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Effect of Anti Elastase Activity and GC-MS Analysis of Different Leaf Extracts of *Lantana camara*

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A B S T R A C T

Traditional medicine is used to cure many diseases. Recent remedy has advanced after natural plant as a source of drugs. The present study is to evaluate the preliminary work of anti-elastase activity and GC MS analysis of methanolic leaf extract of *Lantana camara.* The results showed that the clearly demonstrated that methanolic leaf extract of *Lanatana camara* exhibit more elastase inhibition property when compared to control, aqueous and diethyl ether extracts. In GC MS analysis showed the methanolic leaf extract of *Lanatana camara* have identified 24 bioactive compounds. Out of 24 compounds showed only two compounds of MELcL showed highest peaks value (i) Stigmasterol (17.13%), (ii) Heptadecene-(8)-Carbonic acid-(1) (14.53%), were respectively. Based on these results these beneficial leaf extract of *Lanatana camara* were identified for its potential application as an anti-aging agent in skin creams as well as an anti-proliferation compound against tumor cells. In future, the isolation of above-mentioned bioactive compounds from the methanolic leaf extract of *Lantana camara* would be useful to find out the novel drugs.

***Key words*:** Medicinal plants, Anti elastase, Methanolic leaf extract, *Lantana camara*, GC- MS, Novel drugs

Traditional Medicine is rich source of organic composites indicates are alkaloids, tannins, saponins, steroids and phenolic compounds. These secondary metabolites having many biological properties and major function of developing of new drugs against degenerative diseases namely cancer, cardiovascular and Alzheimer's disease. The Skin aging is generally categorized by wrinkle formation, uneven pigmentation, darkening, thinning, sagging and roughening of skin and they are two mechanisms namely intrinsic or extrinsic skin aging [1]. The Intrinsic skin aging is occurring as age progresses and inevitable. It is due to cumulative effects of oxidative stress and free radicals are produced during normal cellular metabolism and are needed for normal biological functions [2].

However, increase quantities of Reactive Oxidative stress include hydrogen peroxide (H_2O_2) superoxide (O_2-) and also peroxynitrite radicals cause by oxidative damages in skin like DNA, RNA and lipids. Extrinsic skin aging predominantly occurs due to environmental factors such as UV irradiation, physical stress, nutritional deficiency and alcohol consumption. GC - MS is method of analytical combines with the features of Gas chromatography and Mass spectrometry are used to categorize substances present in the leaf plant extract [3-4]. The *Lantana* is a *Verbenaceae* family native to the South America and Ethiopia. *Lantana* is generally native place subtropical and tropical America, Asia and Africa. The stems and branches are sometimes thorny. The leaf is arranged in oval shaped. Flowers are berry like drupe and maturation time flowers turns from green to dark purple. The fruits are clusters, shiny and globose in shape. Despite the many uses of *Lantana camara* there is no data showing the chemical composition of its methanol extract [5-6]. Hence, the present study was investigated the effect of anti-elastase activity and GC MS analysis of different leaf extract of *Lantana camara.*

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MATERIALS AND METHODS

Chemicals

All the chemicals used in the study were purchased from Merck & Co., India.

Preparation of plant particles

The leaves of *Lantana camara* have been collected from Koodapattu, Tirupattur, Tamil Nadu and India. The composed leaves were washed through distilled water, shade dried, finely powdered. The plant authentication (Reg. no of Certificate no: 4133) Institute of Herbal Botany, Plant Anatomy Research Centre, Presidency college, Chennai. The plate 1 shows the plant of *Lantana camara*.

Plate 1 *Lantana camara*

Methods of extraction

10g of *Lantana camara* leaf powder + 100 ml of Methanol (ME), 100 ml of Diethyl et her (DE) and 100 ml of Aqueous (AQ) using Soxhlet apparatus. Extraction was considered to be completed when the Leaves materials become exhausted of their constituents that were confirmed. The filtrates obtained are dried at temperature of 40±2°C to have gummy concentrate of the crude extract. Finally, this extract kept in a suitable vessel with labeling and stored used for further studies.

Inhibition of anti-elastase activity

The determination of human neutrophil elastase activity was performed according to Löser *et al.* [7]. Briefly, 25µl substrate solution (1.4mM N-MeO-Suc-AlaAla-Pro-Val-p-NA in Tris-HCl-buffer, pH 7.5) was added to 25µl Tris-HCl-buffer, pH 7.5, with 25µl different leaf extraction of *Lanatana camara* solution. After the addition of 25 µl enzyme solution the samples were vortexed and then incubated for 1 h. at 37°C. The reaction was stopped by addition of 25µl soybean trypsin inhibitor solution (0.2mg/ml Tris-HCl-buffer, pH 7.5). The samples were then vortexed and the absorbance was measured at 405nm. The inhibitory effect of the selective HNE inhibitor GW 311616A (IC_{50} =0.04 µg/ml) that was considered as a positive control. Anti-elastase activity of the different leaf extraction was calculated using the formula:

$$\text{Percentage inhibition} = \frac{\text{Absorbance control} - \text{Absorbance sample}}{\text{Absorbance control}} \times 100$$

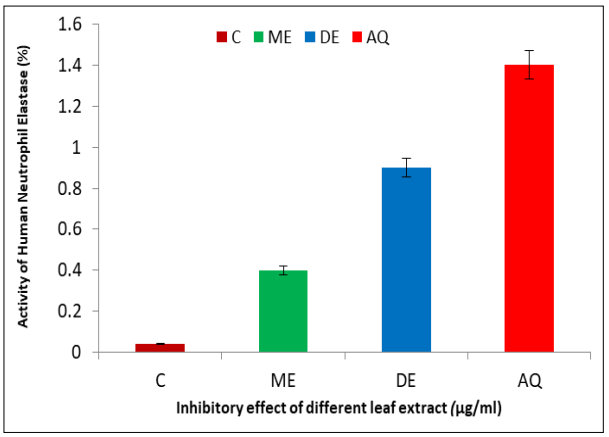
GC-MS analysis

The MELcL sample usually evaluated the occurrence of phytocompounds by Gas chromatograph (GC) Mass spectrometer (MS). The profile is identified by Bishop Heber College, Heber Analytical instrumentation Facility, Tiruchirappalli, India. GC-MS techniques is a thermo trace with a fused silica capillary (DB-5MS) column length Thirty meters, diameter 0.25mm (outside and inside), ultra-equipped and interfaced to a Mass Selective Detector with XCALIBUR software. Gas chromatograph (GC) Mass spectrometer (MS) are usually electron construction with ionization dynamism of Helium gas (-70eVoltage) and flow rate (1ml/min), sample 2µl, temperature 80° to 200°C, isothermal (1min finally) temperature was kept at 250ºC and run time at 40 minutes [8]. The Phytoconsituents was pointed out as percentage (%) with peak area and Identification extract of the compounds was consigned by the comparison of retention time with mass spectra fragmentation patterns in NIST library were respectively.

RESULTS AND DISCUSSION

Anti-elastase activity of various leaf extract of Lantana camara

The methanolic leaf extract of *Lantana camara* have different polarities like methanol (ME), diethyl ether (DE), and aqueous (AQ) extracts screened at concentrations range between 0.1 to 100 µg/ml for their inhibitory effect against the human neutrophil elastase activity. The (Fig 1) shows the activity of Human Neutrophil Elastase various leaf extract of *Lantana camara* as below:



Indicates C- Control; ME- Methanol; DE – Diethyl ether; AQ- Aqueous

Fig 1 Anti Elastase activity of various leaf extract of *Lantana camara*

Among the HNE inhibitory methanolic leaf extract of *Lantana camara* showed maximum active inhibitors with IC_{50} value as 0.4µg/ml when compared diethyl ether (0.9µg/ml), aqueous (1.4 µg/ml) and control HNE inhibitor GW 311616A (0.04 µg/ml) were respectively.

The *in vitro* HNE inhibitions have indicated that among a number of poly phenolic compounds of several medicinal plants used in the cure of inflammation. Because phenolic constituents like flavonoids and caffeic acid esters occur throughout the medicinal plants and also measured HNE inhibitory activity of the tested leaf extracts might be partially attributed to the phenolic compounds [9]. The Human Neutrophil elatase also capable of degrading assortment of soluble proteins like coagulation factors, immunoglobulins, complement and many protease inhibitors [10]. The Human Neutrophil elatase are response to numerous inflammatory activities. Despite this balance between HNE and proteinase inhibitors activities including many human diseases like acute lung injury, fibrosis, ischemic reperfusion injury, rheumatoid arthritis, atherosclerosis and malignant tumors [11].

GC MS analysis of methanolic leaf extract of Lantana camara

The bioactive compounds presences of methanolic leaf extract of *Lantana camara* (MELcL) were identified by Gas chromatograph (GC) Mass spectrometer (MS) Profile (Fig 2).

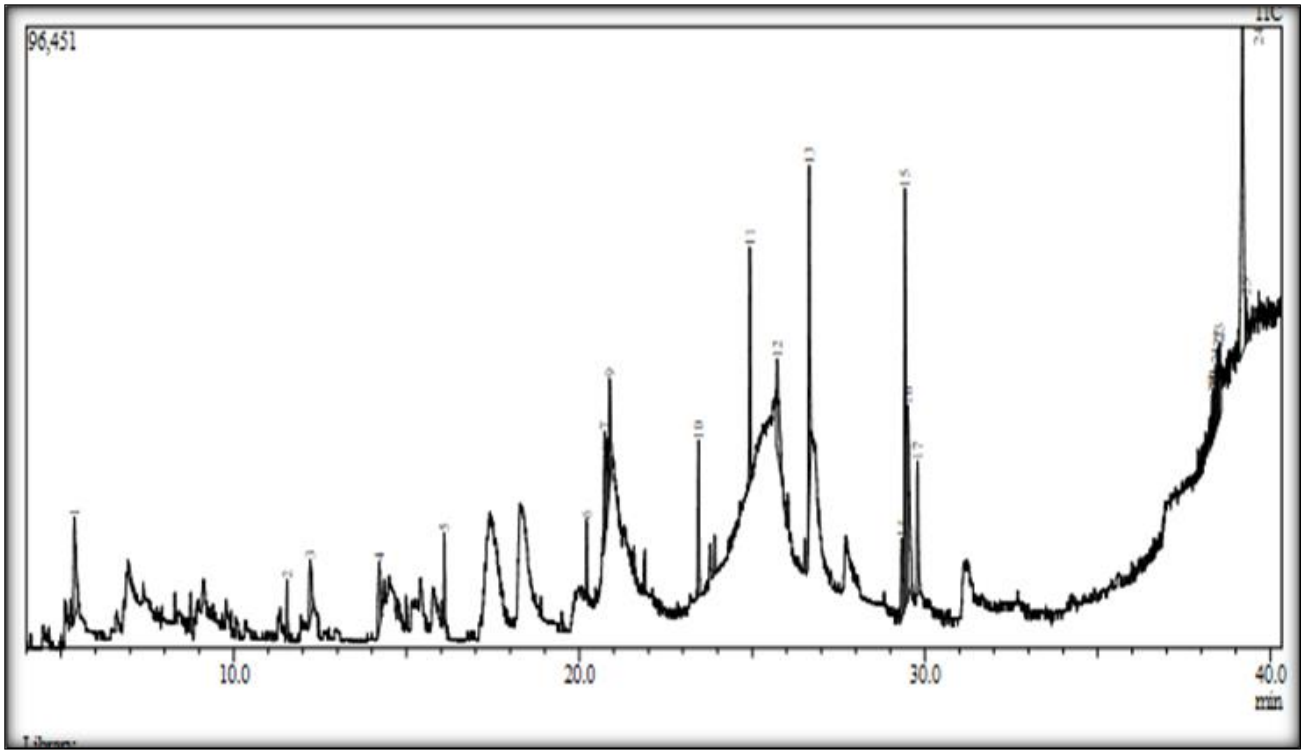


Fig 2 Mass spectrum of MELcL

Table 1 The phytocompounds of MELcL by GC-MS analysis

Retention time	Phytocompounds	Molecular formula	Molecular weight	Peak area (%)
5.38	'2-Cyclopenten-1-one, 2-hydroxy - 2 hydro	$C_5H_6O_2$	98.1	4.66
11.54	Butane, 2,2-dimethyl-	C_6H_{14}	86.81	1.24
12.21	4-vinylphenol	C_8H_8O	120.15	2.81
14.20	4,6-bis(4-ethoxybenzylthio)-5-nitropyrimidine	$C_{22}H_{23}N_3O_4S_2$	457.6	2.64
20.21	Octane	C_8H_{18}	114.23	1.61
20.73	1,1'-bibicyclo[2.1.1]hexane, 4-iodo-	$C_{18}H_{18}$	234.3	2.9
20.79	4-methyl-2-oxotetrahydro-2h-pyran acetate	$C_{11}H_{20}O_3$	200.27	2.89
20.88	Dl-menthol	$C_{10}H_{20}O$	156.27	3.55
23.45	'2(4h)-benzofuranone, 5,6,7, -tetrahydro-6-hydroxy	$C_{11}H_{16}O_3$	196.24	3.25
24.93	'1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester'	$C_{16}H_{22}O_4$	278.34	5.05
25.72	'2h-1,3,2-oxazaphosphorin-2-amine,n-(2-chloroethyl)	$C_7H_{15}Cl_2N_2O_2P$	261.08	4.44
26.65	Decanoic Acid	$CH_3(CH_2)_8COOH$	172.26	8.42
29.33	5-Tetradecen-1-Ol, Acetate, (Z)-	$C_{16}H_{30}O_2$	254.41	2.21
29.42	Heptadecene-(8)-Carbonic Acid-(1)	$C_{17}H_{32}O_2$	268.43	14.53
29.50	1-Tetradecanol	$C_{14}H_{30}O$	214.39	8.85
29.78	Hexadecanoic Acid	$C_{16}H_{32}O_2$	256.43	3.47
38.31	Tetracyclo 1,3 - Undecane	$C_{11}H_{16}$	148.24	0.58
38.34	Cyclohexene, 6-Methyl-1-(1-Methylethenyl)	$C_{15}H_{24}$	204.35	0.82
38.38	1,3,6,10-Dodecatetraene, 3,7,11-Trimethyl	$C_{15}H_{24}$	204.35	0.72
38.42	Methanesulfonic acid, trifluoro-, 2-phenyl-	$C_{14}H_{18}ClF_3N_2O_4S$	402.8	2.07
38.48	Succinic acid, 2-chloro-6-fluorophenyl 4	$C_{19}H_{18}ClFO_4$	364.8	2.05
38.52	Cyanophenyl p-(2-methylbutoxy) benzoate	$C_{19}H_{19}NO_3$	309.4	2.10
39.19	Stigmasterol	$C_{29}H_{48}O$	412.69	17.13
39.29	Chromium(5-cyclopentadienyl)-6-toluene	$C_{12}H_{13}Cr^-$	209.23	0.33

Totally twenty-four bioactive compounds were distinguished in the MELcL. The (Table 1) showed the bioactive compounds are compared with NIST libraries. Totally twenty four bioactive compounds namely Stigmasterol (17.13%), Heptadecene-(8)-Carbonic acid-(1) (14.53%), 1-Tetradecanol (8.85%), Decanoic acid (8.42%), 1,2-Benzenedicarboxylic acid, (2-methylpropyl) ester (5.05%), 2-Cyclopenten-1-one, 2-hydroxy-(4.66%), '2h-1,3,2-oxazaphosphorin'-2-amine, (2-'chloroethyl') tetrahydro-, 2-oxide, (R)- (4.44%), dl-Menthol (3.55%), Hexadecanoic acid (3.42%), 2(4h)-'Benzo - furanone', 5, 6, -'tetrahydro'-6-hydroxy-4,4,7 a-trimethyl- (6S-cis)-9 (3.25%), 1,1'-Bibicyclo [2.1.1] hexane, 4-iodo- (2.9%). 4-Methyl-2-oxotetrahydro-2h-pyran-4-yl acetate (2.89%), 4-Vinylphenol (2.81%), 4,6-bis(4-ethoxybenzylthio)-5-nitropyrimidine (2.64%), 5-Tetradecen-1-ol, acetate, (z)- (2.21%), p-Cyanophenyl p-(2-methylbutoxy) benzoate (2.1%) methanesulfonic acid, trifluoro-, 2-phenyl-1-

propenyl ester, (e)-(2.07%), Succinic acid, 2-Chloro-6-fluorophenyl methoxybenzyl ester (2.05%), Octane (1.68%), butane, 2,2-dimethyl-(1.24%), Cyclohexene,6-methyl-1-(1methylethenyl)-6-(1-methylethyl)-(0.82%), Dodecatetraene,3,7,11-trimethyl (0.72%), Tetracyclo 1,3-Undecane (0.58%) and Chromium(5-cyclopentadienyl)-6-toluene (0.33%) were respectively.

Out of 24 compounds showed only two compounds of MELcL showed highest peaks area namely Stigmasterol (17.13%), Heptadecene-(8)-Carbonic acid-(1) (14.53%), were respectively.

GC-MS showed increasingly valuable identification of hytrophilic and hydrophobic (non-polar and polar) compounds, Volatile oil, FA and lipids [12]. Stigmasterol is otherwise called as Wulzen anti-stiffness factor and it is unsaturated physterol available in different type of plant [13]. A number of chemical processes and utilized to organic compounds for pharmaceutical industry. Stigmasterol is a precursor in the synthesis of progesterone and biosynthesis of hormones and vitamins [14]. The phytocompounds of Heptadecene-(8)-Carbonic Acid-(1) revealed that the uses of various diseases like Respiratory failure, diabetics, diarrhea and edema [15].

CONCLUSION

It can be concluded that the Anti elatase activity of methanolic leaf extract of *Lantana camara* showed maximum active inhibition when compared to diethyl ether, aqueous and control HNE inhibitor were respectively. In GC MS analysis identified 24 bioactive compounds of methanolic leaf extract of *Lantana camara* (MELcL). Out of these twenty-four compounds, only two compounds have maximum peaks percentage like Stigmasterol (17.13%) and Heptadecene-(8)-Carbonic acid-(1) (14.53%) of MELcL may be recommended as various human ailments like skin diseases, diabetes, cancer, cardiovascular diseases and Alzheimer's diseases. In future recommendations, these bioactive compounds can be applied to formulate different drugs to treat innumerable diseases.

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Conflict of interest

The authors have no conflict of interest.

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