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GC-MS analysis of bioactive compounds in methanolic extract of *Holigarna grahamii* (wight) Kurz.

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Abstract
Holigarna grahamii belongs to family Anacardiaceae and commonly known as 'Ranbibba'. It produces white latex which is allergenic and causes contact dermatitis. The present investigation was design to determine the bioactive constituents from various plant parts such as Latex, Stem Bark, Leaves, Mature fruits and Ripened fruits of *H. grahamii* using GC-MS. The GC-MS analysis of the methanolic extract revealed the presence of forty four bioactive compounds with valuable biological activities, including the allergic Melamine. The major chemical constituents are Quinic acid (5.72%), 1,2,3-Benzenetriol (42.25%), Melamine (3.07%), Pentanoic acid, 4-oxo- (1.40%), Myristic acid (3.36%),Oleic Acid (0.49%).

Keywords: GC-MS, *Holigarna grahamii*, Anacardiaceae, Melamine.

1. Introduction
Members of Anacardiaceae family are mostly found in the tropical regions and most of the members are toxic which produce white latex which is turn into black and is highly irritating to skin. Family anacardiaceae known to produce allergenic substances in the resin canals of primary and secondary phloem associated with the veins of leaves and other parenchymatous tissues [1]. Plants are a rich source of secondary metabolites with remarkable biological activities. The secondary metabolites are significant source with a variety of structural arrangements and properties [2]. Natural products which come out from medicinal plants are important for pharmaceutical research and for drug development as a source of therapeutic agents. At presents the demand for herbal or medicinal plant products has increased significantly [3]. GC-MS is the best technique to identify the bioactive constituents of long chain hydrocarbons, alcohols, acids, esters, alkaloids, steroids, amino and nitro compounds etc. [4]. A wide range of medicinal plant parts is used for extract as raw drugs and they possess varied medicinal properties [5].Traditionally used medicinal plants have recently attracted the attention of the biological scientific communities. This has involved the isolation and identification of secondary metabolites produced by plants and their use as active principles in medicinal preparations [6].

2. Material and Methods
2.1 Collection of Plant Material
The plant materials of *H. grahamii* were collected from Dajipur in Kolhapur District. For latex collection stem bark of growing plants were cut and the fluid coming out collected into a clean sterile glass tubes. The identification of the plant was done by using relevant literature.

2.2 Preparation of Powder and Extract
The Leaf, Stem bark, mature and ripened fruit pulp materials were air dried under so as to prevent decomposition of active principle and made fine powder by using mechanical grinder. Then these powders were extracted using Methanol as a solvent. Twenty gram of dried powder was weighed and put in a cheese cloth and subjected to extract successively with 200 ml methanol in Soxhlet extractor until the extract was clear. All the extracts were condensed and preserved in refrigerator in air tight bottles until further use.
Known quantity of fresh latex (1 ml) was mixed with (1 ml) Methanol for preparing methanolic extract. Then mixtures were placed in shaker for overnight and then filtered through the Whatman's filter paper.

2.3 GC-MS analysis of bioactive compounds
The methanolic extract obtained was subjected to Gas Chromatography and Mass

Spectroscopy for the determination of bioactive volatile compounds. GC-MS analysis of the samples was carried out using Shimadzu Make QP-2010 with nonpolar 60 M RTX 5MS Column. Helium was used as the carrier gas and the temperature programming was set with initial oven temperature at 400C and held for 3 min and the final temperature of the oven was 4800C with rate at 100C [min.sup.-1]. A 2 µL sample was injected with split less mode. Mass spectra was recorded over 35-650 amu range with electron impact ionization energy 70 eV. The total running time for a sample is 45 min. The chemical components from the methanolic extracts of plants were identified by comparing the retention times of chromatographic peaks using Quadra pole detector with NIST Library to relative retention indices. Quantitative determinations were made by relating respective peak areas to TIC areas from the GC-MS.

3. Results and Discussion

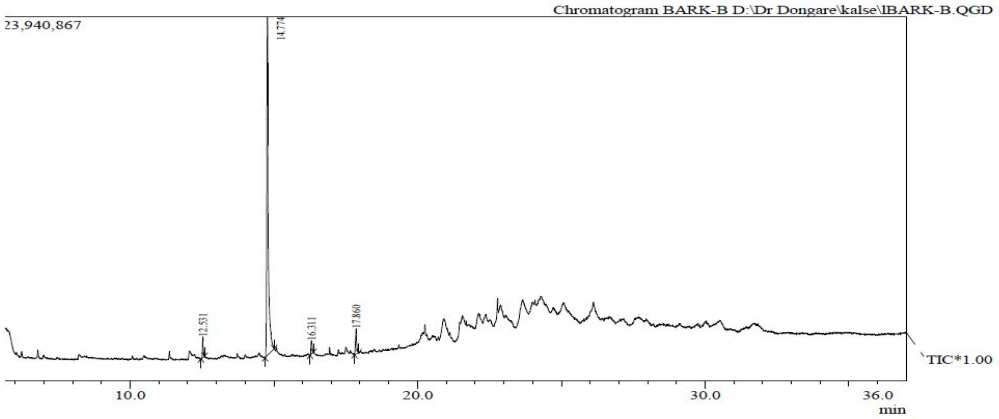

Fig 1: GC-MS chromatogram of methanolic extract of Stem Bark of *H. grahami*

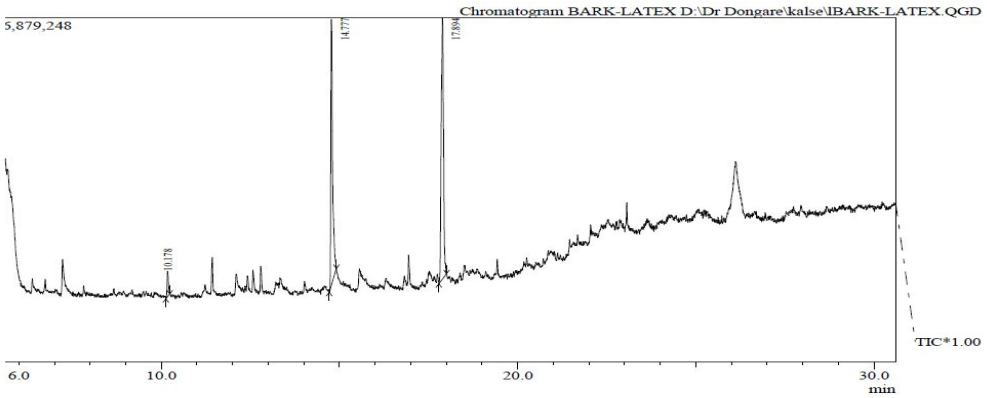

Fig 2: GC-MS chromatogram of methanolic extract of Latex of *H. grahamii*

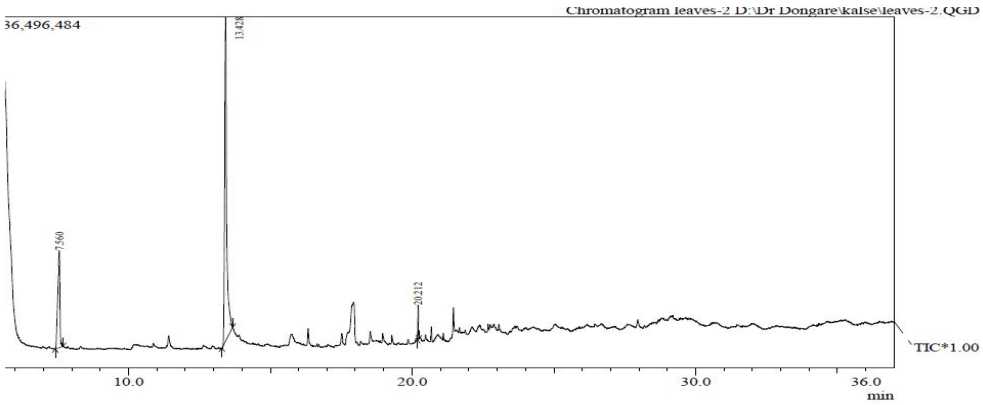

Fig 3: GC-MS chromatogram of methanolic extract of Leaves of *H. grahamii*

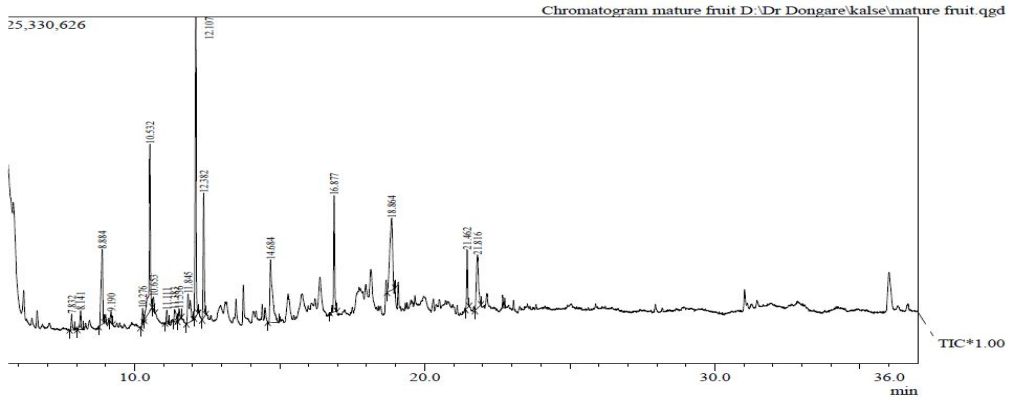

Fig 4: GC-MS chromatogram of methanolic extract of Mature fruits of H. grahamii

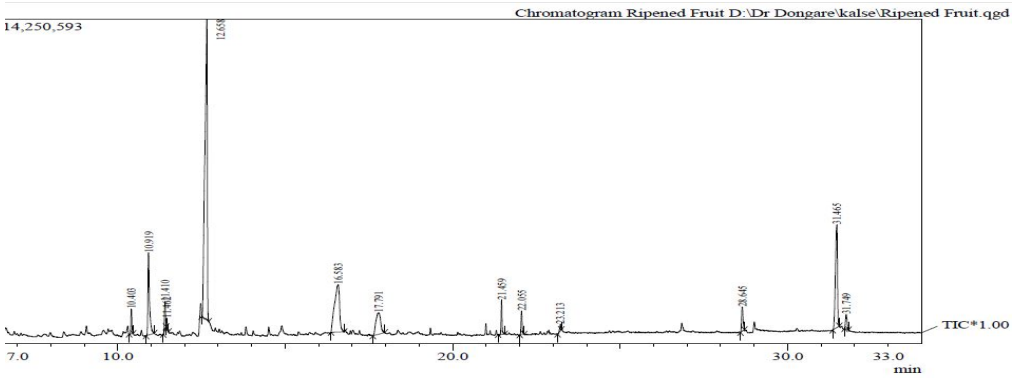

Fig 5: GC-MS chromatogram of methanolic extract of Ripened fruits of *H. grahamii*

Table 1: Bioactive compound detected from methanolic extract of *H. grahamii*

Plant Part	Retention Time	% Peak Area	Compound analyzed	Mol. formula	Mol. weight
Stem Bark	12.531	3.43	2 Furancarboxaldehyde, 5- hydroxymethyl	$C_6H_6O_3$	126
	14.774	88.11	1,2,3, Benzenetriol	$C_6H_6O_3$	126
	16.311	2.75	d-Allose	$C_6H_{12}O_6$	180
			1,6-Anhydro-beta-D-glucopyranose/Leucoglucosan	$C_6H_{10}O_5$	162
			3,4-Altrosan	$C_6H_{10}O_5$	162
	17.860	5.72	Quinic acid	$C_7H_{12}O_6$	192
Latex	10.178	3.07	Cyclopentane, 1-acetyl-1,2-epoxy	$C_7H_{10}O_2$	126
			1,3,5-Triazine-2,4,6-triamine/Melamine/Cyanuramide/ S-Triazine triamine	$C_3H_6N_6$	126
			Melamine	$C_3H_6N_6$	126
			Maltol	$C_6H_6O_3$	126
	14.777	42.25	Pyrogallol	$C_6H_6O_3$	126
	17.894	54.68	Butanoic acid, 2 ethylhexyl ester	$C_{12}H_{24}O_2$	200
Leaves	7.50	23.22	4-Hexen-3-one, 4,5-dimethyl-	$C_8H_{14}O$	126
	20.212	2.50	9-Eicosyne	$C_{20}H_{38}$	278
			Oxirane, tetradecyl	$C_{16}H_{32}O$	240
			Pentadecanal	$C_{15}H_{30}O$	226
Mature Fruits	7.832	1.21	2,5-Hexanedione	$C_6H_{10}O_2$	114
	8.141	1.40	Pentanoic acid, 4-oxo-	$C_5H_8O_3$	116
	9.190	0.63	Succinic acid, monomethyl ester	$C_5H_8O_4$	132
	10.653	0.89	2(3H)-Furanone, dihydro-4-hydroxy-	$C_4H_6O_3$	102
			Formic acid, ethenyl ester	$C_3H_4O_2$	72
			Glycidol	$C_3H_6O_2$	74
	11.111	0.91	(S)-5-Hydroxymethyl-2[5H]-furanone	$C_5H_6O_3$	114
	11.383	1.32	4H-Pyran-4-one, 3,5-dihydroxy-2-methyl	$C_6H_6O_4$	142
	11.845	4.59	Tetrahydrofuran-5-on-2-methanol, .alpha.	$C_{11}H_{16}O_7$	260
	12.382	7.91	1,2,3-Propanetriol, monoacetate	$C_5H_{10}O_4$	134
	18.864	12.36	2-O-Methyl-D-mannopyranosa	$C_7H_{14}O_6$	194
	21.462	3.36	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256
	21.816	7.12	Tetradecanoic acid/Myristic acid	$C_{14}H_{28}O_2$	228
			D-Galactose	$C_6H_{12}O_6$	180

			D-Ribose	$C_5H_{10}O_5$	150
Ripened Fruits	10.403	1.61	Furyl hydroxymethyl ketone	$C_6H_6O_3$	126
	11.462	0.44	3(2H)-Pyridazinone, 6-methyl	$C_5H_6N_2O$	110
	12.658	45.71	3-Furanmethanol	$C_5H_6O_2$	98
			Chlorokojic acid	$C_6H_5ClO_3$	160
	16.583	15.08	1,6-Anhydro-.beta.-D-glucopyranose (levoglucosan)	$C_6H_{10}O_5$	162
			n-Caproic acid/n-Hexanoic acid	$C_6H_{12}O_2$	116
	21.459	2.05	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256
			Nonanoic acid	$C_9H_{18}O_2$	158
	22.055	1.72	3(2H)-Pyridazinone, 6-methyl	$C_5H_6N_2O$	110
	23.213	0.49	Oleic Acid	$C_{18}H_{34}O_2$	282
	28.645	2.05	N-Cyanomethylpiperidine	$C_7H_{12}N_2$	124
	31.465	12.81	1-Cyclohexene-1-acetaldehyde, .alpha.,2-dimethyl	$C_{10}H_{16}O$	152
	31.749	1.35	N-Cyanomethylpiperidine	$C_7H_{12}N_2$	124

Table 2: Activity of Bioactive compound identified in the Methanolic extracts of *H. grahami*

Sr. No	Name of Compound	Activity
1	2 Furancarboxaldehyde, 5- hydroxymethyl	Inhibits the formation of sickled cells in the blood. Antimicrobial, Preservative
2	1,2,3, Benzenetriol	Antiseptic**,** Antioxidant, Antidermatitic, Fungicide Insecticide,
3	3,4-Altrosan	Bacteriostat Fungicide
4	Quinic acid	Quinic acid is used as an astringent
5	1,3,5-Triazine-2,4,6-triamine/Melamine/Cyanuramide/S-Triazinetriamine	Allergenic compound. Irritation-Eye, Nose, Throat, Skin
6	Pyrogallol	It has antiseptic properties.
7	Pentadecanal	Nutrient, Stabilizers, Surfactants and Emulsifier
8	Pentanoic acid, 4-oxo-	Potential biofuels can be prepared. Also used in cigarettes to increase nicotine delivery in smoke and binding of nicotine to neural receptors
9	Succinic acid, monomethyl ester	In Nutraceutical form as a food additive and dietary supplement.
10	Formic acid, ethenyl ester	Can irritate eyes, skin, mucous membranes and the respiratory system of humans and other animals
11	Oleic Acid	It is used as an emulsifying or solubilizing agent in aerosol products, used as emollient
12	Nonanoic acid	In the preparation of plasticizers and lacquers
13	Tetradecanoic acid/Myristic acid	It is used in cosmetic and topical medicinal preparations where good absorption through the skin is desired.

Now a day the study of the organic compounds from plants and their activity has increased. The combination of a best separation technique (GC) with the best identification technique (MS) made GC–MS an ideal technique for qualitative analysis for volatile and semi-volatile bioactive compounds [7]. In present investigation total forty four bioactive chemical constituents were identified in the stem bark, leaves, latex, mature fruits and ripened fruits with important chemical properties. The most abundant components found in the stem bark were 1, 2, 3, Benzenetriol (88.11%) whereas Butanoic acid, 2 ethylhexyl ester (54.68%) found most abundant in latex. In leaves 4-Hexen-3-one, 4, and 5-dimethyl- (23.22%) is most abundant components. In mature fruits 2-O-Methyl-D-mannopyranosa (12.36%) while 3-Furanmethanol (45.71%) found most abundant component in ripened fruits. Present investigation found 1, 3, 5-Triazine-2, 4, 6-triamine or Melamine or Cyanuramide is the allergenic compound which causes Dermatitis (OSHA, United State Dept. of Labor).

In *H. grahamii* methanolic extract of stem bark of shows presence of 2- Furancarboxaldehyde, 5- hydroxymethyl, 1,2,3, Benzenetriol, 3,4-Altrosan, Quinic acid whereas methanolic bark extract of *H. arnottiana* showed the presence of 20 major bioactive compounds. It contains 1-Tetradecene, Tricosane, N-Tetracosane Nonadecane, 9-Octadecanoic acid and 1, 2 dihydroxy benzene which is the allergenic compound Urushiol [8]. The bark of *Odina wodier* L. shoes six different chemical compounds namely Pathalic acid 4-cynophenyl noyl ester, n-Decanoic acid, n-Hexadecanoic acid, 4-Dodecanol, 1,14-Tetradecanediol and silane-trimethyl [5-methoxy-2-(1-methylethyl phenoxy)] [9]. GC-MS analysis of methanolic stem extract of *F. religiosa.* It showed the presence of 1, 2-Benzenediol (9.85%), Caffeine (4.20%) and Stigmasterol, 22, 23-dihydro (1.81%) [10].

Methanolic extract of leaves of *H. grahamii* showed presence of 4-Hexen-3-one, 4, 5-dimethyl-, 9-Eicosyne, Dxirane, tetradecyl, Pentadecanal while of Benzoic acid, Pyrogallol, ferulic acid, gallic acid, vanillic acid found in leaves of *Mangifera indica* [11]. GC-MS analysis for bioactive compounds from methanolic leaf extract of *Hildegardia populifolia* were carried out [12]. The major chemical constituents determined were Squalene (46.44%) and 1-Benzazirene-1-carboxylic acid, 2, 2, 5a-trimethyl-1a-[3-oxo-1-butenyl] perhydro-, methyl ester (43.87%).

The methanolic extract of Latex of *H. grahamii* contain Cyclopentane, 1-acetyl-1, 2-epoxy, 1, 3, 5-Triazine-2, 4, 6-triamine, Melamine, Maltol, Pyrogallol, Butanoic acid, 2 ethylhexyl ester. While 2, 6 dimethyl tetra-1, 5-deacaene, 3, 7,

11-Trime-thyl-2, 6, 10, 12-pentadecatrien-l-ol, ethyl phthalate, di-n-propyl phthalate, phthalic acid diisobutyl ester found in latex of Calotropis *procera* [13]. The GCMS analysis of latex of *Euphorbia caducifolia* showed presence of methyl palmitate, 5, 9-heptadecadienoate, methyl 11 octadecenoate, methyl octadecenoate and 3, 7, 11, 15-tetramethyl- 2-hexadecene-l-ol. [14].

In mature fruits extract of *H. grahamii* showed presence Succinic acid, monomethyl ester, 4H-Pyran-4-one, 3,5-dihydroxy-2-methyl, n Hexadecanoic acid, Myristic acid, D-Galactose whereas Chlorokojic acid, 1,6-Anhydro-.beta.-D-glucopyranose (levoglucosan), n-Caproic acid/n-Hexanoic acid, Oleic Acid, 1-Cyclohexene-1-acetaldehyde, .alpha.,2-dimethyl found in ripened fruits. The analysis of fatty acid from *C. australis* by GC-MS showed that it contains various bioactive constituents including methyl oleate, methyl tricosanoate, methyl pentachlorostearate, and methyl linoleate in major concentration [15]. GC-MS analysis of fruit extract of *Momordica charantia* it shows the presence of Gentisic acid,1-Pentadecyne, Cucurbitacin, B Dihydro, Cis-9-hexadecenal, Hexadecanoic acid, methyl ester, Pentadecanoic acid14-methyl-, methyl ester, β-sitoserol, Stigmasterol, Oleic acid, Stigmastan-3-ol, Ethyl-4,5-dimethyl-phenol and Linoleic acid [16].

4. Conclusion

In the present investigation forty four bioactive compound have been identified from methanolic extract of *Holigarna grahamii* by Gas Chromatogram-Mass spectrometry (GC-MS) analysis. The presence of various bioactive compounds in *H. grahamii* proved that the pharmaceutical importance. Though, further studies will require finding out its bioactivity, toxicity profile.

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