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Analysis of Phytochemical Constituents of Stem Bark Extracts of *Zanthoxylum Tetraspermum* Wight & Arn

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ABSTRACT

The aim of the study was to investigate the Stem bark Extracts of *Zanthoxylum Tetraspermum* Wight & Arn phytochemical compounds of different extracts. The phytochemical compound screened by qualitative and GC-MS method. Qualitatively the different extracts were analyzed the compounds were found to like alkaloids, Saponin, Triterpenoids, Steroids, tannin-phenolic compounds, protein, fat, fixed oils, flavonides and flavones, whereas carbohydrates, glycoside, gum and mucilage, totally absent in this plant. Ethanol and aqueous extract selected for GCMS analysis because of high percentage extractive value. (3.7552% & 4.0632% w/w respectively) In the GC-MS analysis, 12 & 6 bioactive phytochemical compounds were identified in the ethanolic and aqueous extract of *Stem bark of Zanthoxylum Tetraspermum* Wight & Arn .

Key words: *Zanthoxylum Tetraspermum* Wight & Arn, Phytochemical, GC-MS

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INTRODUCTION

Zanthoxylum Tetraspermum wight & arn (Rutaceae) is a thorny, stout, aromatic, climbing shrub, with brown bark having short recurved prickles, found in the western ghats in the nilgiri and annaimalai hills and in kollis hills (namakal district, TN) at attitudes of 1,200 1,800m and in kerala and Karnataka. The wood is yellowish and soft. The plant is credited in srilanka with stimulant, astringent and digestive properties and is prescribed in dyspepsia and diarrhoes [1-3]. 8-acetyldihydroxanthone, 8-acetyldihydroxanthone, Liriodenine, sesamin, Lichexanthone, (+) - piperitol-gamma- dimethylallylether have been reported [4]. The present investigation was undertaken to study the phytochemicals present in the stem bark extracts of this plant by GC-MS method.

MATERIAL AND METHODS

Plant material

The stem bark of *Zanthoxylum Tetraspermum* wight & arn were collected from cholakkadu, a village of kolli hills, 60km away from Namakkal (TN) in the month of August (2007). The plant was identified by local people of that village and authenticated by G.V.S. Murthy, Joint Director, botanical survey of India, southern circle, Coimbatore, (No. Bsi/sc/5/23/07-08/ Tech 715). A herbarium specimen of the plant was preserved in the department of pharmacognosy of our institute for further references.

Chemicals

All the reagents used were of analytical grade obtained from S.D. fine chemicals Ltd, Mumbai.

Preparation of stem bark extracts

The stem barks of *Zanthoxylum Tetraspermum* were washed with water, air-dried at room temperature and then reduced to coarse powder. The dried powder (250mg) was subjected to soxhlet extraction with petroleum ether (40-60), chloroform, ethyl acetate, ethanol and aqueous (in order of increasing polarity) for continuous hot extraction. The extracts were filtered and the filtrates were concentrated under reduced pressure to obtain the extracts as solid residues. The percentage extraction values (% w/w) were 1.25, 1.37, 1.96, 3.75 and 4.06% respectively. The freshly prepared extracts were chemically tested for the presence of different constituents using standard methods. [5- 8]

Principle and Application of Gas Chromatograph -Mass Detector (GC-MS) In Phyto - Chemical Analysis

GC-MS plays a key role in the analysis of unknown components of plant origin. GC-MS ionizes compounds and measures their mass numbers. Ionization method includes EI (Electron

ionization) and CI (Chemical Ionization). Typically, the CI method is used. The EI method produces ions by colliding thermal electrons emitted from a filament with sample gas molecules [9-10]. This method provides high stability in ionization and the obtained mass spectra show good reproducibility. The EI method provides good results for quantitative analysis as well. Quantitation with GC-MS, in which only ions specific to the compounds are measured, is highly selective method without interfering components.

Gas chromatography technique involves the separation of volatile components in a test sample using suitable capillary column coated with polar or non-polar or intermediate polar chemicals. Elite-1 column (100% Dimethyl poly siloxane) is a non-polar column used for the analysis of phytocomponents in medicinal plants and pesticide residues. Elite-5 column (5% phenyl and 95% methyl poly siloxane) is an intermediate column used for the estimation of pesticide residues in soft drinks and food grains. Elite-Wax (Polyethylene glycol) is a polar column used in the estimation of fragrances in rice, alcohol, flowers and fatty acid profile of edible oils. An inert gas such as Hydrogen or Nitrogen or Helium is used as a carrier gas. The components of test sample is evaporated in the injection port of the GC equipment and segregated in the column by adsorption and desorption technique with suitable temperature programme of the oven controlled by software. Different components are eluted from the column based on the boiling point of the individual components. The GC column is heated in the oven between 60 to 270 deg centigrade. The time at which each component eluted from the GC column is termed as Retention time (RT). The eluted component is detected in the Mass detector. The spectrum of the unknown component is compared with the spectrum of the known components stored in the NIST library and ascertains the name, molecular weight and structure of the components of the test materials in GC-MS study. Food grain fragrances, floral fragrances, pesticide residues, Terpenes, steroids, alkaloids & fatty acids are some of the useful components analyzed in the GC-MS study. [11-12].

Plant sample extraction for GC-MS analysis

30ml ethanol extract were taken overnight and filtered in a ash less filter paper with sodium sulphate [2gm] and concentrated the extract to 1ml by bubbling nitrogen into the solution. The extracted material is taken for GC MS analysis [13-14].

RESULTS AND DISCUSSION

The present study was carried out on the *Zanthoxylum Tetraspermum wight & arn* the presence of medical active constituents. The phyto chemical active compounds *Zanthoxylum Tetraspermum wight & arn* were qualitatively analysed and the results were presented in table-1. In GC-MS analysis 12&6 bioactive phytochemical compounds were identified in the ethanolic and aqueous extract of *Zanthoxylum Tetraspermum wight & arn*. The identification of phytochemical compounds is based on the peak area, molecular weight, molecular formula and activity. Activities of Components identified in Ethanolic extract of *Zanthoxylum Tetraspermum wight&arn* results were shown Table-2. Figure-1 shows GC-MS Spectrum of Ethanolic extract of *Zanthoxylum Tetraspermum wight & arn*. The following figures were shown in components

presents in ethanol extract. Figure-2 shows 2-Methoxy-4-vinylphenol, it having 10 largest peaks and the component was used as Antioxidant, anti microbial and anti inflammatory. Synonyms for this compound like (1). (2). Phenol, 2-methoxy-4-vinyl-(3).4-Hydroxy-3-methoxystyrene (4).p- Vinylguaiacol (5).4-Vinylguaiacol. Figure-3 shows Methyl Salicylate, it having 10 largest peaks and the component was used as analgesic. Synonyms for this compound like (1). Benzoic acid, 2-hydroxy-, methyl ester (2). Phenol, 2-methoxy-4-vinyl- (3). 4-Hydroxy-3-methoxystyrene (4). Analgit 5).Betula (6).Betula oil. Figure-4 shows, 3, 4-Altrosan it having 10 largest peaks and the component was used as bacteriostat fungicide. Figure-5 shows, 3', 5'-Dimethoxyacetophenone it having 10 largest peaks. Synonyms for this compound like (1).3,5-Dimethoxyacetophenone(2).Ethanone,1-(3,5-dimethoxyphenyl)- (3).1-(3,5- dimethoxyphenyl) ethanone. Figure-6 shows, 3', 5'-Dimethoxyacetophenone it having 10 largest peaks and the component was used as antioxidant, anti microbial and anti inflammatory. Synonyms for this compound like 1, 2,3,5-Cyclohexanetetrol. Figure-7 shows, 2-Propanone, 1-hydroxy-3-(4-hydroxy-3-methoxyphenyl) - it having 10 largest peaks. Synonyms for this compound like 1-Hydroxy-3-(4-hydroxy-3-methoxyphenyl) acetone. Figure-8 shows, 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol, it having 10 largest peaks and the component was used as antimicrobial agent. Figure-9 shows, Phenol, 4-[2-dimethylamino) ethyl]- it having 10 largest peaks and the component was used as antimicrobial and antiseptic agent. Synonyms for this compound like (1).Phenol, P-[2-(dimethylamino) ethyl (2). P-[2-(Dimethyl amino) ethyl] phenol (3).P-Hydroxy-N,N-dimethylphenethylamine) (4). Anhalin (5). Anhaline (6). Eremursine (7). Hordenin (8). Hordenine (9). Hordetin (10). N, N-Dimethyl-p-hydroxyphenethylamine.Figure-10shows,(+)-1,2,3,4 Tetrahydroisoquinoline, 6,7-dimethoxy-1-phenmethanol-2-methyl - it having 10 largest peaks and the compound was used as anti-inflammatory and antimicrobial agent. Figure-11 shows, N-Dimethylaminomethyl-tert.-butyl-isopropylphosphine, it having 10 largest peaks and the compound was used as antimicrobial agent. Synonyms for this compound like [tert-Butyl (isopropyl) phosphino]-N, N-dimethylmethanamine. Figure-12 shows GC-MS Spectrum of aqueous extract of *Zanthoxylum Tetraspermum wight&arn*. The following figures were shown in components presents in aqueous extract. Figure-13 shows, Gallopamil - it having 10 largest peaks. Synonyms for this compound like (1).5-[(3, 4-Dimethoxyphenethyl) methylamino]-2-isopropyl-2-(3, 4, 5-trimethoxyphenyl) valeronitrile (2). Methoxyverapamil (3). D-600(4). 5-[[2-(3,4-Dimethoxyphenyl)ethyl](methyl)amino]-2-isopropyl-2-(3,4,5-trimethoxyphenyl) pentanenitrile . Figure-14 shows, Propane, 1,1-diethoxy - it having 10 largest peaks. Synonyms for this compound like (1). Propionaldehyde, diethyl acetal (2). Propanaldiethylacetal (3). 1, 1-Diethoxypropane. Figure-15 shows,[1,3]Benzodioxolo[5,6-c]-1,3-dioxolo[4,5-i]phenanthridine it having 10 largest peaks and the component was used as antimicrobial and anti-cancer agent. Synonyms for this compound like (1). Norsanguinarine (2). Sanguinarine, 13-demethyl- (3). 1,3-Dioxolo[i][1,3]dioxolo[4,5]benzo[1,2-c]phenanthridine.

Table-1 Qualitative analysis of Phytochemical compounds

S.No	Phytoconstituents	Petroleum ether extract	Ethyl acetate extract	Chloroform extract	Ethanol extract	Aqueous extract
1	Alkaloids	+	+	-	+	+
2	Carbohydrate	-	-	-	-	-
3	Glycosides	-	-	+	-	-
4	Sterols	+	-	-	-	+
5	Fixed oils and fats	+	+	+	-	-
6	Triterpenoids	+	+	+	+	+
7	Tannins- phenolic compounds	-	-	-	-	+
8	Protein & amino acids	-	-	-	-	+
9	Saponins	+	+	+	+	+
10	Gum and mucilage	-	-	-	-	-
11	Flavones and flavonoids	-	-	-	-	+

+ Presence, - Absence

 Table -2 Activities of Components identified in Ethanolic extract of *Zanthoxylum Tetraspermum* wight & arn

No.	RT	Name of the compound	Molecular Formula	MW	Peak Area %	Compound Nature	**Activity
1.	4.53	Methyl Salicylate	C ₈ H ₈ O ₃	152	11.60	Salicylic acid compound	Analgesic
2.	4.94	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	C ₆ H ₆ O ₃	126	6.25	Aldehyde compound	Antimicrobial Preservative
3.	6.00	2-Methoxy-4-vinylphenol [Synonyms: p-Vinylguaiacol]	C ₉ H ₁₀ O ₂	150	0.56	Phenolic compound	Antioxidant Antimicrobial Anti-inflammatory
4.	8.69	3,4-Altrosan	C ₆ H ₁₀ O ₅	162	1.84	Poly Hydroxy compound	Bacteriostat Fungicide
5.	9.05	3',5'-Dimethoxyacetophenone	C ₁₀ H ₁₂ O ₃	180	1.22	Ketone compound	No activity reported
6.	9.47	Diethyl Phthalate	C ₁₂ H ₁₄ O ₄	222	64.69	Plasticizer compound	Antimicrobial Antifouling
7.	10.36	1,2,3,5-Cyclohexanetetrol, (1à,2à,3à,5à)-	C ₆ H ₁₂ O ₄	148	7.49	Poly Hydroxy compound	Antioxidant Antimicrobial Anti-inflammatory
8.	10.89	2-Propanone, 1-hydroxy-3-(4-hydroxy-3-methoxyphenyl)-	C ₁₀ H ₁₂ O ₄	196	0.96	Ketone compound	No activity reported
9.	11.05	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	C ₁₀ H ₁₂ O ₃	180	0.30	Phenolic compound	Antimicrobial
10.	24.40	Phenol, 4-[2-(dimethylamino)ethyl]- [Synonyms: Anhalin]	C ₁₀ H ₁₅ NO	165	0.97	Alkaloid	Antimicrobial Antiseptic
11.	24.70	(+)-1,2,3,4-Tetrahydroisoquinoline, 6,7-dimethoxy-1-phenmethanol-2-methyl-	C ₁₉ H ₂₃ NO ₃	313	1.09	Alkaloid	Antimicrobial Anti-inflammatory
12.	28.56	N-Dimethylaminomethyl-tert.-butyl-isopropylphosphine	C ₁₀ H ₂₄ NP	189	3.02	Amino compound	Antimicrobial

Table-3 Activity of Components identified in Aqueous extracts *Zanthoxylum Tetraspermum wight & arn*

No.	RT	Name of the compound	Molecular Formula	MW	Peak Area %	Compound Nature	**Activity
1.	3.02	Propane, 1,1-diethoxy-	C ₇ H ₁₆ O ₂	132	1.04	Ether compound	No activity reported
2.	9.31	Diethyl Phthalate	C ₁₂ H ₁₄ O ₄	222	16.36	Plasticizer compound	Antimicrobial Antifouling
3.	9.89	1,2,3,5-Cyclohexanetetrol, (1à,2á,3à,5á)-	C ₆ H ₁₂ O ₄	148	42.86	Poly Hydroxy compound	Antioxidant Antimicrobial Anti-inflammatory
4.	11.02	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	C ₁₀ H ₁₂ O ₃	180	0.78	Phenolic compound	Antioxidant Antimicrobial Anti-inflammatory
5.	19.13	[1,3]Benzodioxolo[5,6-c]-1,3-dioxolo[4,5-i]phenanthridine [Synonyms: Norsanguinarine]	C ₁₉ H ₁₁ NO ₄	317	11.69	Alkaloid	Antimicrobial Anticancer
6.	25.09	Gallopamil	C ₂₈ H ₄₀ N ₂ O ₅	484	27.27	Nitrogen compound	Calcium channel blocker

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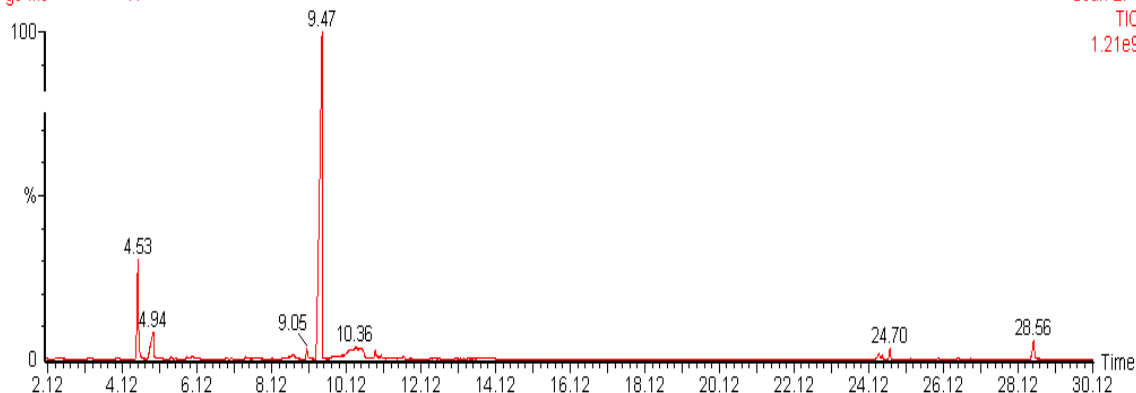


Figure-1GC-MS Spectrum of Ethanolic extract of *Zanthoxylum Tetraspermum wight&arn*

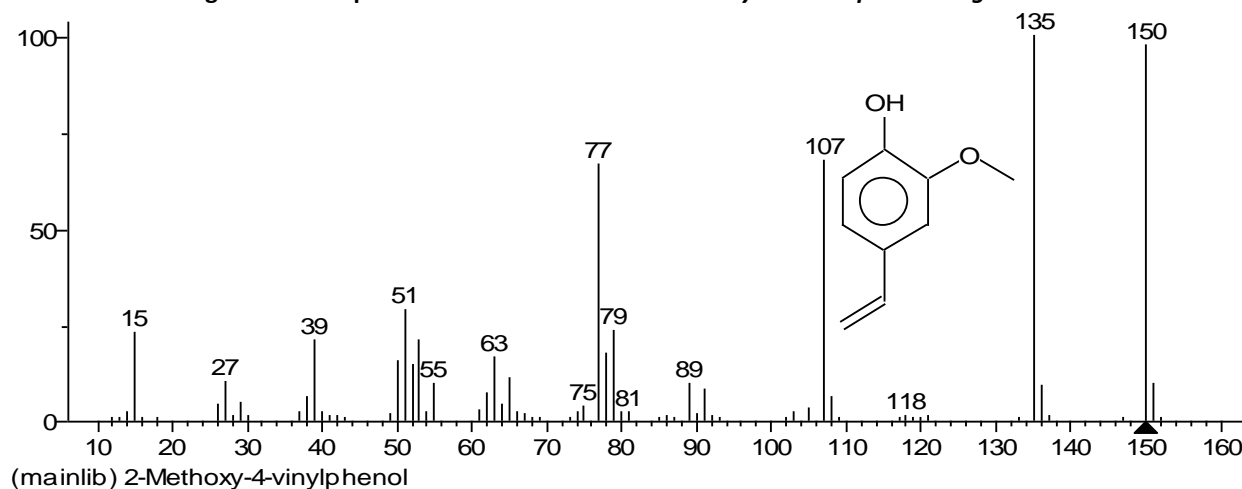


Figure-2 MS spectrum of 2- Methoxy-4-vinylphenol,

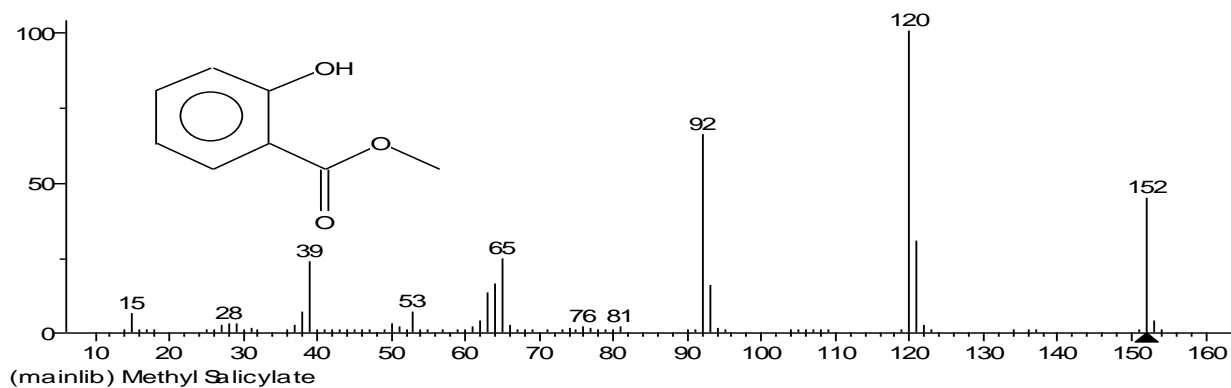


Figure-3 MS spectrum of Methyl salicylate

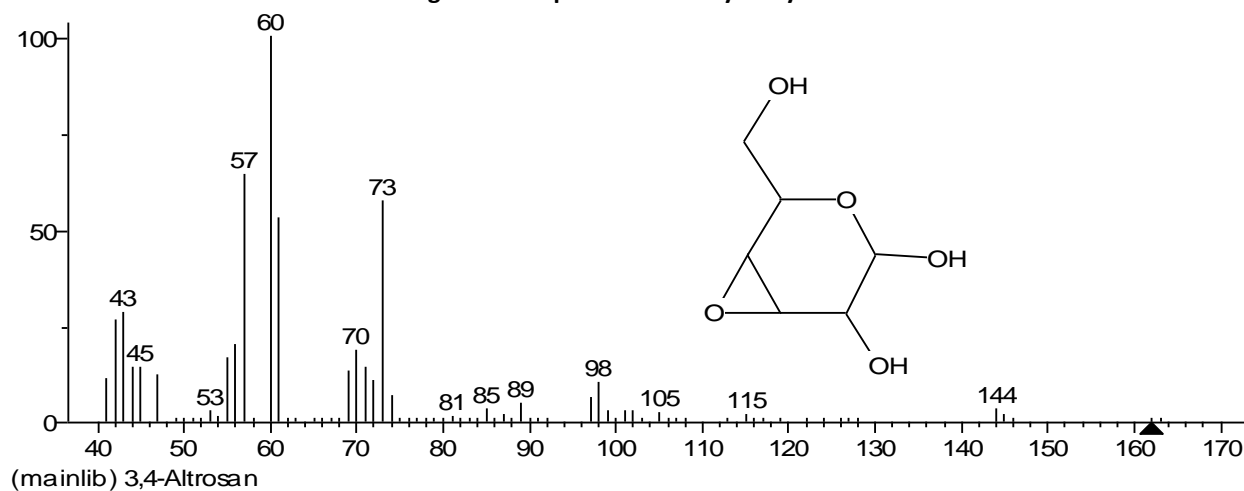


Figure-4 MS spectrum of 3,4-Altrosan

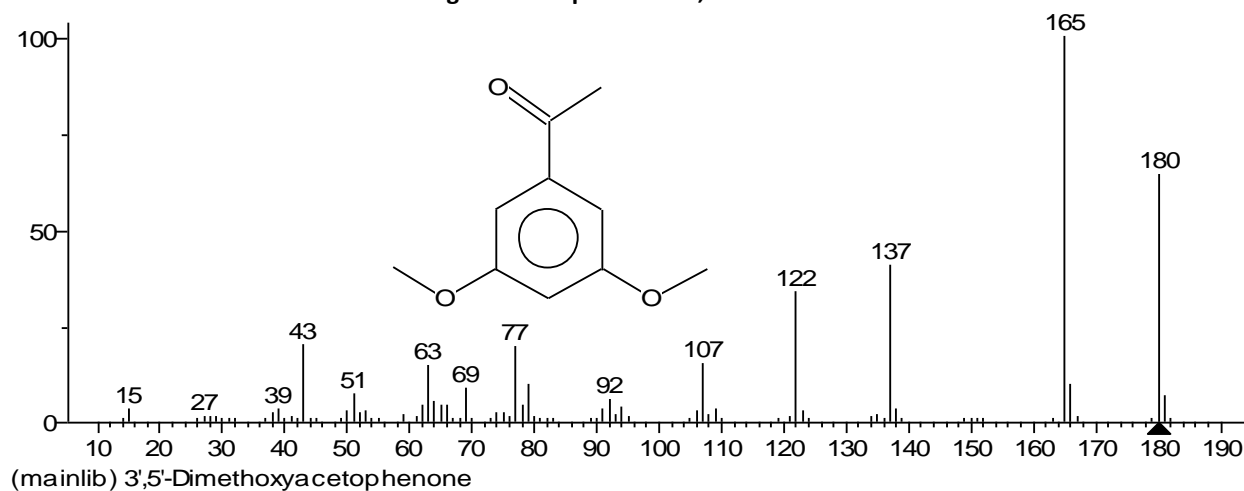


Figure-5 MS spectrum of 3,5-Dimethoxyacetophenone

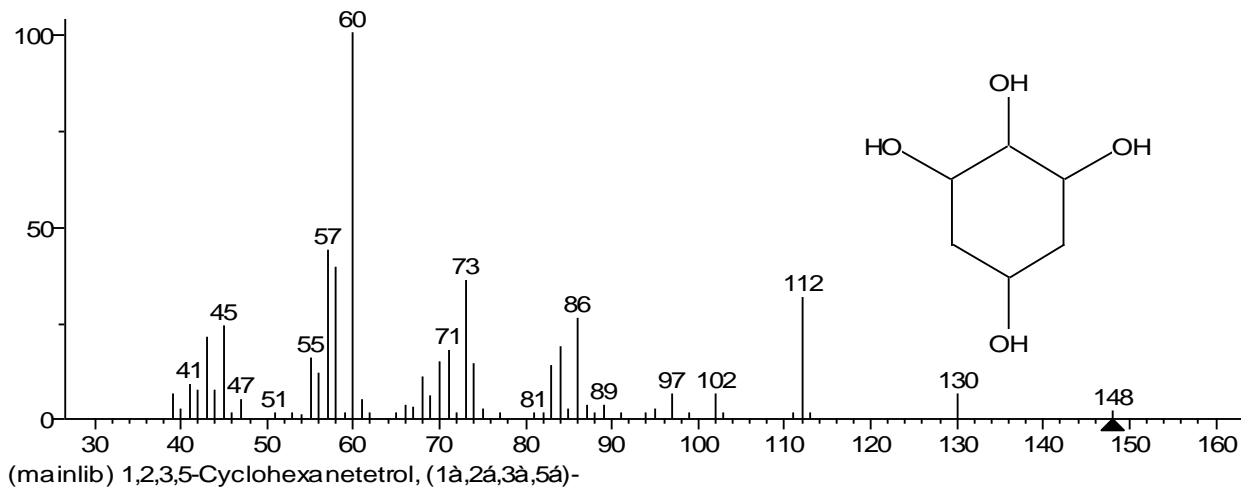


Figure-6 MS spectrum of 1, 2, 3, 5- Cyclohexaneterol

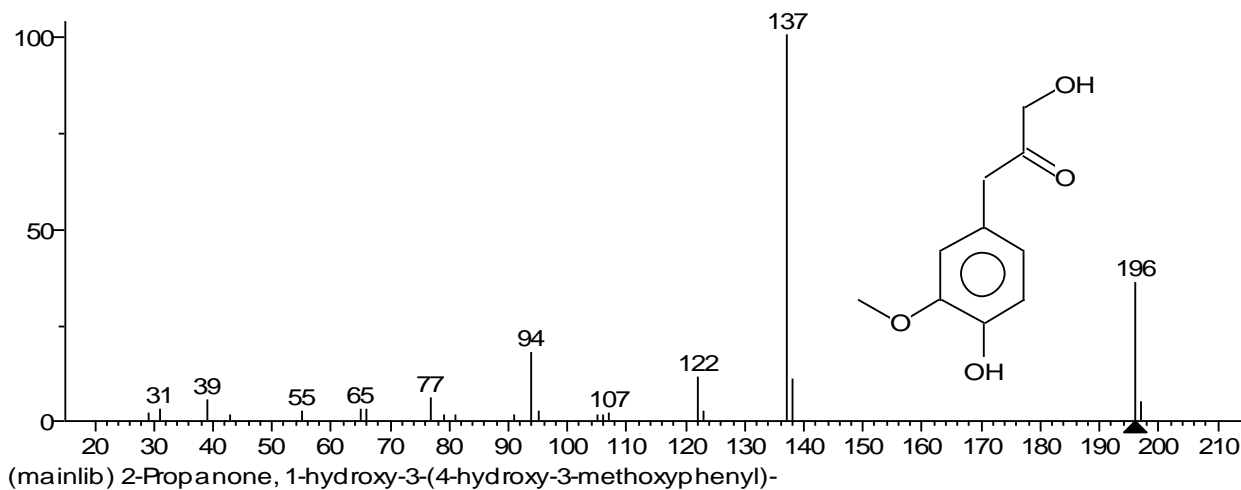


Figure-7 MS spectrum of 2-propanone, 1-hydroxy-3-(4-hydroxy-3-methoxyphenyl)

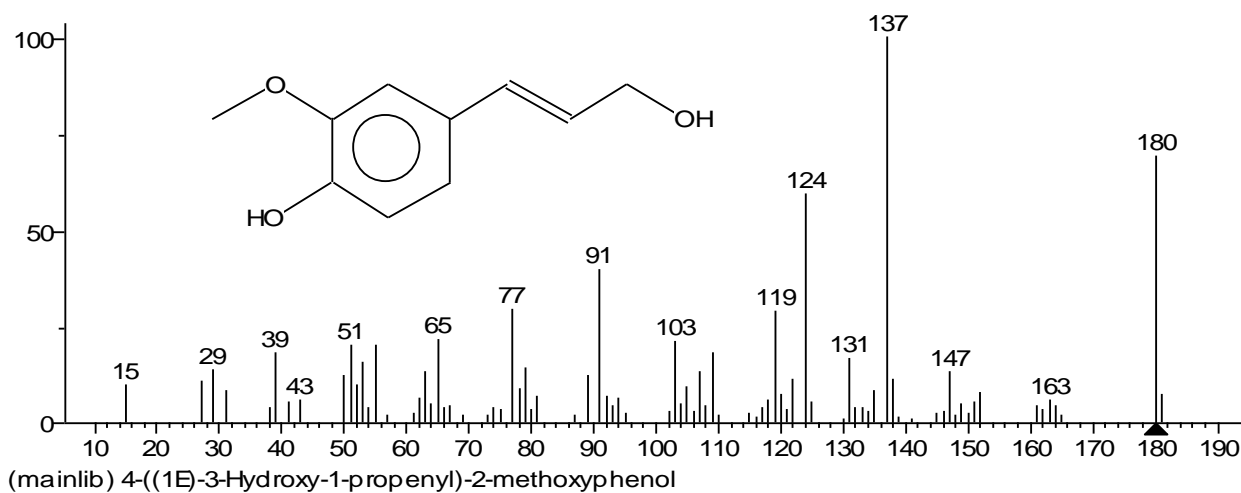


Figure-8 MS spectrum of 4 ((1E) - 3-Hydroxy-1-propenyl)-2- methoxy phenol

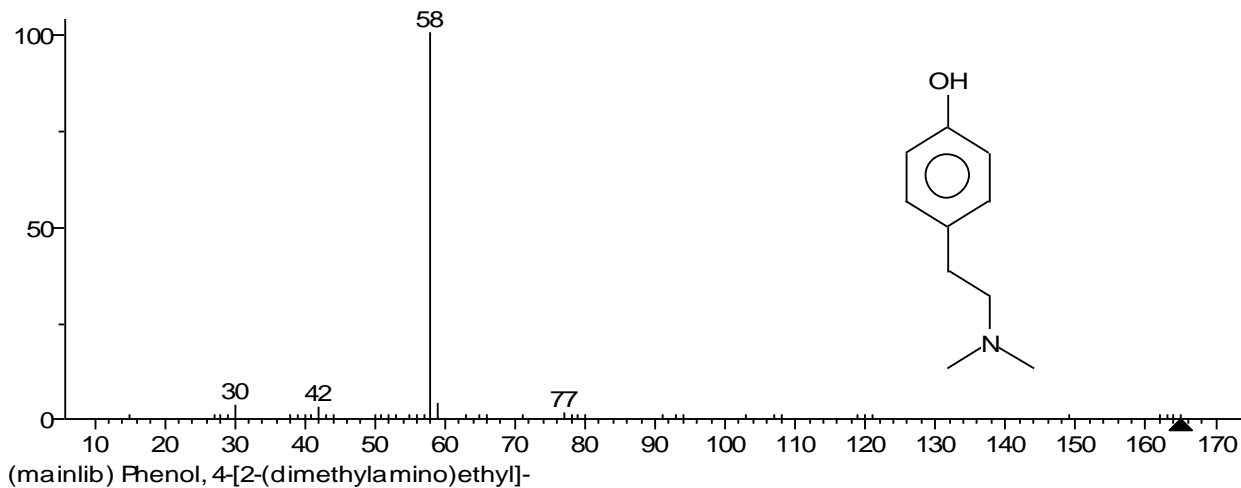


Figure-9 MS spectrum of Phenol, 4[2-(dimethyl amino) ethyl]-

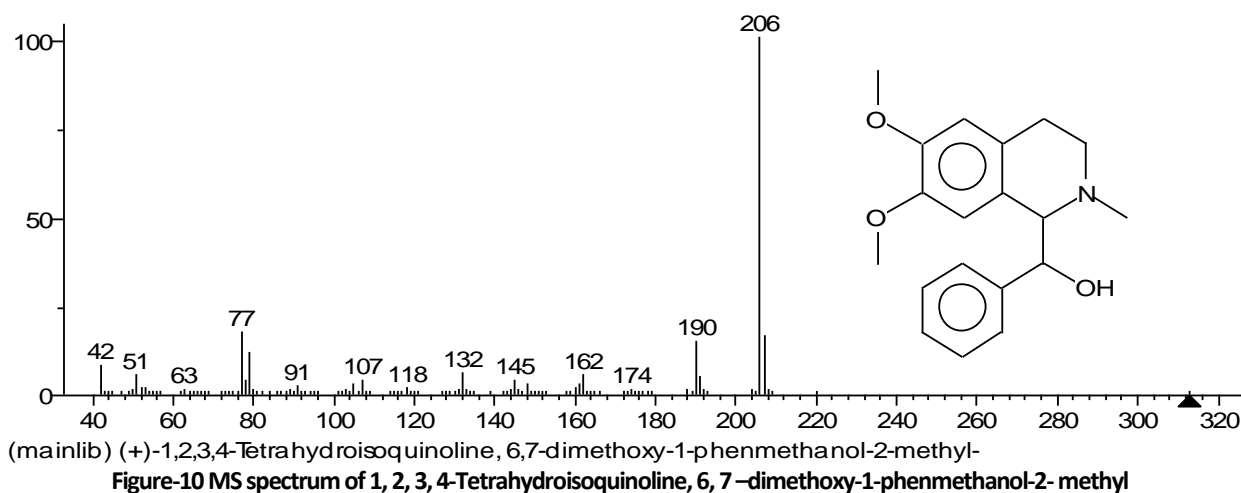


Figure-10 MS spectrum of 1, 2, 3, 4-Tetrahydroisoquinoline, 6, 7-dimethoxy-1-phenmethanol-2- methyl

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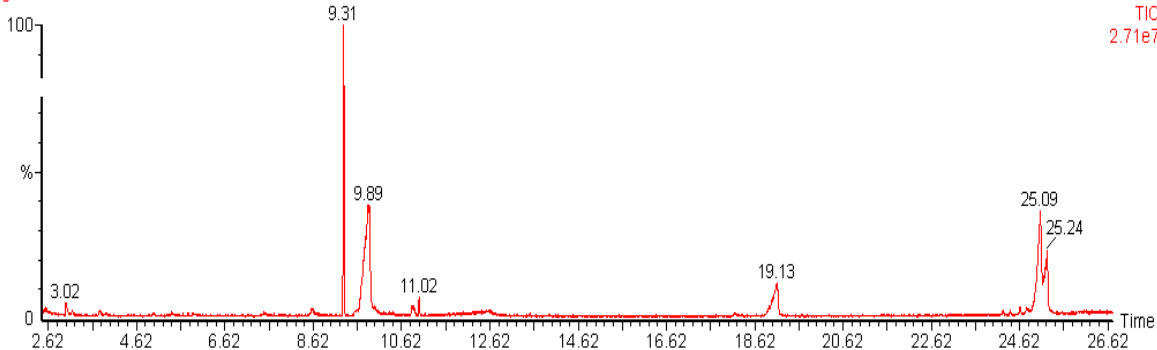


Figure-11GC-MS Spectrum of Aqueous extract of Zanthoxylum Tetraspermum wight&arn

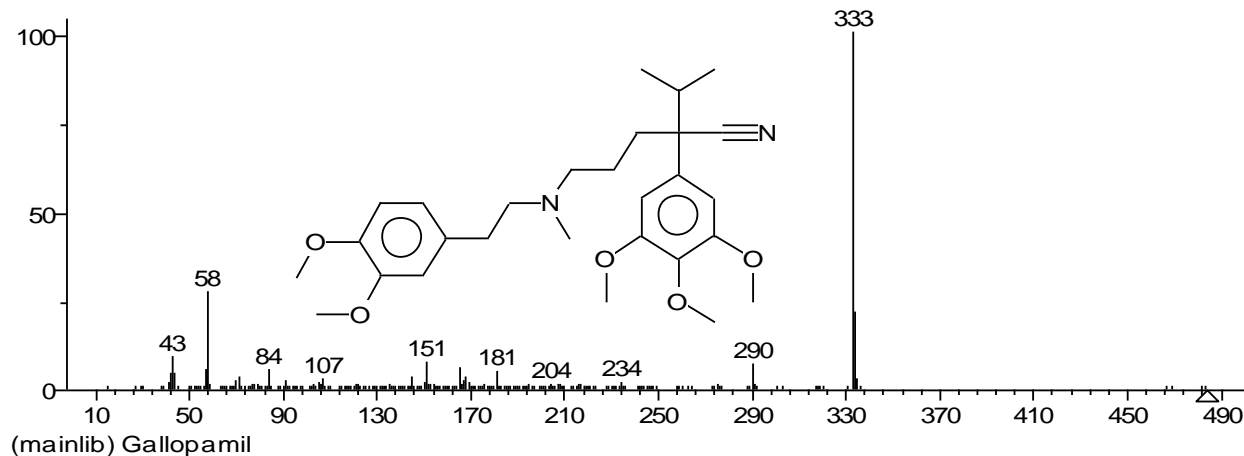


Figure-12 MS spectrum of Gallopamil

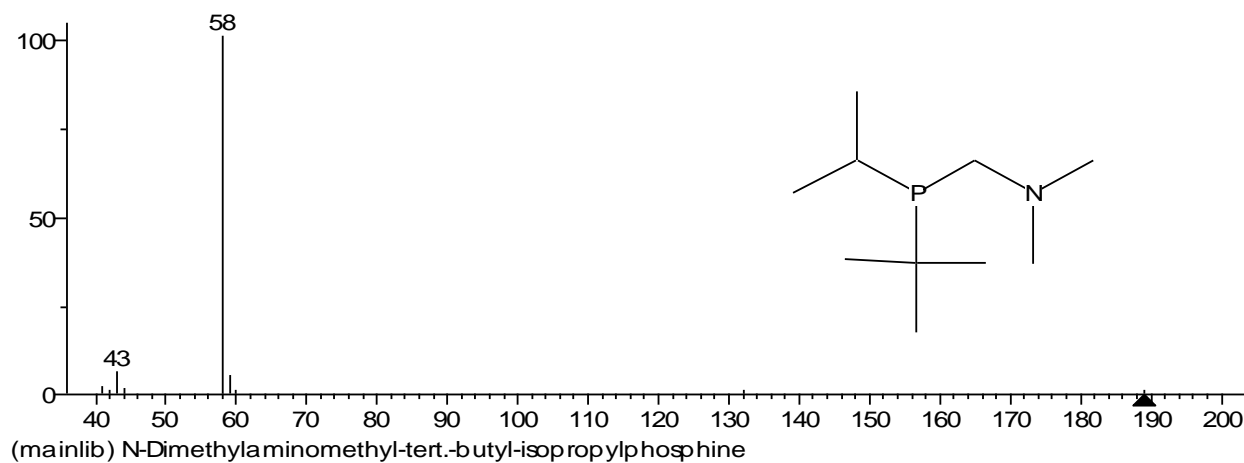


Figure-13 MS spectrum of N-Dimethylaminomethyl-tert-butyl-isopropylphosphine

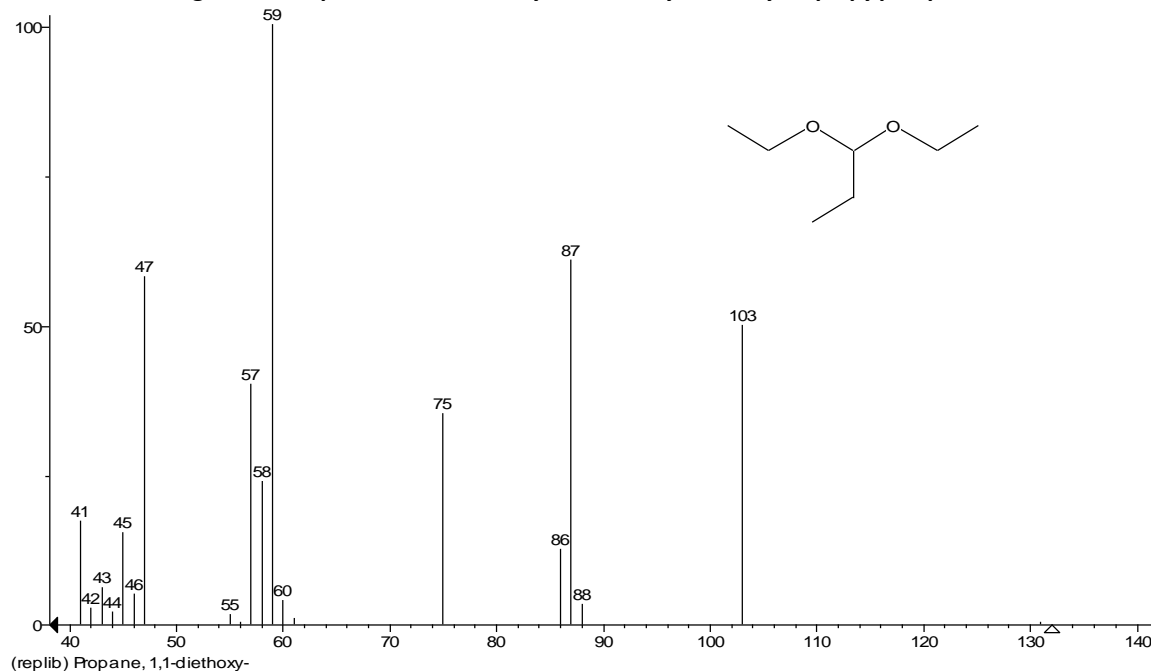


Figure-14 MS spectrum of propane, 1,1-diethoxy

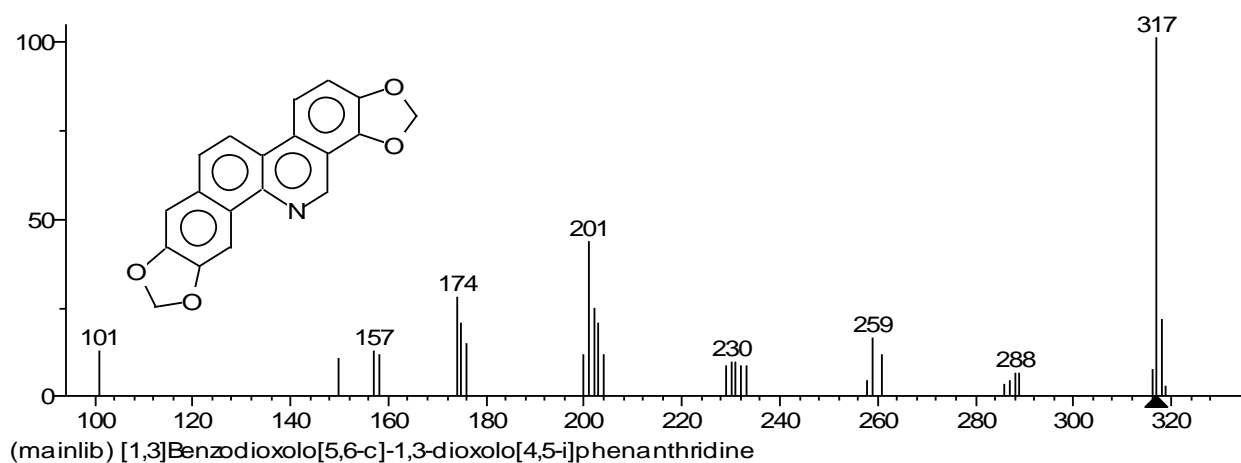


Figure-15 MS [1, 3] Benzodioxolo [5, 6-c]-1,3-dioxolo[4,5-i] phenanthridine

CONCLUSION

This study has revealed the presence of secondary metabolites of various therapeutical active compounds in the bark extract of *Zanthoxylum Tetraspermum* Wight & Arn. It has further confirmed that the bark extract could be used for the treatment of various dental infections. The results credence to the folkloric use, if this plant in treating (microbial infection) and shows that *Zanthoxylum Tetraspermum* Wight & Arn could be exploited for new potent dental care drugs.

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