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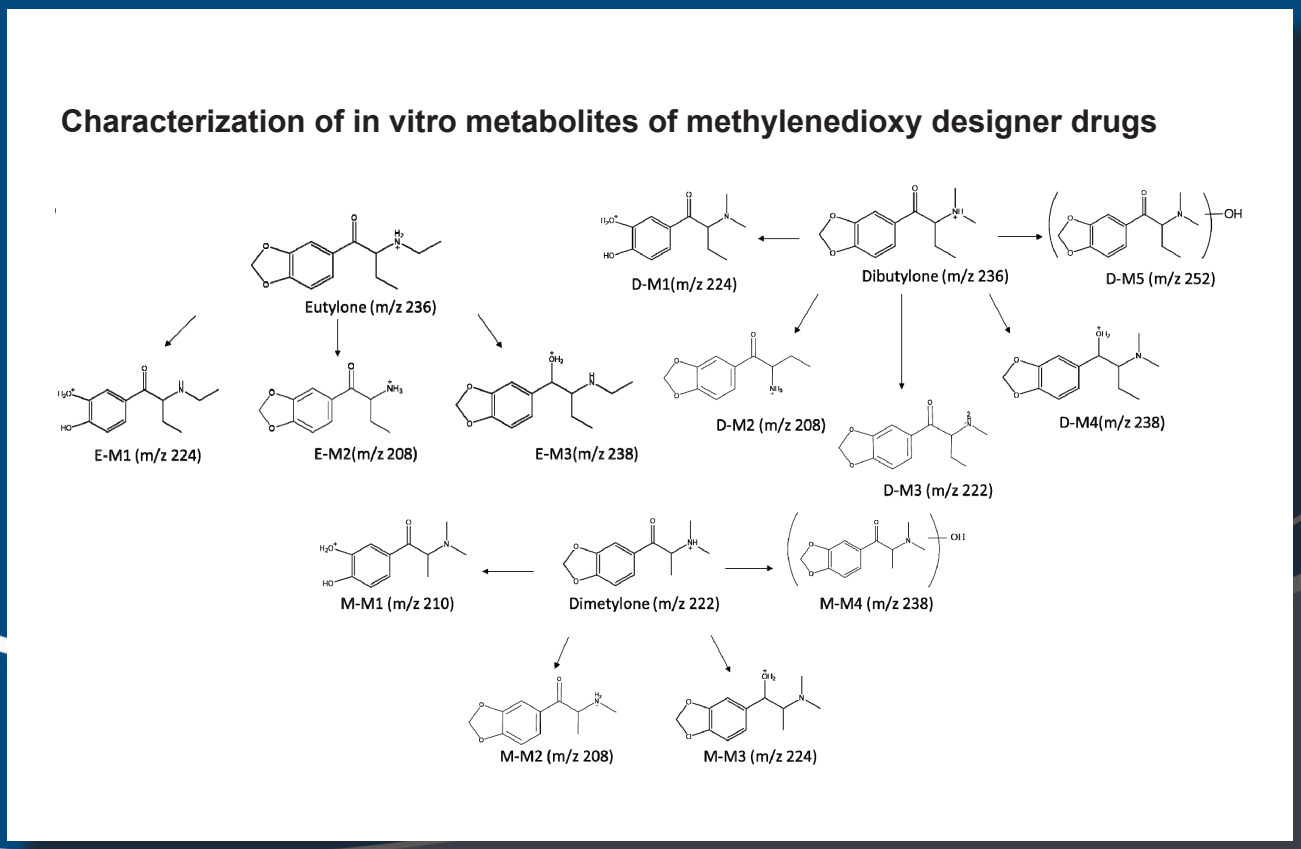

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Aims and Scope

Mass Spectrometry Letters publishes brief letters (maximum length of 4 pages), technical notes, articles, reviews, and tutorials on fundamental research and applications in all areas of mass spectrometry. The manuscripts can be either invited by the editors or submitted directly by authors to the journal editors. Mass Spectrometry Letters topical sections are diverse, covering ion chemistry in a broad sense; gas-phase thermodynamics or kinetics; theory and calculations related with mass spectrometry or ions in vacuum; ion-optics; analytical aspects of mass spectrometry; instrumentations; methodology developments; ionization methods; proteomics and its related research; metabolomics and its related research; bioinformatics; software developments; database development; biological research using mass spectrometry; pharmaceutical research by mass spectrometry; food sciences using mass spectrometry; forensic results using mass spectrometry; environmental mass spectrometry; inorganic mass spectrometry; chromatography-mass spectrometry; tandem mass spectrometry; small molecule research using mass spectrometry; TOF-SIMS, etc. The scope of Mass Spectrometry Letters is not limited to the above-mentioned areas, but includes ever-expanding areas related directly or indirectly to mass spectrometry. Criteria for publication are originality, urgency, and reportable values. Short preliminary or proof-of-concept results, which will be further detailed by the following submission to other journals, are recommended for submission.

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Characterization of *in vitro* Metabolites of Methylenedioxy Designer Drugs

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Abstract : Eutylone, dibutylone, and dimethylone are potential psychotropic designer drugs. The purpose of this study was to investigate the *in vitro* metabolic pathways of synthetic cathinones with methylenedioxy groups. The three methylenedioxy derivatives were incubated with human liver microsomes. The metabolites were characterized based on liquid chromatography and quadrupole-time-of-flight mass spectrometry. Eutylone, dibutylone, and dimethylone were metabolized to yield three, six, and four metabolites, respectively. Reduction and demethylenation were the major metabolic pathways for all three drugs tested. However, dibutylone and dimethylone showed an additional metabolite generated via N-oxidation. These results provide evidence for the *in vivo* metabolism of methylenedioxy synthetic cathinones, and could be applied to the analysis of synthetic cathinones and their relevant metabolites in biological samples.

Keywords : Methylenedioxy derivatives, Designer drug, Metabolism, LC/Q-TOF MS

Introduction

Synthetic cathinones, more commonly known as “bath salts”, are sympathomimetic drugs chemically related to cathinone, a stimulant found in khat plants.[1, 2] Cathinone is a monoamine alkaloid that is chemically similar to ephedrine, cathine, methcathinone, and other amphetamines, and consequently exhibits stimulant effects. Synthetic cathinones can change much more strongly than original products and as a result are, sometimes very dangerous.[3,4] Synthetic cathinones have been widely used in the drug market since the mid-2000s due to their characteristics, which are easy to discover using the internet. Due to the abuse potential of synthetic cathinones, many countries have been trying to regulate laws. However, synthetic cathinones have been newly synthesized to avoid legal control and to increase the stimulant effects.[5-7] As a result of expert evidence from the National Forensic Service, more than 20 kinds of new phenethylamine-like drugs have been detected in field evidence by police and prosecutors. Most of the detected substances are synthetic cathinone and amphetamine analogues.[8,9] Thus, as the abuse cases of new synthetic cathinone analogues have been increasing in recent years, the need for a confirmatory method of cathinone testing has emerged.

When drug users are arrested, they are usually examined through biological sampling. At this point, the parent drug can be analyzed and detected in the biological sample, and the presence of the metabolite in the sample can indicate whether or not the drug was used. Metabolite/drug ratios can sometimes provide estimates of metabolic change. To detect drugs in biological samples, the metabolic fate of the drugs should be studied. Several metabolism screening and confirmation methods have been published for the detection of synthetic cathinones in biological samples.[10-16]

Thus, the ideal analysis for detecting drugs is taking biological samples through drug clinical trials to identify metabolites. However, it is difficult to directly perform the clinical trial of illegal drugs such as synthetic cathinones because of conflict with forensic science. Therefore, *in vitro* metabolite study of synthetic cathinones is necessary to find a biomarker for evidence of drug abuse instead of the clinical trial.

Eutylone, dibutylone, and dimethylone belong to the methylenedioxy group, as their backbone is different from that of synthetic cathinone. As these are illegal drugs such

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as synthetic cathinones, identification of metabolites *in vitro* is needed. Thus, in this study, a metabolism study of eutylone, dibutylone, and dimethylone was conducted. The aim of this study is to identify their phase I metabolites in human liver microsomes using liquid chromatography/ quadrupole-time-of-flight mass spectrometry analysis.

Materials and methods

Chemicals and materials

Eutylone, dimethylone, and dibutylone were supplied by the National Forensic Service Center (Seoul, Korea). Glucose-6-phosphate, β-nicotinamide adenine dinucleotide phosphate (NADP), and glucose-6-phosphate dehydrogenase were purchased from Sigma Chemical Co. (St Louis, MO, USA). Pooled human liver microsomes, were purchased from BD Gentest Corp. (Woburn, MA, USA). Methanol, dimethyl sulfoxide (DMSO), and acetonitrile were purchased from Fisher Scientific (Fair Lawn, NJ, USA). All other chemicals were analytical grade.

Microsomal incubation

A stock standard solution of the drug was prepared at 10 mM in DMSO, respectively. The incubation mixtures consisted of 0.5 mg/mL human liver microsomes, 50 μM of the drug, and an NADPH-generating system (NGS; 0.1 M glucose-6-phosphate, 10 mg/mL $NADP^+$ and 1 U/mL glucose-6-phosphate dehydrogenase) using a total volume of 1000 μL potassium phosphate buffer (0.1 M, pH 7.4). Reactions were initiated by the addition of NGS after a 5-min pre-incubation at 37°C. Incubations were carried out for 0, 0.5, 1, 1.5, and 2 hours, and quenched by the addition of 100 μL of ice-cold 1% formic acid to the reaction mixture. The sample was vortex-mixed for 2 min and applied to the Waters OASIS HLB cartridges (Milford, MA, USA), which were activated with 1 mL of MeOH and equilibrated with 0.1% acetic acid. After sample loading, the cartridges were washed with 2 mL of 0.1% acetic acid (1 mL × 2) and eluted with 1 mL of MeOH. The elutes were dried under N_2 gas at 55°C and reconstituted with 100 μL of 10% mobile phase solvent B. Five microliter-aliquots were injected onto the LC-MS/MS system.

LC/QTOF MS analysis

The HPLC system consisted of an Agilent 1260 series binary gradient pump, a vacuum de-gasser, an autosampler, and a thermostatic column compartment with an Agilent 6530 quadrupole-time-of-flight (QTOF) mass spectrometer equipped with an electrospray ionization source (Agilent Technologies, Inc., Santa Clara, CA, USA). The column used for the separation was a Kinetex C18 (2.1 × 100 mm, 2.6 μm; Phenomenex, Torrance, USA). The HPLC mobile phases consisted of 0.1% formic acid and 0.1% trifluoroacetic acid in water (solvent A), and 90% acetonitrile in solvent A (solvent B). A gradient program was used with a flow rate of 0.3 mL/min. The initial composition of the organic phase (B) was 1.5%; this was increased to 15% in 20 min and again increased up to 95% in 1 min. It was then changed back to its initial condition over 1 min, followed by a 4 min re-equilibration. The total run time was 31 min. The entire column of elutes was directly introduced into an electrospray ionization (ESI) interface. Nitrogen was used as a nebulizing gas, sheath, and collision gas. The drying gas temperature was 300°C at 20 psi, and the sheath gas temperature was 350°C at a flow rate of 10 L/min. The mass spectrometer was operated in the positive ion mode with a 100-1000 *m/z* range, and at a 2 GHz extended dynamic range. For the product ion scan, the monitored mass range was 50-500 Da and the collision energy was set to 15 eV. Data were collected in a centroid format and processed with Mass Hunter software. Mass calibration was performed prior to the sample analysis using a calibration solution provided by the manufacturer. For each analysis, a calibrant solution containing purine (*m/z* 121.0509) and HP-921 (*m/z* 922.0098; Agilent) was delivered through an external isocratic pump as internal calibrants.

Results

In vitro metabolism of eutylone in human liver microsomes

Metabolites of eutylone in human liver microsomes were analyzed through LC-Q/TOF-MS. Three metabolites were identified with a mass accuracy of less than 5 ppm. Metabolites were eluted at 8.7, 15.3, and 17.4 min, with eutylone eluting at 18.8 min (Figure S1, A). The E-M1 was 12 Da lower than eutylone and corresponded to the demethylenation of eutylone. The molecular weight of E-M2 was 28 Da lower than eutylone. The E-M2 was postulated to be an N-dealkylation metabolite. The molecular weight of E-M3 was 2 Da higher than eutylone. The E-M3 was postulated to be a beta-keto reduction metabolite. The accurate mass data for the product ions of the metabolites are summarized in Table 1.

The chemical structures of the detected metabolites were explained by the fragmentation patterns (Figure S2). The structure of each metabolite was characterized by comparison with the MS/MS fragmentation patterns of the parent. The MS/MS analysis of protonated eutylone yielded a major product ion at *m/z* 218, 188, 161, and 86. The ion at *m/z* 218 resulted from the dehydration of eutylone, and the ion at *m/z* 86 resulted from the alpha-cleavage between positions 1 and 2. The ion at *m/z* 188 resulted from the loss of a dioxolane and *m/z* 161 corresponded to the loss of the amine group and demthylenation. The MS spectrum of E-M1 showed major product ions at *m/z* 206, 188, and 86. The ion at *m/z* 206 resulted from the dehydration of E-M1. The characteristic product ions at *m/z* 188 and 86 indicated the same product ion as their parent. The MS spectrum of E-M2 displayed major product ions at *m/z* 190, 160, 149, and 132. The ions at *m/z* 190 indicated the loss of water.

Table 1. Accurate mass data for the product ions of synthetic cathinone derivatives and their metabolites.

Name	RT	Proposed elemental composition	Exact mass $[M+H]^+$	Measured mass $[M+H]^+$	Error (ppm)
Eutylone	18.8	C13H17NO3	236.1281	236.1271	-4.2
		$C13H16NO2^+$	218.1176	218.1165	-5.0
		$C12H14NO^+$	188.1070	188.1059	-5.8
		$C10H9O2^+$	161.0597	161.0586	-6.8
		$C5H12N^+$	86.0964	86.0961	-3.4
E-M1	8.7	C12H17NO3	224.1281	224.1276	-2.2
		$C12H16NO2^+$	206.1176	206.1174	-0.9
		$C12H14NO^+$	188.1070	188.1072	1.0
		$C5H12N^+$	86.0964	86.0968	4.6
E-M2	15.3	C11H13NO3	208.0968	208.0966	-0.4
		$C11H12NO2^+$	190.0863	190.0857	-3.4
		$C10H10NO+^+$	160.0757	160.0764	4.2
		$C8H5O3^+$	149.0233	149.0227	-1.2
		$C9H10N^+$	132.0808	132.0809	1.2
E-M3	17.4	C13H19NO3	238.1438	238.1428	-3.7
		$C13H18NO2^+$	220.1332	220.1337	2.2
		$C11H13NO2^+$	191.0940	191.0944	2.1
		$C10H9O^+$	145.0648	145.0636	-8.2
		$C8H7O2^+$	135.0441	135.0438	-2.2
		$C6H12N^+$	98.0964	98.0967	3.0
Dibutylone	17.7	C13H17NO3	236.1281	236.1280	-0.4
		$C11H11O3^+$	191.0703	191.0704	0.5
		$C10H9O2^+$	161.0597	161.0596	-0.6
		$C8H5O3^+$	149.0233	149.0232	-0.6
		$C5H12N^+$	86.0964	86.0967	3.4
D-M1a	8.5	C12H17NO3	224.1281	224.1274	-3.1
		$C10H9O2^+$	161.0597	161.0597	0.0
		$C9H11O2^+$	151.0754	151.0752	-1.3
		$C7H5O3^+$	137.0233	137.0231	-1.4
		$C7H7O2^+$	123.0441	123.0437	-3.2
		$C5H12N^+$	86.0964	86.0966	2.3
D-M1b	16.2	C12H17NO3	224.1281	224.1287	2.7
D-M2	15.3	C11H13NO3	208.0968	208.0974	2.9
D-M3	16.7	C12H15NO3	222.1125	222.1127	1.3
		$C12H14NO^+$	204.1019	204.1021	1.0
		$C11H12NO^+$	174.0913	174.0915	0.7
		$C10H9O2^+$	161.0597	161.0603	3.8
		$C10H12N^+$	146.0964	146.0967	1.5
D-M4	17.2	C13H19NO3	238.1437	238.1417	-8.3
		$C13H18NO2^+$	220.1332	220.1329	-1.3
		$C11H13NO2^+$	191.0940	191.0933	-3.7
		$C10H9O^+$	145.0648	145.0647	-0.6
		$C8H7O2^+$	135.0441	135.0435	-4.4
D-M5	22.8	C13H17NO4	252.1230	252.1236	2.4
		$C10H9O3^+$	177.0546	177.0558	6.8
		$C8H5O3^+$	149.0233	149.0231	-1.3
		$C5H12N^+$	86.0964	86.0965	1.1

Table 1. Continued.

Name	RT	Proposed elemental composition	Exact mass $[M+H]^+$	Measured mass $[M+H]^+$	Error (ppm)
Dimethylone	13.1	C12H15NO3	222.1125	222.1121	-1.8
		$C10H9O3^+$	177.0546	177.0542	-2.2
		$C9H7O2^+$	147.0441	147.0438	-2.0
		$C4H10N^+$	72.0808	72.0810	2.7
M-M1	5.4	C11H15NO3	210.1124	210.1124	0.0
		$C9H9O3^+$	165.0546	165.0565	11.5
		$C9H7O2^+$	147.0441	147.0451	6.8
		$C8H9O2^+$	137.0597	137.0586	-8.0
		$C4H9O2^+$	89.0597	89.0595	-2.2
		$C4H10N^+$	72.0808	72.0806	-2.7
M-M2	11.8	C11H13NO3	208.0968	208.0967	0.4
		$C11H12NO2^+$	190.0863	190.0855	-4.4
		$C10H10NO^+$	160.0757	160.0753	-1.4
		$C9H7O2^+$	147.0441	147.0433	-5.7
		$C9H10N^+$	132.0808	132.0808	0.0
M-M3	12.3	C12H17NO3	224.1281	224.1273	-3.5
		$C12H16NO2^+$	206.1176	206.1171	-2.4
		$C11H13NO2^+$	191.0940	191.0933	-3.7
		$C9H7O^+$	131.0491	131.0490	-0.7
M-M4	18.0	C12H15NO4	238.1073	238.1073	0.0
		$C13H8N^+$	178.0651	178.0624	-15.2
		$C8H5O3^+$	149.0233	149.0248	10.0
		$C4H10N^+$	72.0808	72.0808	0.0

The ion at *m/z* 160 was generated by the further loss of a dioxolane, respectively. The ion at *m/z* 149 resulted from the loss of the amine group moiety. The ion at *m/z* 132 resulted from the loss of a dioxolane, then following demethylation and reduction. The MS spectrum of E-M3 displayed major product ions at *m/z* 220, 191, 145, 135, and 98. The ions at *m/z* 220 and 135 indicated the loss of water and amine group moiety, respectively. The ion at *m/z* 191 was generated by the further loss of water from the ion in the demethylated form of the parent and then the formation of a radical. The ion at *m/z* 98 resulted from the loss of a 1,3-benzodioxole group.

In vitro metabolism of dibutylone in human liver microsomes

The incubation of dibutylone with pooled human liver microsomes in the presence of an NADPH-generating system generated six metabolites (Figure S1, B). Their $[M+H]^+$ ions were observed at *m/z* 224 for D-M1a and D-M1b by demethylenation, *m/z* 208 for D-M2 by di-demethylation, *m/z* 222 for D-M3 by demethylation, *m/z* 238 for D-M4 by reduction and *m/z* 252 for D-M5 by N-oxidation, respectively. Metabolites D-M1b and D-M4 were found in previous studies. The accurate mass data for the product ions of the metabolites are summarized in Table 1.

The MS/MS analysis of protonated dibutylone yielded a major product ion at *m/z* 191, 161, 149, and 86 (Figure S3). The ion at *m/z* 191 resulted from the deamination of dibutylone and the subsequent loss of alkylation resulted in the generation of the ion at *m/z* 149. The ions at *m/z* 161 generated the loss of water and methylation, and that at *m/z* 86 was formed by alpha-cleavage between the 1 and 2 positions. D-M1 was observed to have a retention time of 8.5 min with a protonated ion at *m/z* 224. The molecular weight of D-M1 was 12 Da lower than that of dibutylone. The MS/MS of the protonated D-M1 generated major product ions at *m/z* 161, 151, 137, 123, and 86. The ions at *m/z* 137 and 86 resulted from the alpha-cleavage between positions 1 and 2. The product ion at *m/z* 161 generated the loss of the amine group and the oxidation and subsequent loss of alkylation resulted in the generation of the ions at *m/z* 151 and 123. D-M2 was observed to have a retention time of 15.3 min, with an $[M+H]^+$ ion at *m/z*

208. The protonated D-M2 was 28 Da lower than that of dibutylone, suggesting that this metabolite was the di-demethylation derivative of dibutylone. However, MS/MS of D-M2 was not generated due to low intensity of metabolite. D-M3 was observed to have a retention time of 16.7 min, with an $[M+H]^+$ ion at *m/z* 222. The protonated D-M3 was 14 Da lower than that of dibutylone, suggesting that this metabolite was the demethylation derivative of dibutylone. The MS/MS spectra of protonated D-M3 showed major product ions at *m/z* 204, 174, 161, and 146. The characteristic product ion at *m/z* 204 resulted from dehydration. The ions at *m/z* 174 resulted from the loss of the dioxolane. The ion at *m/z* 161 was formed by following the deamination and demethylation by reduction. The product ion at *m/z* 146 was formed by the loss of dioxolane, demethylation, and then reduction. D-M4 was observed to have a retention time of 17.2 min with an $[M+H]^+$ ion at *m/z* 238. The protonated D-M4 was 2 Da higher than that of dibutylone, indicating that this metabolite was the hydrogenated derivative of dibutylone. MS/MS spectra showed a major product ion at *m/z* 220 resulting from the loss of water and *m/z* 135 indicated the loss of the amine group and the alkylation group moiety. The ion at *m/z* 191 was generated by the further loss of water from the ion in the demethylated form of the parent and then the formation of radicals. D-M5 was observed to have a retention time of 22.8 min, with an $[M+H]^+$ ion at *m/z* 252. The protonated D-M5 was 16 Da higher than that of dibutylone, suggesting that this metabolite was the N-oxide derivative of dibutylone. The MS/MS spectra of protonated D-M5 showed major product ions at *m/z* 192, 177, 149, 135, 86, and 60. The characteristic product ion at *m/z* 192 resulted from the loss of the amine group. The ions at *m/z* 177 and 149 resulted from the loss of the amine and alkyl groups, and following the loss of the keto group was the *m/z* 135 ion. The ion at *m/z* 86 was formed by alpha-cleavage between the 1 and 2 positions. The product ion at *m/z* 60 was formed by an N-oxide fragment ion.

In vitro metabolism of dimethylone in human liver microsomes

The incubation of dimethylone with pooled human liver microsomes in the presence of an NADPH-generating system generated four metabolites (Figure S1, C). Their $[M+H]^+$ ions were observed at *m/z* 210 for M-M1 by demethylenation, m/z 208 for M-M2 by dealkylation, *m/z* 224 for M-M3 by demethylenation, and *m/z* 238 for M-M4 by hydroxylation, respectively. Metabolite M-1 was found in previous studies. The accurate mass data for the product ions of the metabolites are summarized in Table 1.

The MS/MS analysis of protonated dimethylone yielded major product ions at *m/z* 177, 147, and 72 (Figure S4). The ion at *m/z* 177 resulted from the deamination of dimethylone, and the subsequent loss of methyl and oxidation resulted in the generation of the ion at *m/z* 147. The ions at *m/z* 72 were formed by alpha-cleavage between the 1 and 2 positions. M-M1 was observed to have a retention time of 5.4 min, with a protonated ion at *m/z* 210. The molecular weight of M-M1 was 12 Da less than that of dimethylone. The MS/MS of protonated M-M1 generated major product ions at *m/z* 165, 147, 137, 89, and 72. The ions at *m/z* 165 and 72 resulted from alpha-cleavage between positions 1 and 2. The ion at *m/z* 147 resulted in a loss of the keto and amine groups following the ring. The ion at *m/z* 137 generated the loss of the amine group and oxidation. The ion at *m/z* 89, found only in M-M1, resulted from the cleavage of the benzene ring. M-M2 was observed to have a retention time of 11.8 min, with an $[M+H]^+$ ion at *m/z* 208. The protonated M-M2 was 14 Da less than dimethylone, indicating that this metabolite resulted from the dealkylation. The MS/MS spectra of protonated M-M2 showed major product ions at *m/z* 190, 160, 147, and 132. The ion at *m/z* 190 indicated dehydration. The ion at *m/z* 160 was generated by the loss of dioxolane. The ion at *m/z* 147 resulted from the loss of amino group moiety and reduction. The ion at *m/z* 132 was formed by the loss of dioxolane and reduction. M-M3 was observed to have a retention time of 12.3 min, with an $[M+H]^+$ ion at *m/z* 224. The protonated M-M3 was 2 Da higher than dimethylone, indicating that this metabolite was the reduced derivative of dimethylone. The MS/MS spectra showed a major product ion at *m/z* 206 resulting from the loss of water, and *m/z* 131 indicated the loss of dioxolane moiety. The ion at *m/z* 191 was generated by the further loss of water from the ion in the demethylated form of the parent and then the formation of a radical. M-M4 was observed to have a retention time of 18.0 min, with an $[M+H]^+$ ion at *m/z* 238. The protonated M-M4 was 16 Da higher than dimethylone and was eluted later than the parent, indicating that this metabolite can be postulated to be the N-oxide derivative of dimethylone. The MS/MS spectra of protonated M-M4 showed major product ions at *m/z* 178, 149, 72, and 60. The ion at *m/z* 60 was only found in the N-oxide metabolite fragment ion spectrum. The ions at *m/z* 178, 149, and 72 were observed in other metabolites.

Discussion

In this study, the metabolism of eutylone, dibutylone, and dimethylone was investigated based on accurate mass and MS/MS product ion analyses. The metabolism pathways of them are proposed in Figure 1. The synthetic cathinones included in this study recently became available worldwide. Eutylone, dibutylone, and dimethylone have common metabolites through *O*-demethylenation and the reduction of the keto function in human liver microsomes. N-oxide metabolites were detected only in the reaction samples with dibutylone and dimethylone, which are tertiary amines. The major metabolism of these synthetic cathinones is *O*-demethylenation.

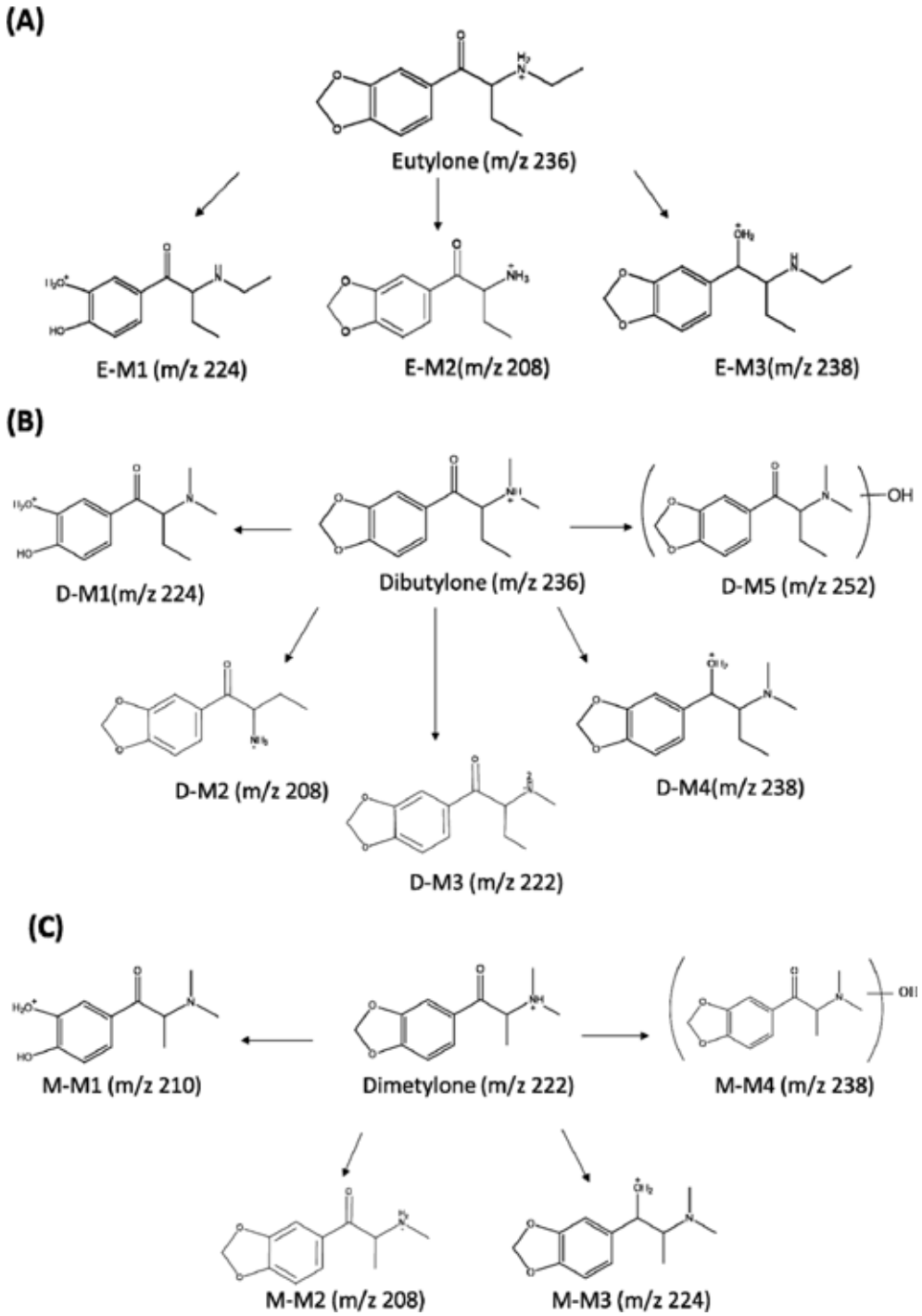

Figure 1. Proposed metabolic pathways of (A) eutylone, (B) dibutylone, and (C) dimethylone.

Compared with reported data, metabolites through demethylenation, demethylation, and hydrogenation were equally identified.[17-19] However, hydroxylation metabolites reported in previous reports were not identified, and the N-oxide metabolites were identified in this study, newly. The reason why the hydroxylation metabolites did not appear in this study was expected that as the pretreatment methods of samples were different, they were lost in the intermediate process such as solid phase extraction, and were not analyzed. From another point of view, in the case of hydroxylation and N-oxide metabolites, there was a possibility that the chemical formula shift is the same and

therefore they could be recognized same metabolites. However, in this study, considering the RT shift of each metabolite with the parent molecule, N-oxide, not hydroxylation, was reasonably judged, and MS/MS fragmentation was also confirmed. Thus, N-oxide metabolites were expected to be generated and further confirmation is needed about hydroxylation metabolites.

Representative compounds of synthetic cathinones include 3,4-methylenedioxymethamphetamine (MDMA) and mephedrone. Meyer et al. and Lim et al. published a metabolism study of MDMA.[10,11,14] MDMA is metabolized through two main pathways. The first is the *O*-demethylenation of the ring followed by methylation, glucuronidation, or sulfatation. The other is the *N*-dealkylation to 3,4-methylendioxyamphetamine (MDA), followed by the oxidation to benzoic acid derivatives. A major metabolism in the human samples was *O*- demethylenation followed by methylation.[10,11,14] The synthetic cathinones investigated in the present study as well as MDMA have common methylenedioxy groups. According to the results, *O*-demethylenation is a major metabolic pathway in synthetic cathinones with methylenedioxy groups.

The metabolism of mephedrone was investigated in human urine samples.[16] Mephedrone was metabolized to yield six Phase I and four Phase II metabolites. The Phase I reactions were *N*-demethylation, a reduction of the keto function, hydroxylation in C_3 and in the benzylic carbon, and the oxidation of C_3 and benzylic methyl into a carboxylic acid. Phase II metabolism occurred mainly via conjugation with glucuronic acid and succinic acid. The synthetic cathinones investigated in the present study as well as mephedrone have common beta-keto function groups. Pozo et al. reported that mephedrone with a ketone group metabolizes to a reduced form. The fragment feature of this metabolite was the loss of water at the most abundant peak in the spectrum, and the subsequent losses of CH_3 and methylamine.[16] The methylenedioxy designer drugs used in this study generated reduced forms of metabolites that exhibited fragment ions through the loss of water, as well as the subsequent losses of CH_3 and methylamine, as shown in the mephedrone metabolite.

Conclusion

This study focused on the metabolism of the newly abused designer drugs eutylone, dibutylone, and dimethylone. The investigation of the metabolism patterns of these methylenedioxy drugs was similar to that of previously reported synthetic cathinones. Demethylenation of the dioxolane group and hydroxylation are common metabolic pathways for most methylenedioxy designer drugs. This information could provide evidence for the *in vivo* metabolism of methylenedioxy designer drugs in biological samples, and would be helpful in appropriate biomarker development for detecting drug use. At this time, information on the metabolism of methylenedioxy designer drugs is limited especially for humans, and further studies are needed to clarify their properties.

Supporting information

Supplementary information is available at https://docs.google.com/document/d/1-qHDmTj4hWzTzvjMpmwHuMg-hLe7r-1G/edit?usp=sharing&ouid=111353140014732050956&rtpof=true&sd=true

Acknowledgements

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Comparative Analysis of Latex Plants by GC-MS using Methanol Extraction

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Abstract : Plants are able to produce a large number of diverse bioactive compounds. Solvent extraction is used for isolation of plant metabolites. The extract yield for plant metabolite extraction strongly depends on the nature of solvent. A review showed the methanol can yield more bioactive compounds. Drying of the sample material is also important for the extraction of plant material. The present study was carried out to analyze the phytocomponents of 5 different latex producing plants. The plants like *Calotropis gigantea, Carica papaya, Nerium oleander, Ficus benghalensis and Plumeria alba* leaves and latex. The GC-MS analysis of the metabolites revealed phytocomponents. *Calotropis gigantea* leaves showed 14 compounds and latex produced 5 compounds out of this 4,4,6A,6B,8A,11,11,14B-Octamethyl-1,4,4A,5,6,6A,6B,7,8,8A,9,10,11,12,12A,14,14A,14B-Octadeca-hydro-2 and 2R- Acetoxymethyl-1,3,3-trimethyl-4T-(3-Methyl-2-Buten-1-Yl)-1T-Cyclohexanol compound was present in both latex and leaf extraction. Beta. -carotene compound was present in both latex and leaf of *Carica papaya*. It was observed that *Ficus benghalensis* contained 2R-Acetoxymethyl-1,3,3-trimethyl-4T-(3-Methyl-2-Buten-1-Yl)-1T-Cyclohexanol was same in latex and leaf extraction.

Key words : Methanol extraction, GC-MS analysis, phytocompounds, latex plants, magnetic stirrer

Introduction

Medicinal plants are the backbone of traditional remedies.[1] The plants may contain many biological components with medicinal values and also be used for application purposes. It is the source for making new drugs.[2] Plants can produce a wide range of phytocomponents that can protect against free radicals that accumulate in fruits and vegetables. Phytochemicals are a collective term for both bioactive and non-nutritive plant substances. Recently, the use of phytochemicals has increased, especially in the areas of functional foods and pharmaceuticals.[3,4] There are about 10% of flowering plants that produce latex. Over 40 families are represented, including *Euphorbiaceous, Apocynaceae*, *Caricaceae*, *Moraceous and Asclepiadaceae*. Latex is a milky white fluid that is secreted by ducts of lactiferous tissue in leaves, stems, fruits and even in roots. some plants, like *Euphorbia hirta, Euphorbia tirucalli, Jatropha gossypifolia, Plumeria rubra, Nerium oleander, Calotropis procera, Ficus benghalensis, Ficus religiosa* and *Carica papaya* latex, were used to identify the phytochemicals present in it but not the methanol extraction study. The naturally synthesised chemical compounds are used to defend against predators like insects, fungi, and herbivorous mammals.[5] Phytochemical screening is the method that has been used to detect antioxidant compounds in plants.[6] Extraction is one method for extracting phytochemicals from the selective plant materials. The phytochemicals yield not only depend on the extraction technique but also depend on the solvent which is used for extraction.[7] There are so many techniques involved in the extraction method, like maceration, infusion, percolation, microwave assisted extraction, the Soxhlet extraction technique, and also other extraction methods like accelerated solvent extraction and supercritical fluid extraction.[8] (Figure 7 and 8) In this research, magnetic stirrer was used for extraction process. The primary goal of extraction is to extract as much of the specific compound as possible while obtaining the highest biological activity from the extract. Many solvents like methanol, ethanol, chloroform, acetone, and water, have been used for extraction depending upon the plant material. A review showed the highest extraction yield by methanol.

It has been proven to be more effective in the extraction of polyphenols with lower molecular weight. Methanol was found to be more effective than ethanol to extract a large number of phenolic compounds.[2] Likewise, (Figure 6) drying the plant material is also important because fresh plant materials may have active enzymes that produce the active constituent's intermediates and metabolic reactions in the plants. Many researchers have done their research by drying plant material in an air-dry process in the shade in a dark room because overheating can cause the volatile

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Figure 1. Latex collection.

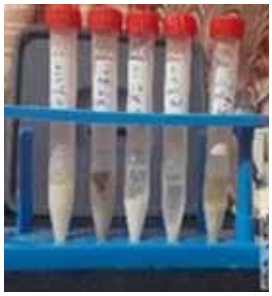


Figure 2. Latex with methanol.

Figure 3. Shade dry.

substance and light-sensitive constituents to be lost.[11] *Calotropis procera*, *Carica papaya* and *Ficus benghalensis* latex were used for extraction by petroleum ether and methanol for antibacterial and antioxidant activity.[12]

Material and methods

Selected plants and collection

The leaves and latex of 5 different latex producing plants like *Calotropis gigantea, Carica papaya, Nerium oleander, Ficus benghalensis, and Plumeria alba* in Vellore district, Tamil Nadu, India.

Extract preparation

For latex, (Figure 1) incisions were made using a sharp, clean knife in the fresh green fleecy stem region of the Calotropis, Nerium, Ficus, Plumeria, and from papaya fruit. The latex was collected in a sterile vial, and immediately, the methanol was added to it to avoid a coagulation in a 1:1 ratio (Figure 2).[12]

For leaf extraction, the collected leaves were shade – dried (Figure 3) for 10 to 15 days to fully dry and then crushed into a powder using an electrical grinder. The dried leaf powder was then dissolved in 50 mL of methanol and placed in a magnetic stirrer set to maximum speed at 60 to 70^{o}C for 3 hours (Figure 4). The setup was kept without disturbance for 24 hours, and then the extract was filtered using normal filter paper. The filtrate was kept there for methanol evaporation in clear petri dishes, and the sample was sent there for GC-MS analysis.[13]

GC-MS analysis

GC-MS analysis of the methanol extract of *Calotropis gigantea, Carica papaya, Nerium oleander, Ficus benghalensis and Plumeria alba* leaves and latex was performed using Clarus 680 GC employed a fused silica column, packed with

Figure 4. A-Leaf sample with methanol, B-Latex sample with methanol.

Elite-5MS (5% biphenyl, 95% dimethylpolysiloxane, 30 m × 0.25 mm ID × 250 μm df). Helium was used as the carrier gas at a constant flow of 1ml/min using an injection volume of 1 μL. The injector temperature was set to 260^{o}C and the ion source temperature to 240^{o}C, with a scan time 0.2 seconds and scan interval of 0.1 seconds. The spectrums of the components were compared with the database of spectra of known components stored in the GC-MS NIST (2008) library.

Result and discussion

The Identification of phytocomponents in plants has become more common in recent years because of their source of availability and their activity as bioactive compounds in various fields. Many studies have been undertaken to identify the compounds from the plants, but there are only a few latex producing plants that were used for analysis with methanol extraction. GC-MS analysis is a very good technique to identify the phytocomponents in the plant extract. In the present study, phytocomponents were taken from 5 different latex producing plants, namely *Calotropis gigantea, Carica papaya, Nerium oleander,*

Ficus benghalensis and Plumeria alba, of both leaf and latex.

Analysis of Calotropis gigantea leaf and latex:

The GC-MS technique revealed the result completely. The *Calotropis gigantea* plant contained 19 compounds, (Table 1) 14 of which were from leaf and (Table 2) 5 from the latex. This 2R-Acetoxymethyl-1,3,3-trimethyl-4T-(3-Methyl-2-Buten -1-YL)-1T-Cyclohexanol (Figure 5A) compound was observed at RT-30.325, with a molecular formula of $C_{17}H_{30}O_3$ and molecular weight of 282, and 4,4,6A,6B,8A,11,14B-Octamethyl-1, 4, 4A, 5, 6, 6A, 6B, 7, 8, 8A, 9, 10, 11, 12, 12A, 14, 14A, 14B Octadecahydro-2 (Figure 5B) was detected at RT 28.854, molecular formula is $C_{30}H_{48}O$, and its molecular weight 424, which were the same in both the leaf and latex methanol extractions and it is the major compound (Figure 5B) present in the latex at a concentration of 48.750% . However, (Figure 5C) the major compound in the leaf extract 3-Tetradecyn-1-ol was found at RT 20.851, molecular formula $C_{14}H_{26}O$ and molecular weight 210 with the area 26.36 %. in leaf.

The *Calotropis gigantea* plant latex and leaf were used for methanol extraction using the Soxhlet apparatus.[12,14] However, the compounds discovered in this study and those mentioned in

Table 1. Phytocomponents identified in the methanolic leaf extract of *Calotropis gigantea* by GC-MS.

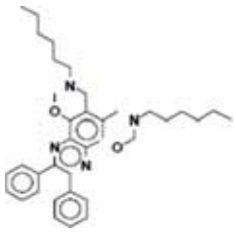
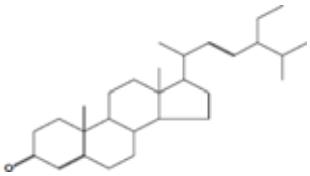
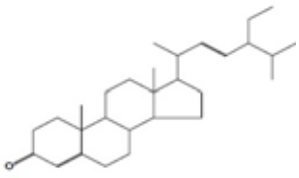
Si.no	RT	Compound name	Molecular formula	Molecular weight	Structure
1	14.693	2,3,4,5,6,7 Hexahydro-3,6-Dihexyl-10,11-Diphenyl-Bis[1,3] Oxazino[6,5-F:5',6	$C_{32}H_{44}O_2N_4$	564	
2	17.059	Heptanal	$C_7H_{14}O$	114	
3	19.285	N-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	
4	19.410	N-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	
5	20.286	Pentadecanoic acid	$C_{15}H_{30}O_2$	242	
6	20.851	3-Tetradecyn-1-ol	$C_{14}H_{26}O$	210	
7	22.542	Hexadecanal	$C_{16}H_{32}O$	240	
8	23.302	Dodecanal	$C_{12}H_{24}O$	184	
9	24.492	Squalene	$C_{30}H_{50}$	410	
10	27.899	4,22-Stigmastadien-3-one	$C_{29}H_{46}O$	410	
11	28.854	4,4,6A,6B,8A,11,11, 14B-Octamethyl-1,4, 4A, 5,6,6A,6B,7,8,8A,9,10, 11,12,12A,14,14A,14B-Octadecahydro-2	$C_{30}H_{48}O$	424	

Table 1. Continued.

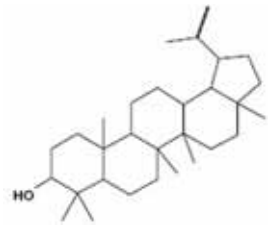
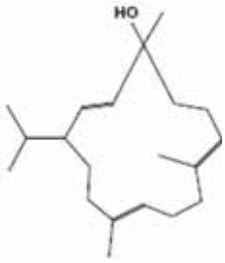
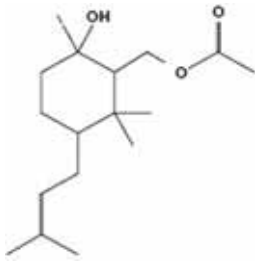
Si.no	RT	Compound name	Molecular formula	Molecular weight	Structure
12	29.284	Lupeol	$C_{30}H_{50}O$	426	
13	29.449	Thunbergol	$C_{20}H_{34}O$	290	
14	30.325	2R-Acetoxymethyl-1, 3,3-trimethyl-4T-(3-Methyl-2-Buten-1-YL)-1T-Cyclohexanol	$C_{17}H_{30}O_3$	282	

Table 2. Phytocomponents identified in the methanolic latex extract of *Calotropis gigantea* by GC-MS.

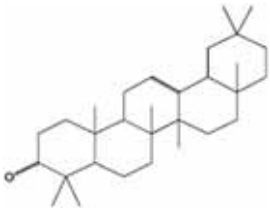
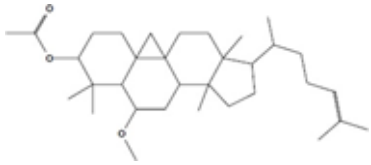
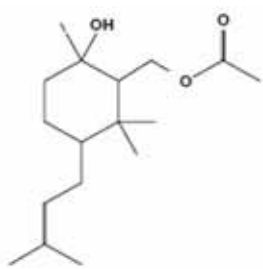
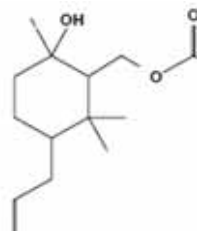
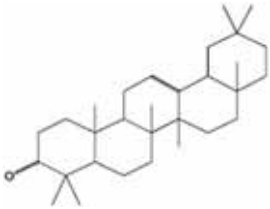
Si.no	RT	Compound name	Molecular formula	Molecular weight	Structure
1	14.693	4,4,6A,6B,8A,11,11,14BOctameth-yl1,4,4A,5,6,6A,6B,7,8,8A,9,10, 11,12,12A,14,14A,14B-Octadecahydro-2	$C_{30}H_{48}O$	424	
2	17.059	3-O-Acetyl-6-Methyoxy-cycloartenol	$C_{33}H_{54}O_3$	498	
3	19.285	2R-Acetoxymethyl-1,3,3-trimethyl-4T-(3-Methyl-2-Buten-1-YL)-1T-Cyclo-hexanol	$C_{17}H_{30}O_3$	282	

Table 2. Continued.

Si.no	RT	Compound name	Molecular formula	Molecular weight	Structure
4	19.410	2R-Acetoxymethyl-1,3,3-trimethyl-4T-(3-Methyl-2-Buten-1-YL)-1T-Cyclo hexanol	$C_{17}H_{30}O_3$	282	
5	20.286	4,4,6A,6B,8A,11,11,14B-Octamethyl-1,4,4A,5,6, 6A,6B,7,8,8A,9,10,11,12, 12A,14,14A,14B-Octa decahydro-2	$C_{30}H_{48}O$	424	

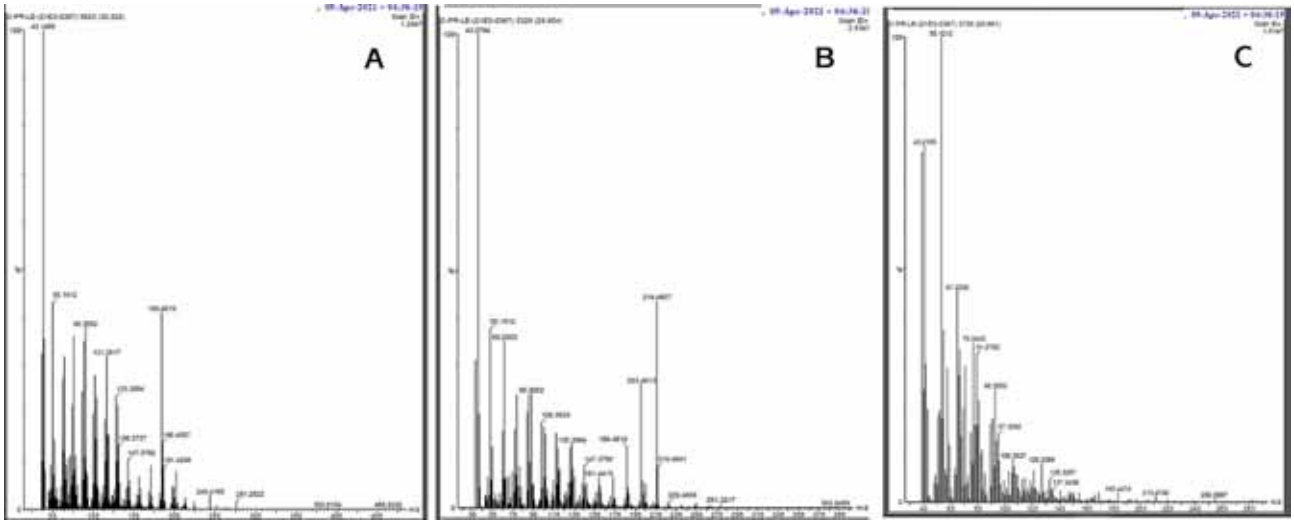


Figure 5. Mass spectra of same and major compound in Calotropis leaf and latex. A)-2R-Acetoxymethyl-1,3,3-trimethyl-4T-(3-Methyl-2-Buten-1-YL)-1T-Cyclohexanol. B) 4,4,6A,6B,8A,11,14B-Octamethyl-1, 4, 4A, 5, 6, 6A, 6B, 7, 8, 8A, 9, 10, 11, 12, 12A, 14, 14A, 14B Octadecahydro-2. C) 3-Tetradecyn-1-ol.

the literature were not the same.[15] has done the extraction of phytochemicals using Calotropis gigantea flowers not the methanol extraction; they identified the hexa-decanoic acid and squalene compounds which were the same in this present study in Calotropis gigantea leaves. These compounds showed anti-oxidant and anti-tumour biological activity.

Analysis of *Carica papaya* leaf and latex

In *Carica papaya*, GC-MS analysis showed total of about 20 compounds, of which (Table 3) 15 compounds were identified in leaf extract and (Table 4) 5 in latex methanol extraction. This Beta- carotene phytochemical had the same molecular formula $C_{40}H_{56}$ and molecular weight 536 in both leaves and latex at RT 29.314. The major compound in the leaf was hexa-decanal, with a RT of 21.156, a molecular formula $C_{16}H_{32}O$, a molecular weight of 240, and an area of 18.507 %. The main compound in latex was (1R,2R,8AS) -2,4,4,7A-Tetramethyl-1-(3-oxobutyl)-trans-hydrindan-2-carboxylic acid with a RT of 27.929, a molecular formula of $C_{18}H_{30}O_3$ and molecular weight of 294 and a peak area of 63.574%.

Analysing the phytochemicals found in *Carica papaya* leaf and latex was one of the research. In the methanol extraction of papaya red lady variety leaf[16] and in this investigation, it was demonstrated that no compound was same. However, the research revealed that n-hexadecanoic acid, which is present in methanol extraction of papaya leaf, is present. The same component was discovered in an aqueous papaya fruit extract that exhibited anti-microbial, anti-cancer, anti-haemolytic and anti-diabetic properties.[17] Squalene was discovered in this study methanol leaf extraction; the same

Table 3. Phytocomponents identified in the methanolic extract of *Carica papaya* leaf by GC-MS.

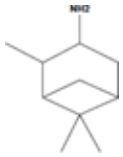
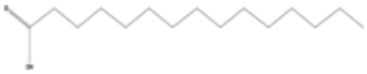
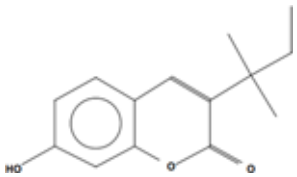
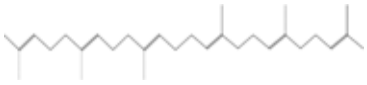
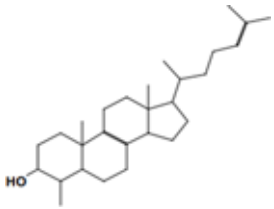
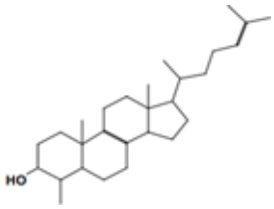
Si.no	RT	Compound name	Molecular formula	Molecular weight	Structure
1	14.758	2,6,6-Trimethyl-bicyclo[3.1.1]hept-3-ylamine	$C_{10}H_{19}N$	153	
2	15.569	Pyrrolidine,1-nitro-	$C_4H_8O_2N_2$	116	
3	17.885	Hexadecanal	$C_{16}H_{32}O$	240	
4	18.335	Phytol	$C_{20}H_{40}O$	296	
5	19.080	Cyclopropanepentanoic acid, 2-undecyl-,methyl ester,trans-	$C_{20}H_{38}O_2$	310	
6	19.785	N-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	
7	19.945	Pentadecanoic acid	$C_{15}H_{30}O_2$	242	
8	21.156	Hexadecanal	$C_{16}H_{32}O$	240	
9	23.337	7-Hydroxy-3-(1,1- Dimethylprop-2-enyl) Coumarin	$C_{14}H_{14}O_3$	230	
10	24.492	Squalene	$C_{30}H_{50}$	410	
11	26.393	Cholesta-8,24-Dien-3 -ol, 4-methyl-,(3.beta., 4.alpha.)-	$C_{28}H_{46}O$	398	
12	26.748	Cholesta-8,24-Dien-3-ol, 4-methyl-,(3.beta., 4.alpha.)-	$C_{28}H_{46}O$	398	

Table 3. Continued.

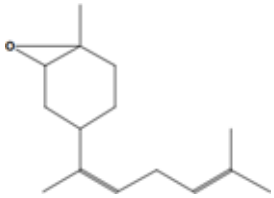
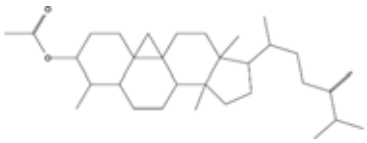
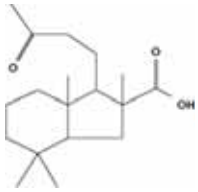
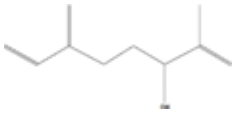
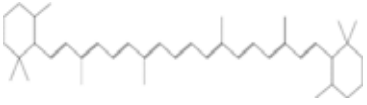
Si.no	RT	Compound name	Molecular formula	Molecular weight	Structure
13	27.909	Trans-z-.alpha.- bisabolene epoxide	$C_{15}H_{24}0$	220	
14	28.649	9,19-Cycloergost-24(28) -en-3-ol,4,14-dimethyl-, acetate,(3.beta.,4.alpha.,5. Alpha	$C_{32}H_{52}O_2$	468	
14	29.314	Beta carotene	$C_{40}H_{56}$	536	

Table 4. Phytocomponents identified in the methanolic extract of *Carica papaya* latex by GC-MS.

Si.no	RT	Compound name	Molecular formula	Molecular weight	Structure
1	16.574	Benzene,1-isocyano-4-methyl	C_8H_7N	117	
2	27.929	(1R,2R,8AS) -2,4,4,7A- Tetramethyl-1-(3-oxobutyl) -trans-hydrindan-2-carboxylic acid	$C_{18}H_{30}O_3$	294	
3	28.794	2-Methyl-6-methylene-octa-1,7-dien-3-ol	$C_{10}H_{16}O$	152	
4	29.424	Beta.carotene	$C_{40}H_{56}$	536	
5	29.789	Arachidonic amide,N-[5-Hydroxy-N-Pentyl]	$C_{25}H_{43}O_2N$	389	

substance had previously been discovered by chloroform extracted latex.[18]

Analysis of *Ficus benghalensis* leaf and latex

GC-MS analysis of *Ficus benghalensis* revealed a total of 16 phytocompounds, (Table 5) 11 of which were found in the leaf and (Table 6) 5 in the latex after methanol extraction. 2R-Acetoxymethyl-1,3,3-trimethyl-4T-(3-methyl-2-buten-1-yl)-1T Cyclohexanol at RT 28.124, with a molecular formula of $C_{17}H_{30}O_3$ and a molecular weight of 282 was found in both the leaf and the latex of Ficus methanol extraction. The major compound in the leaf was N- hexadecanoic acid at RT 21.461, molecular formula $C_{16}H_{32}O_2$ and molecular weight 256, with an area of 20.907 % peak area. The latex main constituent

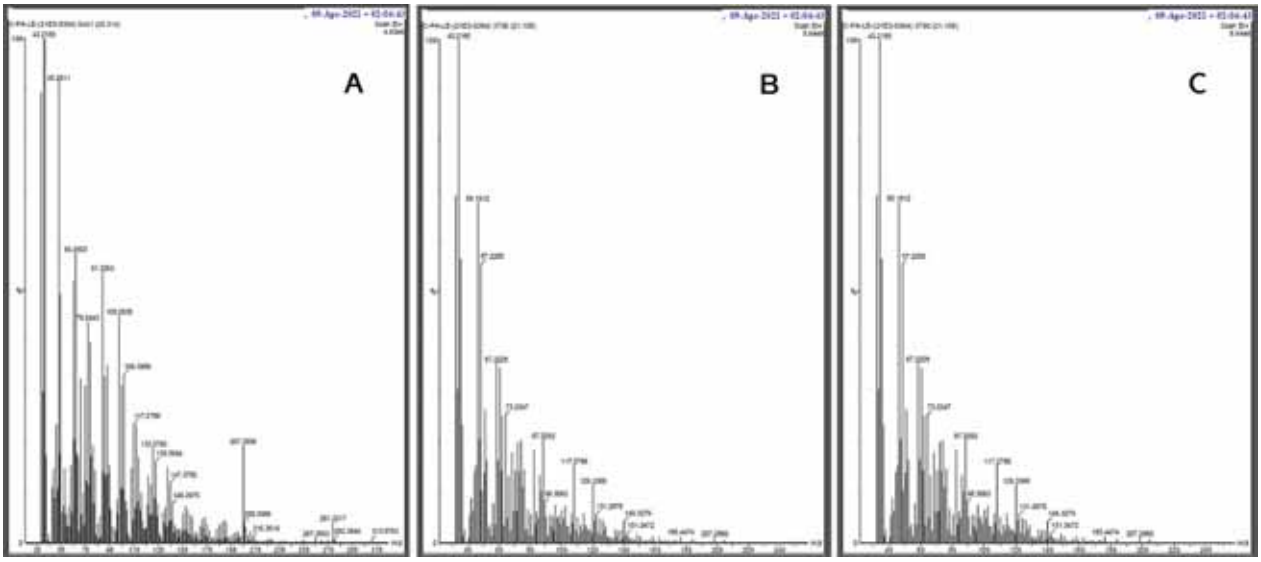


Figure 6. Mass spectra of same and major compound in Carica papaya leaf and latex. (A) Beta- carotene, (B) Hexa-decanal, (C) (1R,2R,8AS) -2,4,4,7A-Tetramethyl-1-(3-oxobutyl)-trans-hydrindan-2-carboxylic acid.

Table 5. Phytocomponents identified in the methanolic extract of *Ficus benghalensis* leaf by GC-MS.

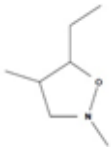
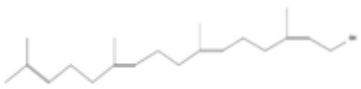
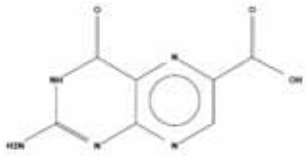
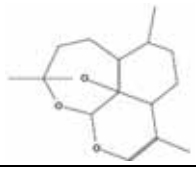
Si.no	RT	Compound name	Molecular formula	Molecular weight	Structure
1	15.394	Methanecarbothiolic Aid	C2H4OS	76	
2	18.280	Isoxazolidine,5-ethyl -2,4-dimethyl-,trans-	C7H15ON	129	
3	20.036	N- hexadecanoic acid	C16H32O2	256	
4	21.461	N- hexadecanoic acid	C16H32O2	256	
5	24.252	Hexadeca-2,6,10,14-tetra-1-ol,3,7,11,16- tetramethyl-,(E,E,E)-	C20H34O	290	
6	24.788	Pterin-6-carboxylic acid	C7H5O3N5	207	
7	26.643	Dihydroartemisinin, 6- Deshydro-5-deshydroxy-3-desoxy	C15H22O3	250	

Table 5. Continued.

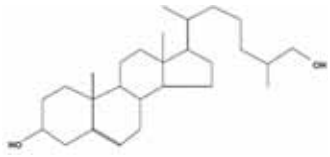
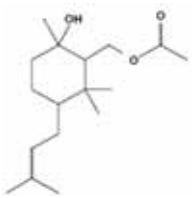
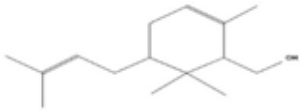
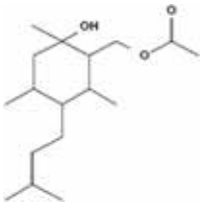
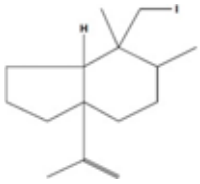
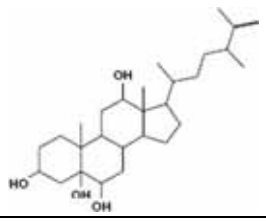
Si.no	RT	Compound name	Molecular formula	Molecular weight	Structure
8	27.334	26-Hydroxycholesterol	C27H4602	402	
9	28.124	2R-Acetoxymethyl-1,3, 3-trimethyl-4T-(3-methyl-2-buten-1-yl)-1T-Cyclo Hexanol	C17H30O3	282	
10	28.444	2,4,4-Trimethyl-3-hydro xymethyl-5A-(3-methyl- but-2-enyl)-cyclohexene	C15H26O	222	

Table 6. Phytocomponents identified in the methanolic extract of *Ficus benghalensis* latex by GC-MS.

Si.no	RT	Compound name	Molecular formula	Molecular weight	Structure
1	27.274	9.19-Cycloergost-24(28) -en-3-ol,4, 14dimethyl-,acetate,(3.beta.,4.alpha., 5.alpha.)-	$C_{32}H_{52}O_2$	468	
2	27.359	2R-Acetoxymethyl-1,3,5-trimethyl-4c-3-(methyl-2-buten-1-yl)-1c-cyclo Hexanol	$C_{17}H_{30}O_3$	282	
3	27.684	6.Beta.bicyclo[4.3.0] nonane.5 beta-iodomethyl-1 beta-isopropenyl-4	$C_{15}H_{25}I$	332	
4	27.894	1,6,10,14,18,22-Tetra cosahexaen-3-ol, 2,6,10,15, 19,23-hexamethyl-,(ALL-E)-	$C_{30}H_{50}O$	426	
5	28.354	Ergost-25-ene-3,5,6,12- tetrol, (3.beta.,5.alpha.,6.beta.,12.beta.)-	$C_{28}H_{48}O_4$	448	

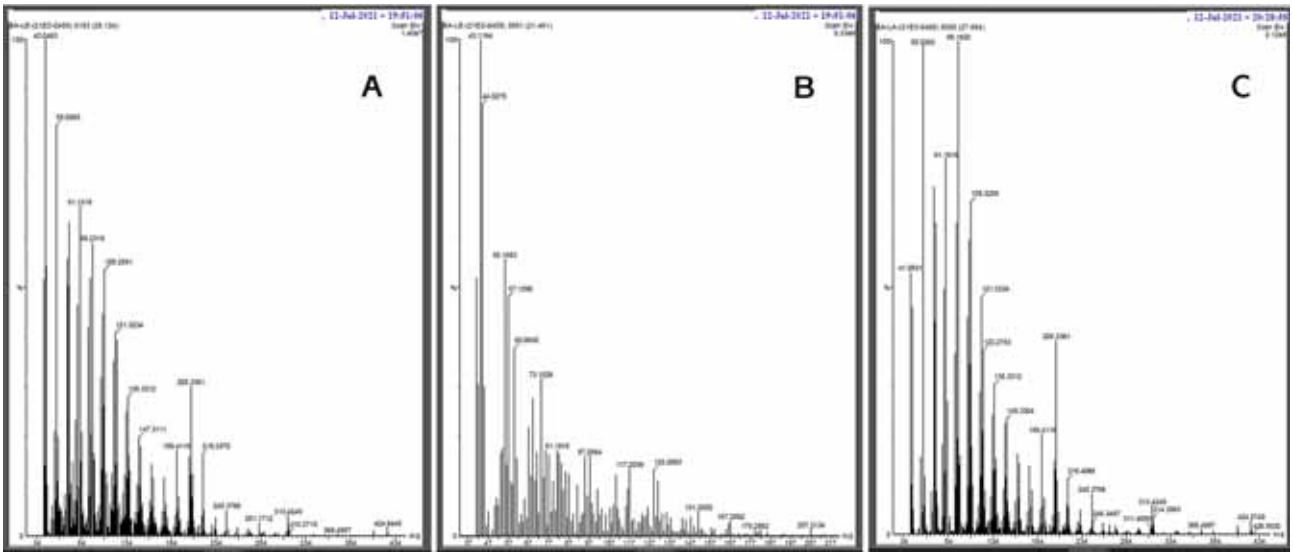


Figure 7. Mass spectra of same and major compound in Ficus leaf and latex. A) 2R-Acetoxymethyl-1,3,3-trimethyl-4T-(3-methyl-2-buten-1-yl)-1T Cyclohexanol, B) N- hexadecanoic acid, C) 6. Beta-bicyclo [4.3.0] nonane.5 beta-iodomethyl-1 beta-isopropenyl-4.

was 6. Beta-bicyclo [4.3.0] nonane.5 beta-iodomethyl-1 beta-isopropenyl-4 with a RT 27.684, a molecular formula of $C_{15}H_{25}I$, and a molecular weight of 332 with a peak area of 53.278%.

One of the study examined the phytocomponents in a methanol extract of *Ficus benghalensis* leaf. N-hexadecanoic acid was same in both studies in this as well. They state that this substance has anti-seborrheic, anti-inflammtory, cytoprotectant and anaesthetic properties.[19] There are no studies was carried out using methanol extraction of *Ficus benghalensis* latex.

Analysis of Nerium oleander

GC-MS analysis of *Nerium oleander* revealed a total of 11 phytocompounds, (Table 7) 7 of which were found in the leaf and (Table 8) 4 compounds in the latex after methanol extraction. There is no similar compound found in Nerium methanol extraction leaf and latex. The major compound in the leaf was myo-inositol,4-c-methyl-at RT 20.185, molecular formula $C_7H_{14}O_6$, and molecular weight 194, with an area of 87.718% peak area. Lupeol was the major compound in the latex, with a RT of 28.474, a molecular formula of $C_{30}H_{50}O$,

Table 7. Phytocomponents identified in the methanolic extract of *Nerium oleander* leaf by GC-MS.

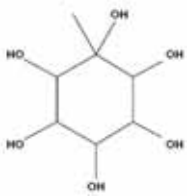
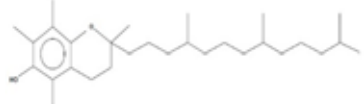
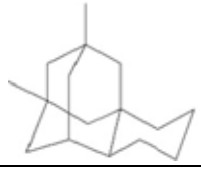
Si.no	RT	Compound name	Molecular formula	Molecular weight	Structure
1	20.185	Myo-inositol,4-c-methyl-	$C_7H_{14}O_6$	194	
2	23.917	Squalene	$C_{30}H_{50}$	410	
3	26.238	D1-alpha-Tocopherol	$C_{29}H_{50}O_2$	430	
4	27.723	9,11-Dimethyl tetracylo [7.3.1.0(2.7).1(7.11)]tetradecane	$C_{16}H_{26}$	218	

Table 7. Continued.

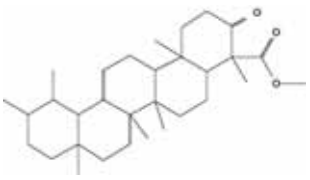
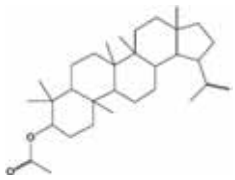
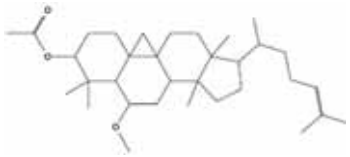
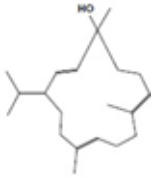
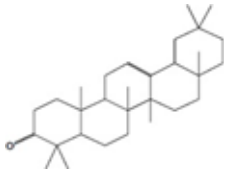
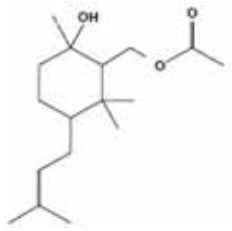
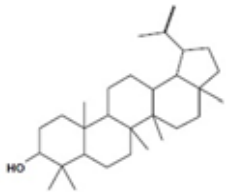
Si.no	RT	Compound name	Molecular formula	Molecular weight	Structure
5	28.199	Urs-12-en-24-oic acid,3-oxo-methyl, ester, (+)-	$C_{31}H_{48}O_3$	468	
6	28.584	Lup-20(29)-en-3-ol, acetate,(3.beta.)-	$C_{32}H_{52}O_2$	468	
7	28.669	3-O-Acetyl-6-methoxy-cycloartenol	$C_{33}H_{54}O_3$	498	

Table 8. Phytocomponents identified in the methanolic extract of *Nerium oleander* latex by GC-MS.

Si.no	RT	Compound name	Molecular formula	Molecular weight	Structure
1	27.904	Thunbergol	$C_{20}H_{34}O$	290	
2	28.029	4,4,6A,6B,8A,11, 11,14B-Octamethyl-1, 4,4A,5,6,6A,6B,7,8, 8A,9,10,11,12,12A,14, 14A,14B-Octa Decahydro	$C_{30}H_{48}O$	424	
3	28.399	2R-Acetoxymethyl-1, 3,3-Trimethyl-4T-(3- Methyl-2-buten-1-yl)- 1T-Cyclohexanol	$C_{17}H_{30}O_3$	282	
4	28.474	Lupeol	$C_{30}H_{50}O$	426	

a molecular weight 426, and an area of 55.852% peak area.

This research's dl-alpha-tocopherol chemical compound, which is also found in the leaf extract, was compared to a study that examined the phytocomponent in *Nerium oleander*

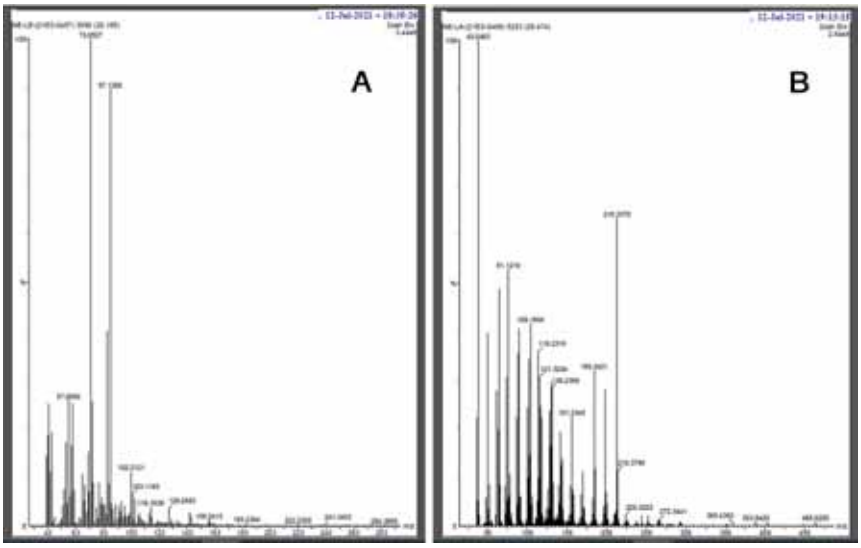


Figure 8. Mass spectra of same and major compound in Nerium leaf and latex. A) myo-inositol,4-c-methyl, B) Lupeol.

leaf part methanol extraction. They also emphasised the biological effects of this chemical, including its anti-oxidant, immunological, anti-cancer, anti-inflammtory properties.[20] There are no studies was carried out using methanol extraction of *Nerium oleander* latex.

Analysis of *Plumeria alba*

In *Plumeria alba*, GC-MS analysis showed total of 16 phytocompounds, of which (Table 9) 10 were found in the leaf and (Table 10) 6 compounds in the latex after methanol extraction. 2,4,4-Trimethyl-3-hydroxymethyl-5A-(3-Methyl-but-

Table 9. Phytocomponents identified in the methanolic leaf extract of *Plumeria alba* leaf by GC-MS.

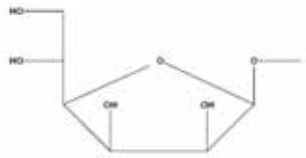
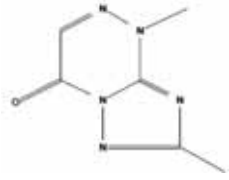
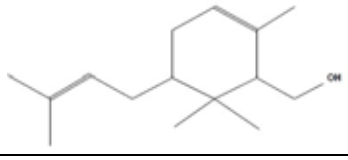
Si.no	RT	Compound name	Molecular formula	Molecular weight	Structure
1	18.610	Eicosanoic acid, methyl ester	$C_{21}H_{42}O_2$	326	
2	19.160	Tridecanoic acid	$C_{13}H_{26}O_2$	214	
3	20.676	Beta.-D-Manno furanoside, methyl	$C_7H_{14}O_6$	194	
4	24.127	Squalene	$C_{30}H_{50}$	410	
5	26.528	2,4-Dimethyl-7-Oxo-4, 7-dihydro-triazolo (3,2-c)triazine	$C_6H_7ON_5$	165	
6	28.069	2,4,4-Trimethyl-3- hydroxymethyl -5A-(3-Methyl-but-2-enyl) -cyclohexene	$C_{15}H_{260}$	222	

Table 9. Continued.

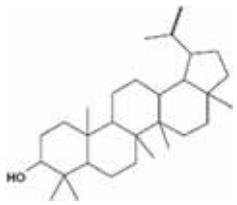
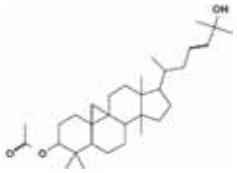
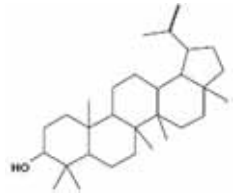
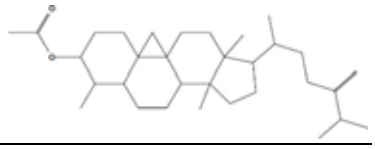
Si.no	RT	Compound name	Molecular formula	Molecular weight	Structure
7	28.284	Lupeol	$C_{30}H_{50}O$	426	
8	28.454	9,19-Cyclolanost-23-ene-3,25-diol, 3-Acetate, (3.beta.,23E)-	$C_{32}H_{52}O_3$	484	
9	28.789	Lupeol	$C_{30}H_{50}O$	426	
10	29.009	9,19-Cycloergost-24(28)-en-3-ol,4, 14- dimethyl-,acetate (3.beta.,4.alpha.,5.alpha.)-	$C_{32}H_{52}O_2$	468	

Table 10. Phytocomponents identified in the methanolic leaf extract of *Plumeria alba* latex by GC-MS.

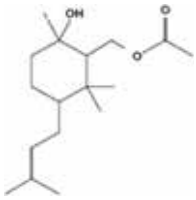
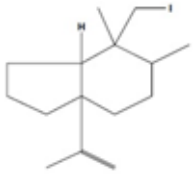
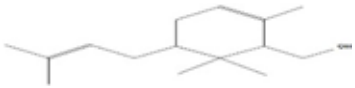
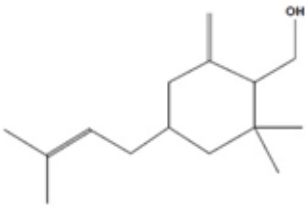
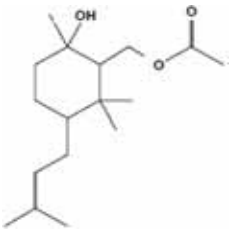
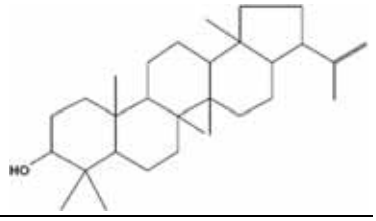
Si.no	RT	Compound name	Molecular formula	Molecular weight	Structure
1	27.554	2R-Acetoxymethyl-1,3,3-trimethyl-4t-(3--1t-cyclohexanol	$C_{17}H_{30}O_3$	282	
2	27.654	6.Beta.bicyclo[4.3.0] nonane,5.beta-iodomethyl -1.beta-isopropenyl-4.alpha.,5.alpha.-dimethyl	$C_{15}H_{25}I$	332	
3	27.854	2,4,4-Trimethyl-3-hydro xymethyl-5A-(3-methy l-but-2-enyl)-cyclohexene	$C_{15}H_{26}O$	222	
4	27.919	1-Methylene-2B-Hydroxy methyl-3, 3-dimethyl-4B-(3-Methylbut-2-enyl)-cyclo Hexane	$C_{15}H_{26}O$	222	

Table 10. Continued.

Si.no	RT	Compound name	Molecular formula	Molecular weight	Structure
5	28.424	2R-Acetoxymethyl-1,3,3-trimethyl-4t-(3-methyl-2-buten-1-yl)-1t-cyclo Hexanol	$C_{17}H_{30}O_3$	282	
6	28.630	Hop-22(29)-En-3.beta-ol	$C_{30}H_{50}O$	426	

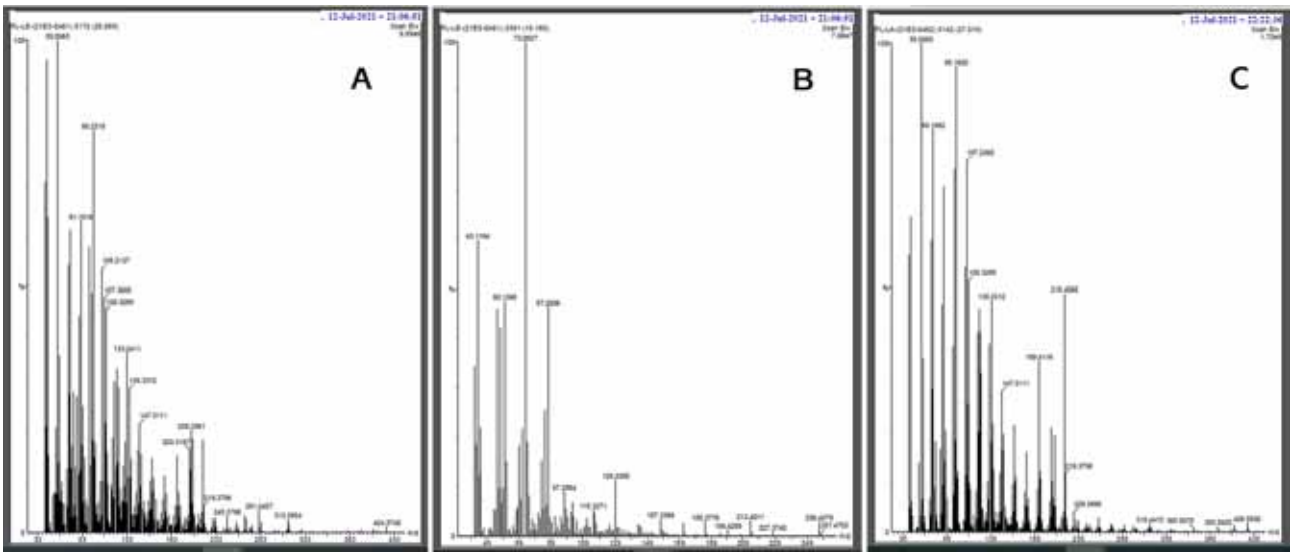


Figure 9. Chromatogram of same and major compound in Plumeria leaf and latex. A) 2,4,4-Trimethyl-3-hydroxymethyl-5A-(3-Methyl-but-2-enyl)-cyclohexene, B) tridecanoic acid, C) 1-Methylene-2B-Hydroxymethyl-3,3-dimethyl-4B-(3-Methylbut-2-enyl)-cyclohexane.

2-enyl)-cyclohexene at RT 28.069, with a molecular formula of $C_{15}H_{26}0$ and a molecular weight of 222 was found in both the leaf and the latex of Ficus methanol extraction. The major compound in the leaf was tridecanoic acid at RT 19.160, molecular formula $C_{13}H_{26}O_2$ and molecular weight 214, with a peak area of 53.664 %. At RT 27.919, molecular formula $C_{15}H_{26}0$, and molecular weight 222, the major compound in the latex was 1-Methylene-2B-Hydroxymethyl-3,3-dimethyl-4B-(3-Methylbut-2-enyl)-cyclohexane with the area 52.524% peak area.

One research did not analyse the methanol extraction, just the phytochemicals found in *Plumeria alba* flower. They named the substance Squalene.[21] The *Plumeria alba* leaf in the current investigation contained the same chemical, as determined by methanol extraction.

Conclusion

The investigation in this study involves the leaves and latex of five different plants. As per the literature, this shows the presence of more identified phytocomponents and a few compounds that showed biological activity. The *Calotropis gigantea* plant latex and leaf were used for methanol extraction using the Soxhlet apparatus.[13,14] But the compounds in this study and those mentioned in the literature were not the same.[2] It has done the extraction of phytochemicals using *Calotropis gigantea* flower, not the methanol extraction; they identified the hexa-decanoic acid and squalene compounds which were found in this study in *Calotropis gigantea* leaf, and *Carica papaya* leaf, and Plumeria leaf. These compounds showed the antioxidant and anti-tumour

biological activities. Beta carotene, which is present in the *Carica papaya* leaf as well as in the latex, the was shown in the review to play a dynamic role in delaying aging, reducing inflammation, and preventing certain cancers.[19] Literature showed that identified compound phytol in ethanolic leaf extraction of *Calotropis gigantea* that showed anti-inflammatory and anti-cholesteric activity, the same was identified in this study in *Carica papaya* leaf. The comparison of latex and leaf methanolic extraction reveals a large number of compounds, with some compounds present in both leaf and latex.

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