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RESEARCH ARTICLE

ESSENTIAL OIL STUDIES OF THE GENUS *VITEX* L. (VERBENACEAE).

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Abstract

The present study documents the characterization of essential oils obtained from different species of the genus *Vitex* L. of the family Verbenaceae. The oils were analyzed by gas chromatography-mass spectrometry (GC-MS). The coeffiecient of similitude, percentage yield and percentage area of occurrence were calculated. The study showed the richness of volatile oils in the genus *Vitex.*

Introduction:-

The family Verbenaceaeis a conglomerate ofdicotyledonous plants with prolific applications in economic, medicinal, ethno-botanical and decorative fields. The family is distributed throughout tropical and sub-tropical regions and hardly seen outside tropical regions. *Callicarpa*, *Clerodendrum*, *Lantana*, *Lippia*, *Priva*, *Verbena* and *Vitex* are found almost anywhere. *Lantana*, *Lippia*, *Clerodendrum* and *Vitex* occur in the Asian and African regions. *Callicarpa, Clerodendrum, Gmelina, Premna, Tectona* and *Vitex* are abundant in South East Asia. *Stachytarpheta* flourishes in Brazil and *Citharexylum* and *Duranta* are found further north.

Plants offer a variety of flavors and fragrances which find applications in everyday life. Around 3000 plants contain essential oils of which only 10 percent are commercially important. Essential oils or volatile oils or ethereal oils are aromatic liquids obtained from plant sources, generally liquid at ordinary temperatures and volatile without decomposition (Parry, 1922; Sangwan *et al*.,2001; Ahmadi *et al*., 2002; Morone-Fortunato *et al*., 2010). They are stored in special structures in plants such as glands, secretory hairs, secretory ducts, secretory cavities etc. Plant families containing large amounts of essential oils include Annonaceae, Asteraceae, Apiaceae, Lamiaceae, Lauraceae, Liliaceae, Myrtaceae, Magnoliaceae, Pinaceae and Rutaceae. The content of essential oil is generally very low in plants and rarely exceeds 1% (Bowles, 2003).

Essential oils and their constituents find use for their therapeutic properties as well as in food, flavor, cosmetics and perfume industries. They serve as natural defense mechanisms against predation by microbes, insects and herbivores (Bakkali *et al*., 2008). The principal ingredients of essential oils includes mono and sesquiterpenes including carbohydrates, phenols, alcohols, ethers, aldehydes and ketones which are known for their aromatic and medicinal uses as well as for their redolence. Many of these constituents of essential oils have shown antibacterial, antifungal and antioxidant properties (Lee and Ahn, 1998).

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Materials And Methods:-

Essential oil extraction

The mature plants of *Vitex altissima*, *Vitex leucoxylon*, *Vitex negundo* var. *negundo*, and *Vitex trifolia* were selected for the essential oil extraction. Fresh leaves were collected, dried in shade, cut into small pieces and subjected to hydro-distillation in Clevenger apparatus at 100ºC for 4h (Clevenger, 1928). The distillate was extracted with diethyl ether, dried with anhydrous sodium sulphate and ether was removed by evaporation at room temperature. The quantity of the essential oil was measured and it was transferred into small amber coloured bottles covered with black paper and stored at 4ºC. The percentage of oil was calculated on a dry weight basis.

Gas chromatography- mass spectrometry (GC-MS) analysis

The volatiles of the different plant parts were analyzed by the hyphenated system, GC-MS.The Gas chromatography-Mass Spectrometry (GC-MS) analyses were performed on a Hewlett Packard (HP) 6890 GC interfaced with a Hewlett Packard 5973 Mass Selective Detector (MSD) system operating at 70 eV and 250^{o}C, equipped with a split-less injector. A cross-linked 5% phenyl methyl siloxane column (HP-5), with 320 µm x 30 m and 0.25 µm film thickness, was utilized. Helium was used as the carrier gas at a flow rate of 1.4 ml/min. The temperature program for the HP-5 column was set at 60°C - 243°C, at a rate of 3°C/min. Run time was 61min. Identification of individual components was done using the NIST MS Search literature.

Chemotaxonomic evaluation

The data obtained from the qualitative analysis were subjected to numerical analysis to understand the chemical affinity among the different parts of the plant by arriving at a numerical constant, the coefficient of similitude (CS) using the following formula proposed by Sneath and Sokal (1973).

$$CS = \frac{\text{Number of similar compounds}}{\text{Total number of components}} \text{ x } 100$$

Results:-

Using a conventional hydro-distillation process with a Clevenger apparatus, the leaves of *Vitex trifolia*, *Vitex altissima*, *Vitex leucoxylon* and *Vitex negundo* var. *negundo* yielded mobile, fragrant, pale yellow to light brown oil with pungent spicy fragrance.

Screening for desirable qualities like essential oil yield and composition becomes significant during characterization of essential oils. The essential oil isolated by hydro-distillation of leaves of different species of *Vitex* showed difference in yield based on the dry weight of the sample while the percentage of essential oil obtained also varied. The percentage yield of essential oil was found to be 0.7 (v/w) for *Vitex leucoxylon*, 0.4 (v/w) for *Vitex altissima*, 0.6 (v/w) for *Vitex trifolia* and 0.9 (v/w) for *Vitex negundo* var. *negundo*. The GC-MS pattern was distinctly different in all cases. The area percentage occurrence of the components in the leaf essential oil of selected *Vitex* sp. are also given. Number of compounds identified in *Vitex trifolia* is 16, *Vitex altissima* is 15, *Vitex leucoxylon* is 10 and *Vitex negundo* var. *negundo* is 9. The components identified by GC-MS analysis with their percentage composition are listed (Table 1, 2, 3 & 4).

The samples of the oil varied in colour from pale yellow to light brown. *Vitex trifolia* leaf essential oil was pale yellow, *Vitex altissima* leaf essential oil was brown, *Vitex leucoxylon* leaf essential oil was dark yellow and *Vitex negundo* var. *negundo* leaf essential oil was light brown in colour. The analysis of the oil samples showed a wide range of variation in their constituents.

The components which showed the most abundant percentage area were caryophyllene (38.36%) and eucalyptol (25.72%) in *Vitex trifolia*, (+)-epi-bicyclosesquiphellandrene (23.07%), caryophyllene (17.41%) and alpha cedrene (16.21%) in *Vitex altissima*, resorcinol (45.14%) in *Vitex leucoxylon* and isoaromadendrene epoxide (40.85%) and caryophyllene (23.78%) in *Vitex negundo* var. *negundo*. Alpha-terineol acetate (9.60%), Alpha Pinene (7.85%), Copaene (7.71%), 3-Cyclohexen-1-ol,4-5-methyl-1-(1-methyl ethyl)- (12.79%), Beta-phellandrene (8.21%), Cis-alpha-bisabolene (10.02%) and Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-,[S-(R*,S*)- (13.12%) were also present in four species of *Vitex*. Caryophyllene is present in all the species of *Vitex* with marked variation in the area percentage i.e., 38.36% in *Vitex trifolia*, 17.41% in *Vitex altissima*, 6.13% in *Vitex leucoxylon* and 23.78% in *Vitex negundo* var. *negundo*. In the present study, GC-MS analyses revealed minimum 9 components in each sample. The total number of chemical components detected by GC-MS in all the plant parts was found to be 39. A comparative account of essential oil compounds present in selected *Vitex* sp. is given in Table 5. The coefficient of similitude between the leaf essential oils was found to be 2.56%. The high terpenoid content in *Vitex* sp. is significant for further medicinal applications.

Table 1:-Chemical composition of leaf essential oil of *Vitex trifolia* (VT)

Sl. No.	Retention time	Compound	Percentage area of occurrence
1	4.094	1R alpha Pinene	1.24
2	4.215	Alpha Pinene	7.85
3	6.888	Eucalyptol	25.72
4	7.495	Gamma-terpinene	1.93
5	8.398	(+)-4-Carene	0.50
6	11.827	(-)-Terpinen-4-ol	4.49
7	18.876	Alpha-terpineol acetate	9.60
8	20.032	Beta-damascenone	0.47
9	21.943	Caryophyllene	38.36
10	22.771	Alpha-caryophyllene	2.10
11	23.853	Copaene	3.41
12	24.509	Alpha-muurolene	0.33
13	27.094	Trans-nerolidol	0.19
14	27.637	Caryophyllene oxide	1.52
15	29.846	Tau-muurolol	0.88
16	30.347	Alpha-cadinol	1.33

Table 2:-Chemical composition of leaf essential oil of *Vitex altissima* (VA)

Sl. No.	Retention time	Compound	Percentage area of occurrence
1	18.416	Alpha-cubebane	0.61
2	19.460	Copaene	7.71
3	19.786	Alpha-Bourbonene	0.74
4	21.253	Caryophyllene	17.41
5	22.506	Alpha-Caryophyllene	1.22
6	23.101	Alpha-Cedrene	16.21
7	23.769	(+)-epi-bicyclosesquiphellandrene	23.07
8	24.924	Cis-alpha-bisabolene	10.02
9	25.050	Cyclohexene, 1 methyl 1-4-(5-methyl-1-methylene-4-hexenyl)-,(S)-	3.20
10	25.579	Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-,[S-(R*,S*)-	13.12
11	27.021	Nerolidol 2	0.80
12	27.372	(-)-Spathulenol	0.77
13	27.875	Isoaromadendrene epoxide	1.71
14	29.744	Tau-cadinol	1.92
15	36.967	2-pentadecanone,6,10,14-trimethyl	1.31

Table 3:-Chemical composition of leaf essential oil of *Vitex leucoxylon* (VL)

Sl. No.	RT	Compound	Percentage area of occurrence
1	3.954	p-benzoquinone	8.24
2	17.616	Resorcinol	45.14
3	21.143	Caryophyllene	6.13
4	24.285	1,3-Cyclohexadiene,5-(1,5-dimethyl-4-hexenyl)	8.75
5	24.577	Cis-alpha-bisabolene	4.55

6	25.329	Cedrene	6.02
7	27.292	(-)-spathulenol	4.81
8	30.142	1-naphthalenol,decahydro-1,4a-dimethyl-7-1	6.46
9	36.941	2-pentadecanone,6,10,14 trimethyl-	4.87
10	45.297	Phytol	4.98

Table 4:-Chemical composition of leaf essential oil of *Vitex negundo* var. *negundo* (VN)

Sl. No.	Retention time	Compound	Percentage area of occurrence
1	4.962	Beta-phellandrene	8.21
2	5.973	(+)-4-Carene	1.23
3	7.182	1,4-Cyclohexadiene,1-methyl-4-(1-methylethyl-	4.45
4	11.470	3-Cyclohexen-1-ol,4-5-methyl-1-(1-methyl ethyl)-	12.79
5	11.825	3-Cyclohexene-1-methanol,.alpha.,alpha.4-trimethyl	1.04
6	20.989	Caryophyllene	23.78
7	22.507	1,6,10-Dodecatriene,7,11-dimethyl-3-methylene-(E)-	5.35
8	27.036	Caryophyllene oxide	2.25
9	27.875	Isoaromadendrene epoxide	40.85

Table 5:-Comparative account of chemical composition of leaf essential oils of *Vitex* sp.

Sl. No.	Compound	VT (%)	VA (%)	VL (%)	VN (%)
1.	Caryophyllene	38.36	17.41	6.13	23.78
2.	Eucalyptol	25.72	-	-	-
3.	Alpha-terpineol acetate	9.60	-	-	-
4.	Alpha Pinene	7.85	-	-	-
5.	(-)-Terpinen-4-ol	4.49	-	-	-
6.	Copaene	3.41	7.71	-	-
7.	Alpha-caryophyllene	2.10	1.22	-	-
8.	Gamma-terpinene	1.93	-	-	-
9.	Caryophyllene oxide	1.52	-	-	2.25
10.	Alpha-cadinol	1.33	-	-	-
11.	1R alpha Pinene	1.24	-	-	-
12.	Tau-muurolol	0.88	-	-	-
13.	(+)-4-Carene	0.50	-	-	1.23
14.	Beta-damascenone	0.47	-	-	-
15.	Alpha-muurolene	0.33	-	-	-
16.	Trans-nerolidol	0.19		-	
17.	Isoaromadendrene epoxide	-	1.71	-	40.85
18.	3-Cyclohexen-1-ol,4-5-methyl-1-(1-methyl ethyl)-	-	-	-	12.79
19.	Beta-phellandrene	-	-	-	8.21
20.	1,6,10-Dodecatriene,7,11-dimethyl-3-methylene-(E)-	-	-	-	5.35
21.	1,4-Cyclohexadiene,1-methyl-4-(1-methylethyl-	-	-	-	4.45
22.	3-Cyclohexene-1-methanol,.alpha.,alpha.4-trimethyl	-	-	-	1.04
23.	Resorcinol	-	-	45.14	-
Sl.No.	**Compound**	**VT (%)**	**VA(%)**	**VL(%)**	**VN(%)**
24.	1,3-Cyclohexadiene,5-(1,5-dimethyl-4-hexenyl)	-	-	8.75	-
25.	p-benzoquinone		-	8.24	-
26.	1-naphthalenol,decahydro-1,4a-dimethyl-7-1	-	-	6.46	-
27.	Cedrene	-	-	6.02	-

28.	Phytol	-	-	4.98	-
29.	2-pentadecanone,6,10,14 trimethyl-	-	1.31	4.87	-
30.	Cis-alpha-bisabolene	-	10.02	4.55	-
31.	(+)-epi-bicyclosesquiphellandrene	-	23.07	-	-
32.	Alpha-Cedrene	-	16.21	-	-
33.	Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-,[S-(R*,S*)-	-	13.12	-	-
34.	Cyclohexene, 1 methyl 1-4-(5-methyl-1-methylene-4-hexenyl)-,(S)-	-	3.20	-	-
35.	Tau-cadinol	-	1.92	-	-
36.	Nerolidol 2	-	0.80	-	-
37.	(-)-Spathulenol	-	0.77	4.81	-
38.	Alpha-Bourbonene	-	0.74	-	-
39.	Alpha-cubebane	-	0.61	-	-

Discussion:-

The complex make-up of the essential oils and the variety of chemical structures of their constituents with a wide range of biological activities create compounding interest in the areas of human and animal health. Many essential oils and their components have traditionally been used for their antimicrobial properties which have long been acknowledged (Cheng *et al*., 2009). The insecticidal (Ayvaz *et al*., 2010); antibacterial (Orhan *et al*., 2011); antiviral (Orhan *et al*., 2012); antimycotic (Mota *et al.,* 2012) and antiparasitic (Pillai *et al.,* 2012) properties of essential oils are staggeringly investigated. The application of essential oils in medicine, pharmacology, phytopathology, treating diseases, and food preservation are well established globally. Moreover, essential oils are employed as raw materials for perfumes, cosmetics, aromatherapy, phytotherapy and nutrition too (Buchbauer, 2000).

The results of the present study show difference in chemical composition in four selected species of *Vitex*. The detailed chemical composition of the leaf essential oils of *Vitex* sp. showed the presence of 39 compounds with varying percentage area of occurrence. In *Vitex trifolia* the major component caryophyllene (38.36%) was followed by eucalyptol (25.72%). The chemical composition of the essential oil obtained from *Vitex trifolia* conform to the findings of Suksamrarn *et al*. (1991). In both studies, caryophyllene had been reported as the major sesquiterpene component. However the main monoterpene component eucalyptol was different to previous reports. Alpha-terpineol acetate and alpha pinene were present in chromatographic profiles of both studies. Caryophyllene, eucalyptol and alpha-pinene possess significant anti-inflammatory and analgesic activity. Xin *et al*. (2006) reported the anti-inflammatory and analgesic activity from the same species. Eucalyptol can be used in the treatment of human leukemia HL-60 cells and the treated cells showed fragmentation of DNA indicating an induction of apoptosis (Moteki *et al*., 2002). The studies of Calcabrini *et al.* (2004) and Takahashi *et al.* (2003) established the anticancerous and antioxidant potential of terpinen-4-ol and gamma-terpinen components. Alpha-copaene (25.26%) was the predominant constituent of *Vitex negundo* leaf essential oil as reported by Singh *et al.* (2010) and the copaene content in the present analysis of *Vitex trifolia* was found to be 3.41%.

The chief components in *Vitex altissima* were (+)-epi-bicyclosesquiphellandrene (23.07%), caryophyllene (17.41%) and alpha cedrene (16.21%). The antifungal and antibacterial activity of (+)-epi-bicyclosesquiphellandrene was presented by Cheng *et al.* (2005) and Skaltsa *et al.* (2003).

Resorcinol was the major component in leaf essential oil of *Vitex leucoxylon* with an area percentage of 45.14%.. Shukla *et al.* (2010) reported hypoglycemic, anti-inflammatory and antipyretic activity of extracts of *Vitex leucoxylon*. A copolymer synthesized from resorcinol and melamine was tested for its inhibitory action against pathogenic bacteria and fungi (Rahangdale, 2012). The chemical composition of the essential oil of leaf isolated from *Vitex negundo* (Taneja *et al*., 1979) and other *Vitex* species have been reported by several researchers in the past (Jirovetz *et al.*, 1998). The studies of Feng *et al.* (1999) provided caryophyllene (33.01%), eucalyptol (13.3%), alloaromadendrene (6.58%) and beta-farnesene as major constituents in the leaf essential oil of *Vitex negundo*. The result of the present study presented isoaromadendrene epoxide (40.85%) and caryophyllene (23.78%) as major compounds in leaf essential oil of *Vitex negundo* var. *negundo.*

The other important compounds present in significant quantities in four species of *Vitex* include alpha-terpineol acetate (9.60%), alpha Pinene (7.85%), copaene (7.71%), 3-cyclohexen-1-ol,4-5-methyl-1-(1-methyl ethyl)-(12.79%), beta-phellandrene (8.21%), cis-alpha-bisabolene (10.02%) and cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-,[S-(R*,S*)- (13.12%). Considering the samples as a whole, caryophyllene was the only common compound that was present in all species of *Vitex*. But, there is noted variation in the percentage of occurrence of caryophyllene in various *Vitex* species; 38.36% in *Vitex trifolia*, 17.41% in *Vitex altissima*, 6.13% in *Vitex leucoxylon* and 23.78% in *Vitex negundo* var. *negundo*. The environmental factors such as temperature, luminosity, soil, etc. play a key role which results in variation of the chemical compounds present in the essential oils extracted from the plants of different geographic origins (Mello and Silva-Filho, 2002).

The demand of novel anti-infective agents has led to the discovery of new sources of potential antimicrobials (Carson and Riley, 2003). Among them, the plants offer a wide range of molecules of immense value for the pharmaceutical industry. The results of the present study showed that the tested *Vitex* species contain higher percentage of some different constituents of essential oil and these can be applied to many therapeutic, perfumery and flavoring functions.

Conclusion:-

Essential oils are volatile aromatic compounds isolated from plants and the pharmaceutical properties of aromatic plants are partially attributed to essential oils. They are considered as the 'chemical weapons' since their compounds may have insect deterrent and antimicrobial properties. They are also regarded as 'plant pheromones' and oxygenated molecules of essential oils can serve as chemical messengers for cells. The present study was undertaken to examine the chemical composition of essential oil isolated from the leaves of *Vitex* species.

The results of the present study indicate that the selected *Vitex* species are capable of producing essential oils with variable antifungal activity. The essential oils of different species of *Vitex* showed difference in yield that varied from 0.4-0.9 (v/w). *Vitex negundo* var. *negundo* has the best essential oil yield of 0.9 (v/w). The total number of compounds detected in the essential oil from the leaves in four species of *Vitex* was 39 with a minimum of 9 components in each sample. Maximum number of compounds was found in *Vitex trifolia* while minimum in *Vitex negundo* var. *negundo*. The essential oil of the genus *Vitex* contains mostly sesquiterpenes. Along with that the essential oil also contains monoterpenes, alcohols and small amount of ketones.

The main components in the essential oil of *Vitex* species as established by GC-MS analysis were caryophyllene (*Vitex trifolia*), (+)-epi-bicyclosesquiphellandrene (*Vitex altissima*), Resorcinol (*Vitex leucoxylon*) and isoaromadendrene epoxide (*Vitex negundo* var. *negundo*). Caryophyllene was present as a common compound in all the species of *Vitex* with an area percentage range of 6.13% in *Vitex leucoxylon* and 38.36% in *Vitex trifolia*. The coefficient of similitude was found to be 2.56%. The chemical composition of all the species indicates that a number of chemicals are present in large percentage, which may find potential use in the pharmaceutical industry.

The type and composition of essential oil, yield and bioavailability and the concentration required to attain efficacy are all aspects that need to be considered for use as a therapeutic molecule. More efforts are necessary for an in vivo study of their single or combinational use as an alternative to antibiotics. Essential oils can also play an important role in determining and solving the taxonomic ambiguities involved in chemo-taxonomic classifications.

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