

ANTIOXIDANT PROPERTY AND GC MS PROFILING OF THE METHANOLIC LEAF EXTRACT OF *PLUMBAGO ZEYLANICA*

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ABSTRACT

Plants have been a natural source with the ability to cure many inflammatory diseases and disorders. The roots of the *Plumbago spp* have been reported for a numerous therapeutic properties and have been extensively investigated for its phyto compounds. The present study was used to investigate the total phenolics, free radical scavenging activity and the GC- MS profiling of the methanolic leaf extract of *Plumbagozeylanica*. The total phenolics was estimated to be 1219±2.41 gallic acid equivalent (GAE) g⁻¹. The free radical scavenging activity was equivalent to that of the standard ascorbic acid indicating a potential anti-oxidant property. The GC- MS analysis showed a number of compounds belonging to sterols, terpenes, alkanes and aromatic hydrocarbons many of which have been reported for potential bioactive property.

INTRODUCTION

The production of free radicals is a normal physiological process that results from various metabolic reactions carried out by the cells in the body. Their presence can have an effect on the cells affecting the nucleic acids, proteins, carbohydrates and lipids influencing its biological functions [1-3]. With the ability to affect the homeostasis of the body they are responsible for inducing oxidative stress ultimately leading to a number of inflammatory diseases and accelerates the process of aging [4-8]. The body's own natural mechanism of eliminating the free radicals is through the production of enzymes (Superoxide dismutase, catalase and glutathione enzymes) and antioxidant substances (Glutathione, uric acid and Melatonin) in the cells [9-18]. Antioxidants are obtained through dietary sources such as Beta carotene (Vitamin A), Ascorbic acid (Vitamin C), tocopherols and tocotrienols (Vitamin E) [19-23]. Very often the dependence on this natural mechanism is affected by factors such as the age and lifestyle induced stress [1, 24-25].

Synthetic antioxidants were once the preferred alternatives and added as supplement in various food sources. Owing to its benefits they also had its applications in other industries such as cosmetics and therapeutics. Commercially used synthetic anti-oxidants are phenolic derivatives such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA). However studies have shown that these synthetic anti-oxidants have harmful effects on the human health [26].

A new area of interest is the search of antioxidants from natural sources which are nontoxic and abundantly available. Numerous investigations were done in search of anti-oxidants in fruits, vegetables and plants with medicinal properties [27-29]. Green tea and black teas are currently the most preferred for its health benefits and studies have shown high anti-oxidant properties owing to its presence of phenolic compounds [30]. Indian Medicinal plants that have been investigated for its anti-oxidant properties are *Aloevera* (Indian aloe), *Azadirachta indica* (Neem), *Bacopamonniera* (Brahmi), *Curcuma longa* (Turmeric), *Emblica officinalis* (Indian gooseberry), *Murrayakoenigii* (Curry leaf), *Ocimum sanctum* (Holy basil) and *Withaniasomifera* (Winter cherry) [31].

Plumbago spp commonly known as the Leadwort is a perennial flowering plant belonging to the family Plumbaginaceae. Commonly seen in India, it is the most grown ornamental plant for its aesthetic form. All the species of the plant namely *P. auriculata*, *P. europaea*, *P. zeylanica* and *P. rosea* have been reported in traditional use for its medicinal properties [32-38]. The present study was used to investigate the phyto constituents in the leaves of *P. zeylanica* and its potential as an antioxidant. This part of the plant is less explored as more studies have been done on the root part of the plant reported for its traditional medicinal properties.

MATERIAL AND METHODS

MATERIALS

All the chemicals and reagents were of analytical grade. 2,2- diphenyl- 1- picrylhydrazyl (DPPH) was procured from SISCO Research Laboratories, Maharashtra (India), Gallic acid were obtained from Hi Media Laboratories Pvt. Ltd, Mumbai, India. Petroleum ether, Chloroform, sodium carbonate, Ascorbic acid were obtained from SD Fine Chemicals Limited, Mumbai (India), Methanol from Spectrum Chemical Private Limited, Cochin (India), Folin–Ciocalteu reagent was from MERCK Specialties Private Limited, Mumbai (India).

Collection of Plant Material

The leaves of *P.zeylanicawas* collected from the garden of Institute of Wood Science &Technology, Bangalore, Karnataka (India). The authentication of the plant was done at Department of Botany, Maharani Lakshmi Ammanni College for Women and at Department of Microbiology & Biotechnology, Bangalore University, Jnana Bharathi Campus, and Bengaluru.

Preparation of plant extract

The leaves thoroughly washed with tap water followed by distilled water were left for shade drying and later pulverized to a fine powder. It was subjected to sequential soxhlation with the solvents in the order of petroleum ether, chloroform and Methanol for 16hrs each. The methanolic extract which was evaporated to dryness in a water bath at 40°C was later used for the study [39].

Total Phenolic content (TPC)

TPC of methanolic extract of *P.zeylanicawas* analyzed by FolinCiocalteu method [40]. In a Microtitre well 50 µl of different concentrations of the extract (10-50 µg mL⁻¹) was taken with equal quantities of FC reagent previously diluted with 1:1 with distilled water. The mixture was allowed to stand for 5 mins and then mixed with 100µl of Sodium carbonate solution (2%). After 10mins the absorbance was measured at 730nm. TPC of the extract was determined using standard curve and expressed as µggallic acid equivalent (GAE)/mg extract using the formula $T = [C \times V]/M$. Where T is the TPCs in µg/mg of the extracts as GAE, C is the concentration of gallic acid in µg/mL, V is the volume of the extract in mL, and M is the weight in mg of the extract. All the assays were carried out in triplicates.

Antioxidant activity

DPPH Radical Scavenging Activity

The free radical scavenging activity of the extract was determined using DPPH. [40] 0.006% of DPPH solution was prepared in 95% methanol. Different concentrations of the extract was treated with equal volumes of freshly prepared DPPH and left in dark for 30mins. The absorbance was read at 517nm in UV spectrophotometer. All the tests were carried out in duplicates, Ascorbic acid was used as standard and 95% methanol was used as blank. Percent scavenging of the DPPH free radical was measured using the formula:

$DPPH \text{ scavenging effect } (\%) = (A_o - A_1) / A_o \times 100$; where, A_o was the absorbance of the control and A_1 was the absorbance in the presence of the sample (methanolic leaf extract of *P.zeylanica*)

GAS CHROMATOGRAPHY–MASS SPECTROMETRY (GC MS) ANALYSIS OF P.ZEYLANICA METHANOLIC LEAF EXTRACT

The Clarus 680 GC was used in the analysis employed a fused silica column, packed with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm ID × 250µmdf). 1µl of the sample was used for injection and the components were separated using Helium as carrier gas at a constant flow of 1 ml/min. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS NIST (2008) library.

STATISTICAL ANALYSIS

All the experiments were carried out in duplicates and the results were expressed as mean ± standard error of the mean. The data were statistically analyzed using Microsoft Office Excel 2007.

RESULT

Plants have been a natural source for a number of phytochemicals with the potential to treat diseases and disorders in humans. Their therapeutic properties are well documented since the ancient medicinal practice and the knowledge on their potentiality now continues to interest researchers to explore the phytochemicals to be used a potential drug considered to be safe with no side effects.

In the present study, an attempt was made to estimate the total phenolic, anti-oxidant property and to analyze the phyto compounds by GC MS analysis. The total phenolic as estimated by FC method was found to be 1219±2.41

gallic acid equivalent (GAE) g^{-1} . The free radical scavenging activity of the extract was evaluated by its ability to inhibit the decolourization of DPPH. The present study showed the potential of the extract as an effective anti-oxidant as its free radical scavenging activity was equivalent to that of the standard anti oxidant namely ascorbic acid. The Fig1 shows the comparison of the DPPH assay of the methanolic extract and Ascorbic acid.

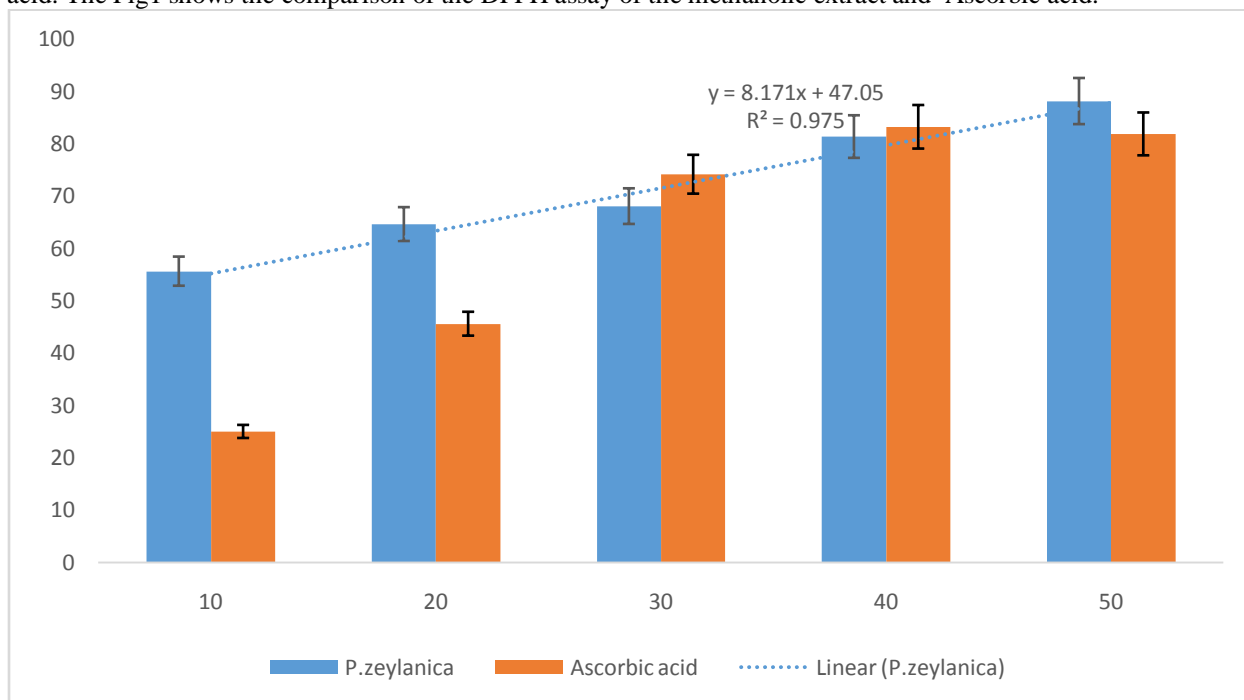


Fig 1. Comparison of the DPPH activity of Methanolic extract of *P.zeylanica* and Ascorbic acid

The GC MS analysis of the extract showed a diverse range of compounds, the predominant being the occurrence of phytosterols and terpenoids. Table 1 shows the characteristics of the phyto compounds detected in the methanolic extract of *P. zeylanica* detected by GC- MS analysis.

The Fig 2 shows the Chromatogram of the compounds detected in the GC MS analysis.

The sterols and terpenes that were detected are Pregnan-20-one, 3-hydroxy- ; Cholesta-8,24-dien-3-ol, 4-methyl-, (3.beta.,4.alpha.)- ; 3-Chloro-5-cholestene; 26-Hydroxycholesterol; Stigmasteryltosylate; 7-dehydrocholesteryl isocaproate; 22,23-dibromostigmasterol acetate; Ergosta-7,22-dien-3-ol, acetate, (3.beta.,5.alpha.)- ; Cholesterol 3-o-[[2-acetoxy]ethyl]-, stigma sterol ; Ethyl iso-allocholate ; Lanost-7-en-3-one, (9.beta.,13.alpha.,14.beta.,17.alpha.)- ; 9, 19-Cyclolanostan-3-ol, acetate, (3.beta.)- ; 30-Norlupan-28-oic acid, 3-Hydroxy-21-methoxy-20-oxo-, methyl ester; androstane-17-carboxylic acid, 3,11-bis(acetyloxy)-12-hydroxy-, methyl and phytol.

Alkanes of varying chain length were the next most prominent compounds detected in the analysis. Some of the compounds observed were Tetradecane, 1-chloro- ; Hexadecane, 1-chloro- ; 1-Octadecanesulphonyl chloride; Nonadecane, 1-chloro- ; Dodecane, 1-chloro- ; 4-Propionyloxytridecane ; 2-Pentadecanone,6,10,14-trimethyl-; Hexatriacontane; Dotriacontane ; Tritetracontane ; Tetratetracontane ; Heptacosane, 1-chloro- ; Octacosane ; Tetracosane, 11-decyl- ; Heneicosane, 11-(1-ethylpropyl)- ; 1-Chloroeicosane ; behenyl chloride ; Z-5-methyl-6-heneicosen-11-one,

Few Fatty aldehyde compounds that was detected were 7-Hexadecenal, (z)-; Hexadecanal, 2-methyl-; and fatty acids such as Hexadecanoic acid, 2-oxo-, methyl ester; Eicosanoic acid ; Oleic acid,3-(octadecyloxy)propyl ester; Vinyl decanoate; 3-(Octadecyloxy)propyl ester; 2,2-dimethylpropanoic acid, oct-3-en-2-yl ester and fatty acid derivative Geranyl isovalerate were also observed.

Some of the aromatic hydrocarbons detected were 2-Piperidinone, n-[4-bromo-n-butyl]- ; beta. carotene ; 2(3h)-Benzofuranone, hexahydro-4,4,7a-trimethyl- ; Pseudosarsasapogenin-5,20-dien; 7-hydroxy-3-(1,1-dimethylprop-2-enyl)coumarin ; 1-Coprosten-3-one 2,4-dinitrophenylhydrazone and 1-naphthalenepropanol, .alpha.-ethyldecahydro-5-(hydroxymethyl)-. Ethylene oxide derivative also known as oxirane, tetradecyl- was also detected. Fig 3 shows the structure and mass spectrum of some of the compounds detected in the methanolic leaf extract of *P.zeylanica*

Table 1 shows the characteristics of the phyto compounds detected in the methanolic extract of *P. zeylanica* detected by Gas chromatography- Mass spectrometry analysis.

Compound Name	M.W.	. Formula	REV	FOR	Retention time	Peak Area %	CAS
Cholest-22-ene-21-ol, 3,5-dehydro-6-methoxy-, pivalate	498	C ₃₃ H ₅₄ O ₃	824	637	27.413	16.273	900124-60-2
			784	637	28.064	9.008	
Total= 25.281							
Cholesta-8, 24-dien-3-ol, 4-methyl-, (3.beta.,4.alpha.)-	398	C ₂₈ H ₄₆ O	834	643	28.679		7199-92-0
1-methylene-2b-hydroxymethyl-3,3-dimethyl-4b-(3-methylbut-2-enyl)-cyclohexane	222	C ₁₅ H ₂₆ O	914	393	26.563	11.814	900144-10-6
Tetradecane, 1-chloro-	232	C ₁₄ H ₂₉ Cl	842	611	27.213	11.276	2425-54-9
Octadecane, 1-chloro-	288	C ₁₈ H ₃₇ Cl	864	637	26.853	7.779	3386-33-2
Hexatriacontane	506	C ₃₆ H ₇₄	917	709	26.313	6.047	630-06-8
1-Hexyl-2-Nitrocyclohexane	213	C ₁₂ H ₂₃ O ₂ N	848	482	29.289	5.747	118252-04-3
Dotriacontane	450	C ₃₂ H ₆₆	915	699	25.768	4.585	544-85-4

PL-M-(19ES-1162) NR+Sm (Mn, 2x4)

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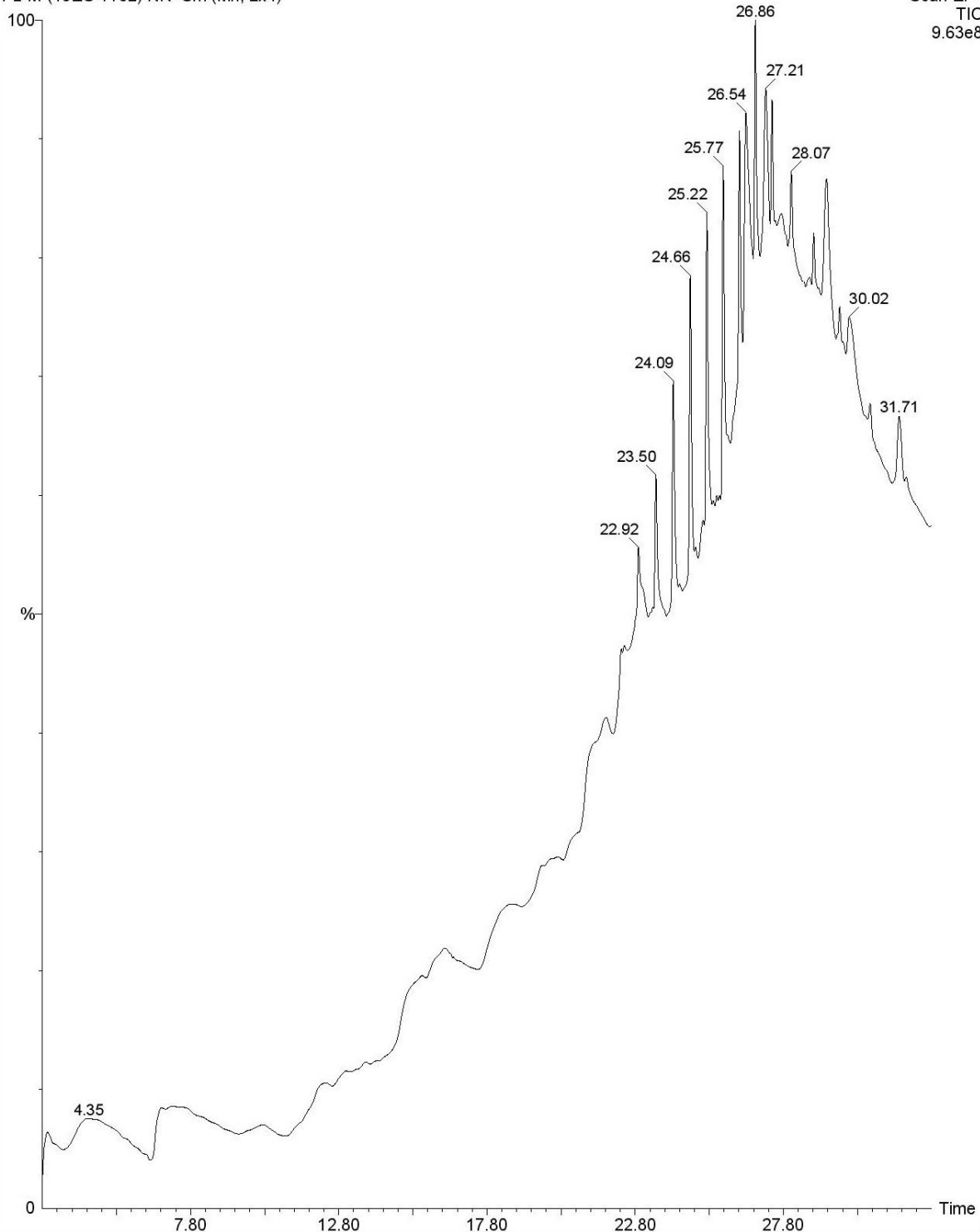


Fig 2. Chromatogram of the Methanolic extract of *P.zeylanica*

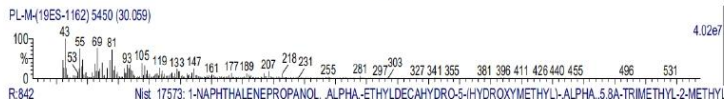
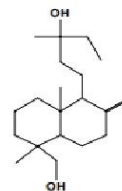
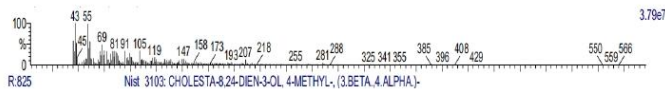
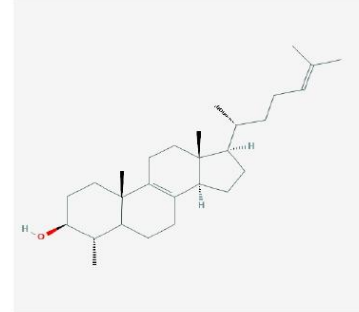
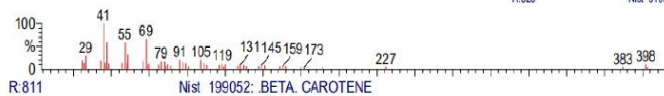
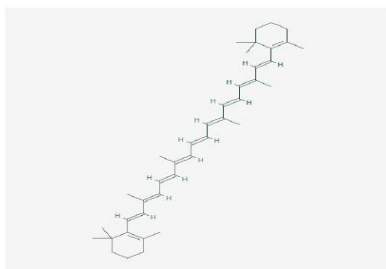
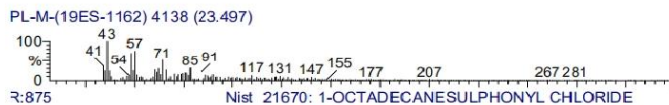
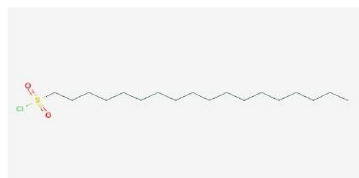
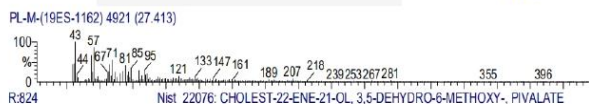
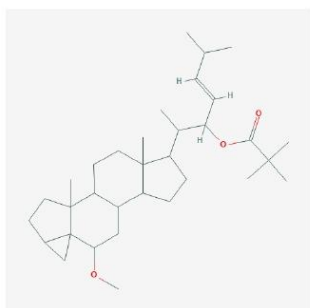
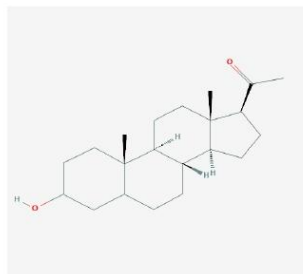
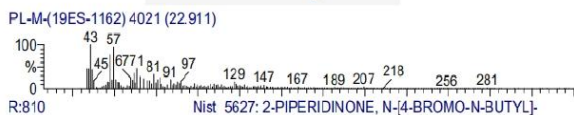
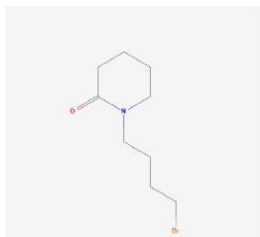


Table 2 showing the chemical nature and reported bioactive active property of the compounds that were detected in the methanol extract of *M.elengi*

COMPOUND NAME	CHEMICAL NATURE	REPORTED BIOACTIVE PROPERTY	REFERENCE
Cholest-22-ene-21-ol, 3,5-dehydro-6-methoxy-, pivalate	Phytosterol	Antimicrobial activity	41
1-methylene-2b-hydroxymethyl-3,3-dimethyl-4b-(3-methylbut-2-enyl)-cyclohexane	Sesquiterpene alcohol	Antimicrobial, Anti-inflammatory, Anti hyperlipidemic	42
Tetradecane, 1-chloro-	Alkane	Antimicrobial activity	43
Octadecane, 1-chloro-	Alkane	Anti-oxidant, antibacterial	44
Hexatriacontane	Alkane	Anti-oxidant	45
1-Hexyl-2-Nitrocyclohexane	Aromatic hydrocarbon	Antimicrobial activity	46
Dotriacontane	Alkane	Antimicrobial activity, hypercholesterolemic, Antioxidant, antispasmodic.	47, 48
Hexadecanoic acid, 2,3-bis[(trimethylsilyl)oxy]propyl ester	Fatty acid	Anti-inflammatory, Antioxidant, antipsychotic, hypocholesterolemic, nematocidal, pesticide, anti androgenic flavor, hemolytic, 5-Alpha reductase inhibitor, potent mosquito larvicide.	49,50,51
Stigma sterol	Phytosterol	Anti-cancer, antioxidant, hypoglycemic and thyroid inhibiting properties	52
Ethyl iso-allocholate	Sterol derivative	Anti-cancer	53
30-Norlupan-28-oic acid	Triterpenoid	Anti- HIV activity	54
Phytol	Diterpene	Precursor for synthetic Vitamin E and K1 manufacture; activate the transcription factors PPAR-alpha involved in fatty acid metabolism, fragrance industry	55,56, 57,58
Octacosane	Alkane	Act as good phase change materials	59,60
Heneicosane	Alkane	Antibacterial, Inhibit larva growth	61
2-Piperidinone, n-[4-bromo-n-butyl]-	Lactam	Antimicrobial activity	62
β-carotene	Terpenoid	Vitamin a precursor, Anti-oxidant	63

DISCUSSION

Plumbagozeylanica is well documented for its medicinal property and has been widely used in the Indian Medicinal practices. The roots of the plant is widely used and have been reported for various treatment but had to be cautiously used since it is equally poisonous and considered to be an abortifacient [64]. The pharmacological property of the plant root has been attributed to its phyto compounds such as alkaloids, glycosides, tannin, saponins, terpenoids, steroids and most importantly Plumbagin (Naphtha quinone)[65].

The present study showed that leaf extracts of *P. zeylanica* possess phyto compounds that may play a major role in therapeutics and overcome the toxicity associated with the roots of the plant. The methanolic leaf extract showed a good antioxidant potential with percentage inhibition of 80% and was almost equivalent to that of the Ascorbic used as standard.

The GC MS analysis of the extract showed a high amount of sterol compounds with Cholest-22-ene-21-ol, 3, 5-dehydro-6-methoxy-, pivalate being the most abundant as determined from the chromatogram with peak area percentage of 25.281. This compound has been earlier documented as a bioactive compound known for

antimicrobial activity[41]. Other compounds detected were also seen to possess similar activity as shown in Table 2. In vitro antimicrobial studies of methanol leaf extracts of *P. zeylanica* showed both antibacterial and antifungal activity when tested against both human and agricultural pathogens [66]. The presence of a triterpenoid is very promising as it has been documented for anti HIV activity. Some sterols and sterol derivatives detected have been documented for anti-cancer activity.

The high anti-oxidant activity of the extract can be attributed to the phyto compounds such as carotenes, aromatic compounds and alkenes that have been detected through the GC MS analysis. The phytols that have been detected could be a source for synthetic preparations of Vitamin E which is a potent antioxidant.

CONCLUSION

This study clearly indicates the potential of the leaves of *P. zeylanica* for various therapeutic purposes. With numerous compounds detected with the potential of anti oxidant, anti microbial, anti cancer and anti inflammatory activity, the leaves can be equally important like the roots of *P. zeylanica* for therapeutic use. They can be source for phytol extraction which has huge application in the commercial process of synthetic vitamins like Vitamin E and Vitamin K1. Investigations need to be done on the anti HIV potential of the leaf since there is a detection of an antiviral compound 30-Norlupan-28-oic acid. This finding can be very significant to be used against the virus HIV.

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