the final paper by peer-reviewers.

Final Points:

A purpose of organizing a research paper is to let people to interpret your effort selectively. The journal requires the following sections, submitted in the order listed, each section to start on a new page.

The introduction will be compiled from reference matter and will reflect the design processes or outline of basis that direct you to make study. As you will carry out the process of study, the method and process section will be constructed as like that. The result segment will show related statistics in nearly sequential order and will direct the reviewers next to the similar intellectual paths throughout the data that you took to carry out your study. The discussion section will provide understanding of the data and projections as to the implication of the results. The use of good quality references all through the paper will give the effort trustworthiness by representing an alertness of 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 the 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

Mistakes to evade

- Insertion a title at the foot of a page with the subsequent text on the next page
- Separating a table/chart or figure - impound each figure/table to a single page
- Submitting a manuscript with pages out of sequence

In every sections of your document

· Use standard writing style including articles ("a", "the," etc.)

· Keep on paying attention on the research topic of the paper

· Use paragraphs to split each significant point (excluding for the abstract)

· Align the primary line of each section

· Present your points in sound order

· Use present tense to report well accepted

· Use past tense to describe specific results

· Shun familiar wording, don't address the reviewer directly, and don't use slang, slang language, or superlatives

· Shun use of extra pictures - include only those figures essential to presenting results

Title Page:

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

Abstract:

The summary should be two hundred words or less. It should briefly and clearly explain the key findings reported in the manuscript--must have precise statistics. It should not have abnormal acronyms or abbreviations. It should be logical in itself. Shun citing 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 approach 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. Yet, use comprehensive sentences and do not let go readability for briefness. You can maintain it succinct by phrasing sentences so that they provide more than 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 maintain the initial two items to no more than one ruling each.

- Reason of the study - 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 quantitative data; results of any numerical analysis should be reported
- Significant conclusions or questions that track from the research(es)

Approach:

- Single section, and succinct
- As a outline of job done, it is always written in past tense
- A conceptual should situate on its own, and not submit to any other part of the paper such as a form or table
- Center on shortening results - bound background information to a verdict or two, if completely necessary
- What you account in an conceptual must be regular with what you reported in the manuscript
- Exact spelling, clearness 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 to comprehend and calculate the purpose of your study without having to submit to other works. The basis for the study should be offered. Give most important references but shun difficult to make a comprehensive appraisal of the topic. In the introduction, describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will have no attention in your result. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here. Following approach can create a valuable beginning:

- Explain the value (significance) of the study
- Shield the model - why did you employ this particular system or method? What is its compensation? You strength remark on its appropriateness from a abstract point of vision as well as point out sensible reasons for using it.
- Present a justification. Status your particular theory (es) or aim(s), and describe the logic that led you to choose them.
- Very for a short time explain the tentative propose and how it skilled 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 with every section. If you make the four points listed above, you will need a least of four paragraphs.

- Present surroundings information only as desirable in order hold up a situation. The reviewer does not desire to read the whole thing you know about a topic.
- Shape the theory/purpose specifically - do not take a broad view.
- As always, give awareness to spelling, simplicity and correctness of sentences and phrases.

Procedures (Methods and Materials):

This part is supposed to be the easiest to carve if you have good skills. A sound written Procedures segment allows a capable scientist to replacement 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 for the least amount of information that would permit another capable scientist to spare 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 object, mention the specific item describing a way but draw the basic principle while stating the situation. The purpose is to text 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:

- Explain materials individually only if the study is so complex that it saves liberty this way.
- Embrace particular materials, and any tools or provisions that are not frequently found in laboratories.
- Do not take in frequently found.
- If use of a definite type of tools.
- Materials may be reported in a part section or else they may be recognized along with your measures.

Methods:

- Report the method (not 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 - details how procedures were completed not how they were exclusively performed on a particular day.
- If well known procedures were used, account the procedure by name, possibly with reference, and that's all.

Approach:

- It is embarrassed or not possible to use vigorous voice when documenting methods with no using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result when script up the methods most authors use third person passive voice.
- Use standard style in this and in 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.

Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part a 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. Carry on to be to the point, by means of statistics and tables, if suitable, to present consequences most efficiently.You must obviously differentiate material that would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matter should not be submitted at all except requested by the instructor.

Content

- Sum up your conclusion in text and demonstrate them, if suitable, with figures and tables.
- In manuscript, explain each of your consequences, point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation an exacting study.
- Explain results of control experiments and comprise 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 in manuscript form.

What to stay away from

- Do not discuss or infer your outcome, report surroundings information, or try to explain anything.
- Not at all, take in raw data or intermediate calculations in a research manuscript.
- Do not present the similar data more than once.
- Manuscript should complement any figures or tables, not duplicate the identical information.
- Never confuse figures with tables - there is a difference.

Approach

- As forever, use past tense when you submit to 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 part.

Figures and tables

- If you put figures and tables at the end of the details, make certain that they are visibly distinguished from any attach appendix materials, such as raw facts
- Despite of position, each figure must be numbered one after the other and complete with subtitle
- In spite of position, each table must be titled, numbered one after the other and complete with heading
- All figure and table must be adequately complete that it could situate on its own, divide from text

Discussion:

The Discussion is expected the trickiest segment to write and describe. A lot of papers submitted for journal are discarded based on problems with the Discussion. There is no head of state for how long a 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 implication of the study. The purpose here is to offer an understanding of your results and hold up for all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of result should be visibly 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 with prospect, and let it drop at that.

- Make a decision if each premise is supported, discarded, or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."
- Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work
- You may propose future guidelines, such as how the experiment might be personalized to accomplish a new idea.
- Give details all of your remarks as much as possible, focus on mechanisms.
- Make a decision if the tentative design sufficiently addressed the theory, and whether or not it was correctly restricted.
- Try to present substitute explanations if sensible alternatives be present.
- One 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:

- When you refer to information, differentiate data generated by your own studies from available information
- Submit to work done by specific persons (including you) in past tense.
- Submit to generally acknowledged facts and main beliefs in present tense.

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Topics	Grades		
	A-B	**C-D**	**E-F**
Abstract	Clear and concise with appropriate content, Correct format. 200 words or below	Unclear summary and no specific data, Incorrect form Above 200 words	No specific data with ambiguous information Above 250 words
Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
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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