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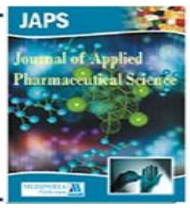

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Extraction and Phytochemical Evaluation of *Litsea Glutinosa* Bark Methanolic Extract

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ABSTRACT

The present investigation deals with the extraction of bark powder of *Litsea glutinosa* and its preliminary phytochemical screening. The bark powder was subjected to methanolic extraction and further explored for its phytochemical constituents using TLC and GC MS. Preliminary phytochemical screening reveals the presence of alkaloids, flavonoides, glycosides, phenols, tannins and saponins. The extract was further subjected to separation using TLC and fractions were evaluated using GC MS. GC MS analysis of the total methanolic extract showed the presence of Oliec acid, tricosene, erucic acid, tetra decanoic acid, pyrrolidinone, piperidine, eicosanoic acid like major phytochemicals. Alkaloid fraction was found to be rich in therapeutically potential compounds like Eicosane, Pieprizine, pyridine, thio-coumarin, tetrahydroisoquinoline. Apart from this various Androstane, Androsta-trione, pregnene like phytoestrogens were also observed in this plant, justifying its aphrodisiac and osteoprotective effect. TLC of various subfractions of alkaloids revealed that this plant is rich in variety of potential therapeutic phytochemicals, hence justifying its ethnomedical usage.

Keywords: *Litsea Glutinosa*, Phytochemical screening, extraction.

INTRODUCTION

Litsea is a medium sized evergreen tree, up to 25 m high and 1.5 m in girth and a clean bole of around 5 – 6 m. It is found throughout India ascending up to an altitude of 1400 m in the Himalayas (Sukh Dev, 2006). Litsea has been described as promoter of longevity, promoter of semen generation and emollient. Sap of fresh bark or its decoction is prescribed as a remedy for diarrhea, dysentery, rheumatism, and as an aid to longevity. In addition, in current usage, a paste prepared by grinding bark with water is used as a plaster in cases of sprain, bruises, wounds, inflammation, back pain, rheumatic and gouty joints, bone fractures etc. It has analgesic, antiseptic and emollient effects (Devi and Meera, 2010). Methanol extract of bark showed highly significant antibacterial action against micrococcus luteus (Sukh Dev, 2006). In another investigation, methanol extract demonstrated to be as effective as chloramphenicol against sixteen tested organisms (Mandal et al., 2000). Bark extract has useful antifungal activity against several organisms (Sukh Dev, 2006). Enough research data shows that aporphine alkaloids have highly significant antioxidant activity. Litsea glutinosa is found to be rich in these alkaloids. Apart from this, ethanolic extract of the bark also showed aphrodisiac effect in male rats (Sukh Dev, 2006).

Though most of the biological activities had been carried out on the bark extract, very scanty data is available regarding the phytochemical constituents present in the bark, and their effects on pathogens. Thus, the present study was undertaken to characterize and isolate the phytochemicals from this plant. As most of the studies indicated that alkaloids are abundant in the bark of *L. glutinosa*, alkaloids were further subjected for TLC purification and GC MS analysis.

MATERIALS AND METHODS

Plant Material

The bark powder of *L. glutinosa* was purchased from local drug market and checked for any type of bacterial or fungal infection. The dried powdered plant material (20gms) was extracted successively with methanol by soxhlet's apparatus for 72 hours at a temperature not exceeding the boiling point of the solvent. The filtrate was evaporated at 60º C on water bath to yield semi solid paste. This paste was later stored in -20º C.

Test for various phytochemicals

Flavonoid

Test for flavonoids were carried out as described previously (Sharstry *et al.,* 2010; Devmurari *et al.,* 2010).

Terpenoids

Test for terpenoids were carried out as described previously (Daneil, 1991).

Phenol

Test for phenols were carried out as described previously (De *et al.,* 2010).

Tanin

Test for tannins were carried out as described previously (Daneil, 1991).

Saponin

Test for saponins were carried out as described previously (Daniel, 1991).

Glycosides

Test for glycosides were carried out as described previously (Kodangala *et al.,* 2010; Sahu *et al.,* 2010).

Test for Cardiac Glycosides

Test for cardiac glycosides were carried out as described previously (Daneil, 1991).

Alkaloids

Tests for alkaloids were carried out as described previously (Kam *et al.,* 2001). Briefly, 20 mg of plant crude extract was added to 10 ml methanol and placed in a sonic bath to dissolve. The extract was then filtered using a Wattman No.1 filter paper; 2 ml of filtrate was taken and mixed with 1 % HCl. Three different tests were performed for Alkaloids.

1. Mayers' test: To 1 ml of mixture, 6 drops of Mayer's reagent, was added leading to the formation of a yellowish creamish precipitate.
2. Wagner's Test: To 1 ml of mixture, 6 drops of Wagner's reagent was added forming bronish red precipitates indicating the presence of alkaloids.
3. Dragendroff's test: To 1 ml of mixture, 6 drops of Dragendroff's reagent was added, forming an orange precipitate indicating the presence of alkaloids.

Isolation and TLC of Alkaloid

Alkaloids were isolated by the method described previously (Daneil, 1991). Briefly, 20gm bark powder was soaked in 100 ml 10% ammonical ethanol for 24 hours. Filtrate was collected and concentrated to yield a semi solid paste, which is further subjected to TLC. TLC was carried out on silica gel G using Chloroform-Methanol-tetraethylamine system developed at the ratio of 75:22:3. After running the sheet for 45 min in a chromatigraphic chamber on a 20 cm long precoated silica plate, the plate was visualized in *uv* for the separation of bands. Separated bands were cut from the plate and extracted in methanol for analysis of absorption maxima and GC MS.

UV-VIS IR analysis

For carrying out absorption maxima analysis, 8 fractions obtained through TLC were dissolved in methanol and scanned from 200 to 1000 nm using Perkin Elmer automatic analyzer.

GC MS analysis

GC/MS analysis was carried out using Perkin Elmer auto system XL with turbo mass system equipped with PE 5 MS 30m X 250 micron silica capillary. Injector and detector temperatures were 250 º and 300º C, respectively. The temperature started from 70 º C for 5 min and then rose to 290 ºC at the rate of 10 º C per minute. Helium was used as carrier gas. The MS was taken at 70 eV. Scanning speed was 0.84 scans s−1 and the scanning period was from 40 to 550 s. sample volume was kept 3 µL.

RESULTS

The result of phytochemical screening of plants gave positive results for alkaloids, flavonoids, glycosides, phenols, tannins and saponins. However, these preliminary tests were negative for terpenoids, amines and anthocyanins. Our results supported previous study conducted by Yan *et al.,* (2000) who reported the presence of alkaloids in this plant. Results are presented in Table 1.

TLC of alkaloids

TLC of alkaloids resulted in 8 *uv* active bands. These bands are shown in the table 4 according to their Rf values (Table 2).

Table. 1: Phytochemical analysis of *Litsea glutinosa*.

Fraction		Test	Presence/Absence
Alkaloids		Mayers	+
		Wagners	+
		Dragondorff's	+
Flavonoids		N-lead acetate	-
		Zinc dust	-
		NaOH	+
Terpenoids		H2SO4	-
		Chl+H2so4	-
Amines		Na-Nitro	-
		Dragondorff's	-
		Ehlrich	-
Glycosides	**cynogenic**	Molisch	+
		Cold H2SO4	+
	cardiotonic	Kedde	-
		Keller	-
Phenols		N-FeCl3	+
		FeSO4	+
Anthocynins		Na acetate	-
		Na2CO3	-
Tannins		Gelatin	+
		K2Cr2O7	+
		Iodine	-
		Lead acetate	-
Saponin		Water	+
		Lead acetate	+

Table. 2: showing Rf values of various alkaloid fractions.

Band	A	B	C	D	E	F	G	H
Rf value	0.93	0.71	0.62	0.61	0.58	0.42	0.34	0.18

uv vis IR scan of alkaloids

uv scan of alkaloid fraction revealed that all most of the constituents are colorless and not having any absorption in the visible range (Figure 1). All the fractions showed absorption maxima in the range of 200 – 300 nm. Fraction A was found to be having absorption in visible region, and it yielded a yellow crystalline powder, suggestive of presence of colored flavinoids. Rest all fractions yielded a white crystalline powder. These results supported the study of Yang *et al.*, (2005) who isolated two new aporphine alkaloids from *Litsea* bark and reported one's absorption maxima at 218, 282 and 306, while the other compound has absorption maxima at 278 and 306.

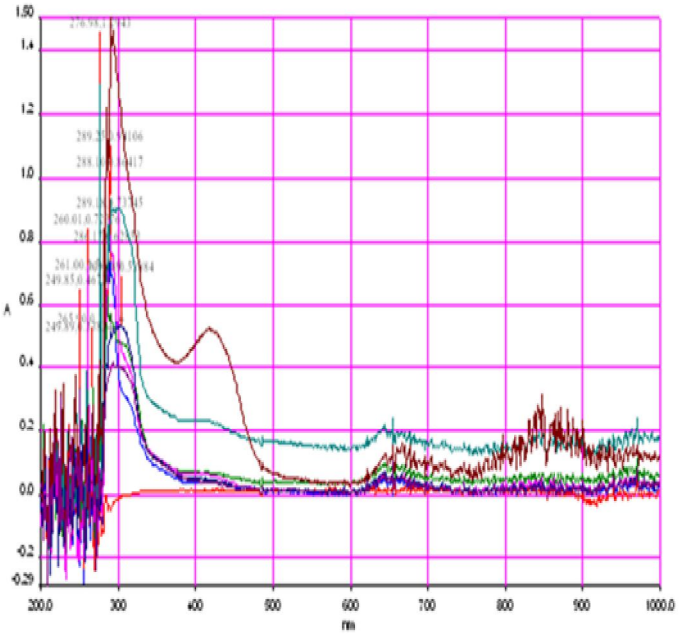

Fig. 1: showing *uv* vis scan of alkaloid fractions A to H.

GC MS ANALYSIS

GC spectrum analysis of methanolic extract of the plant is presented in Figure 2. MS analysis of the total methanolic extract showed presence of various phytochemicals listed in Table 3. GC MS analysis of Alkaloid sub fractions revealed presence of various phytochemicals listed in Table 4 and presented in figures 2(a-n).

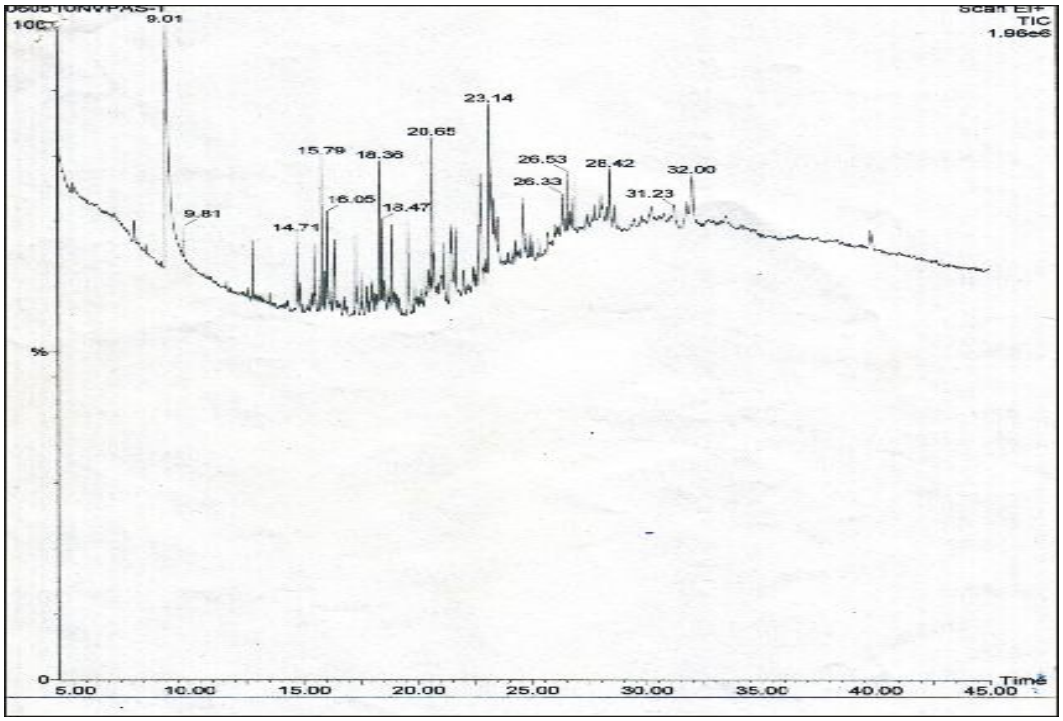

Fig. 2: GC MS Scan of methanolic extract of plant.

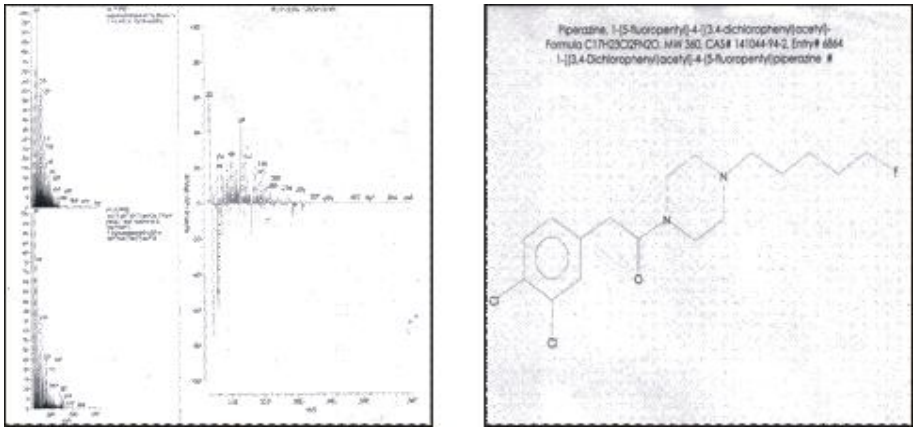

Fig. 2a : MS analysis and probable presence of Piperzine derivatives.

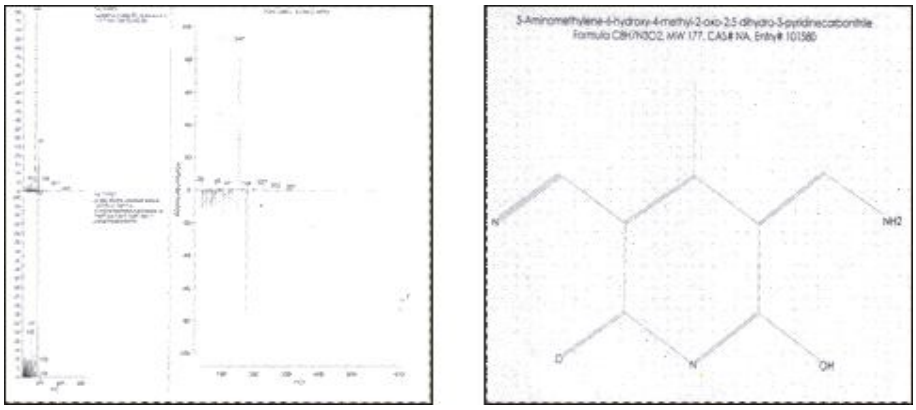

Fig. 2b: MS analysis and probable presence of Piperzine carobnitrile.

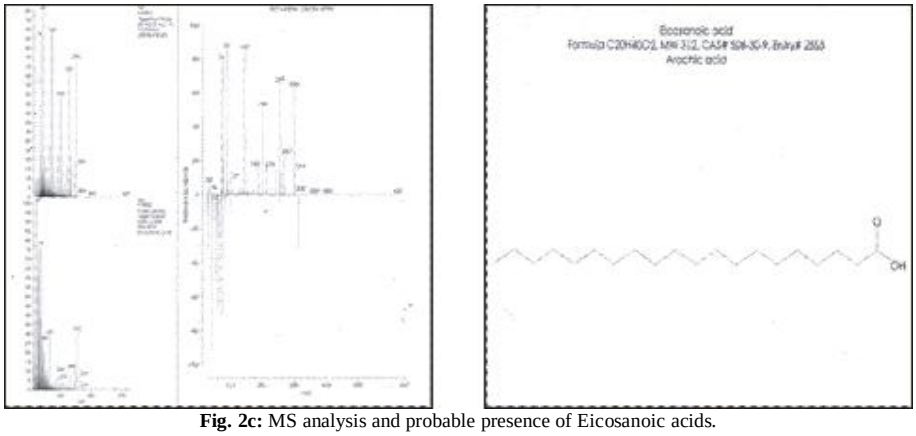

Fig. 2c: MS analysis and probable presence of Eicosanoic acids.

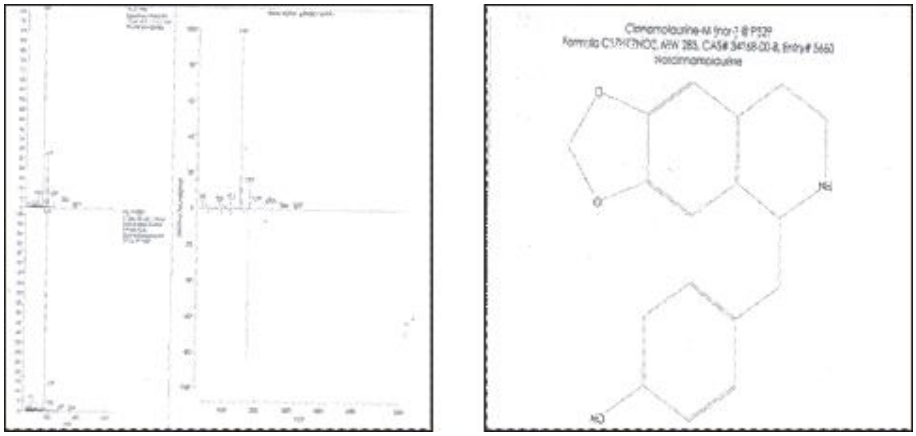

Fig.2d: MS analysis and probable presence of Cinnamolaurine derivatives

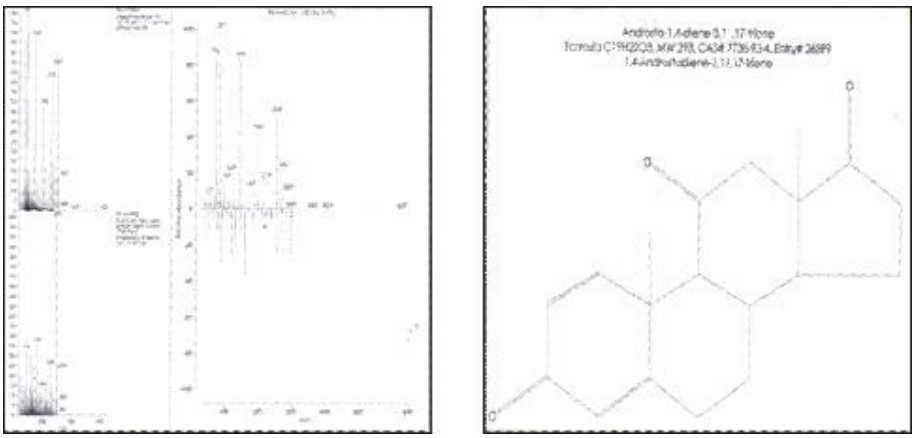

Fig. 2e: MS analysis and probable presence of Androstane derivatives.

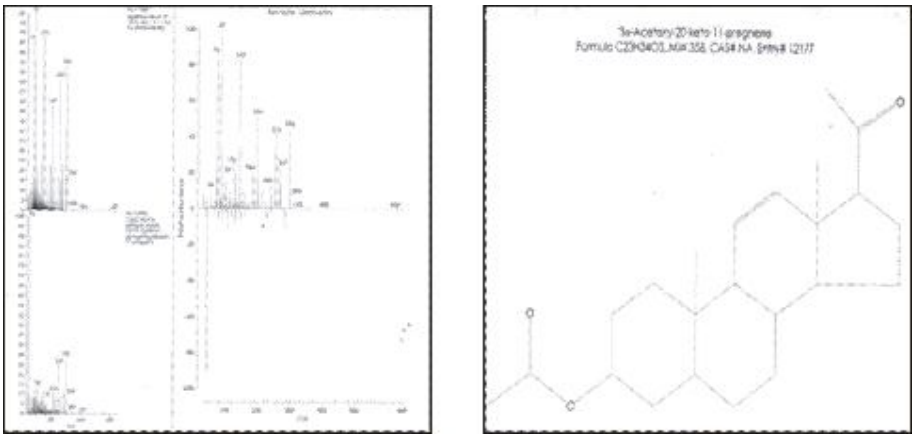

Fig. 2f: MS analysis and probable presence of Pregnane derivatives.

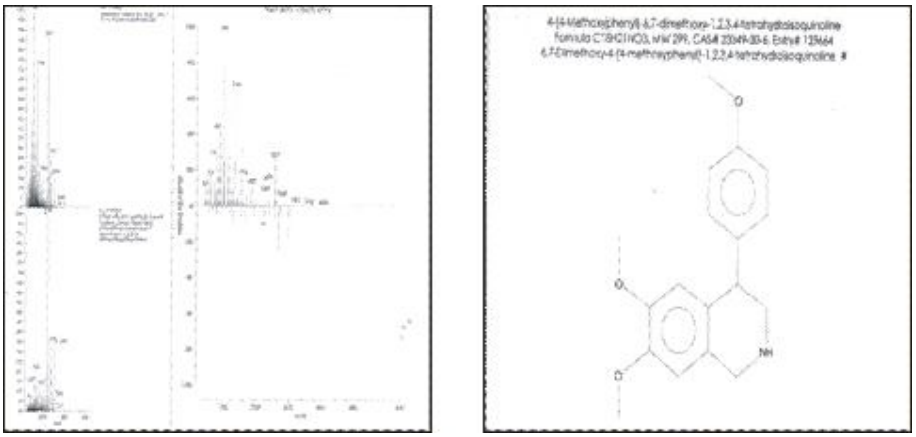

Fig. 2g: MS analysis and probable presence of Quinoline derivatives.

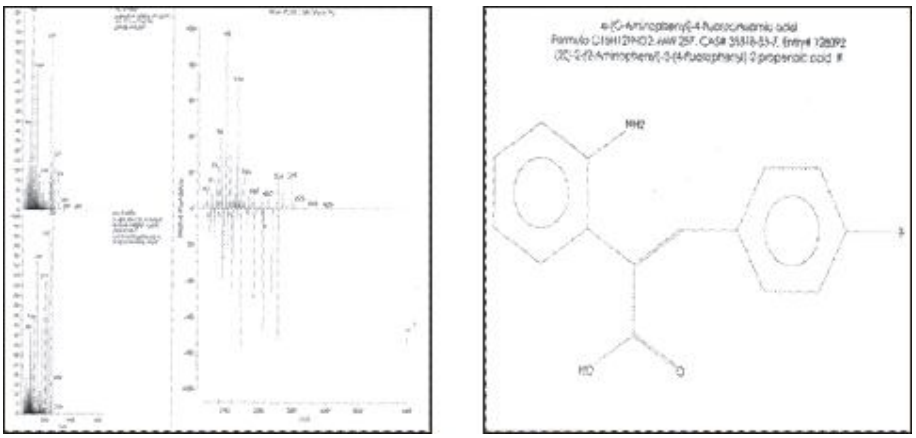

Fig. 2h: MS analysis and probable presence of Cinnamic acid derivative.

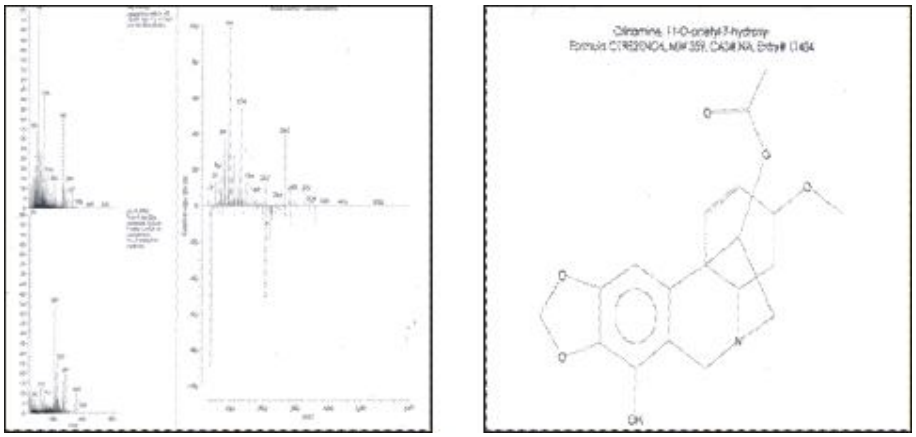

Fig. 2i: MS analysis and probable presence of Crinamine derivatives.

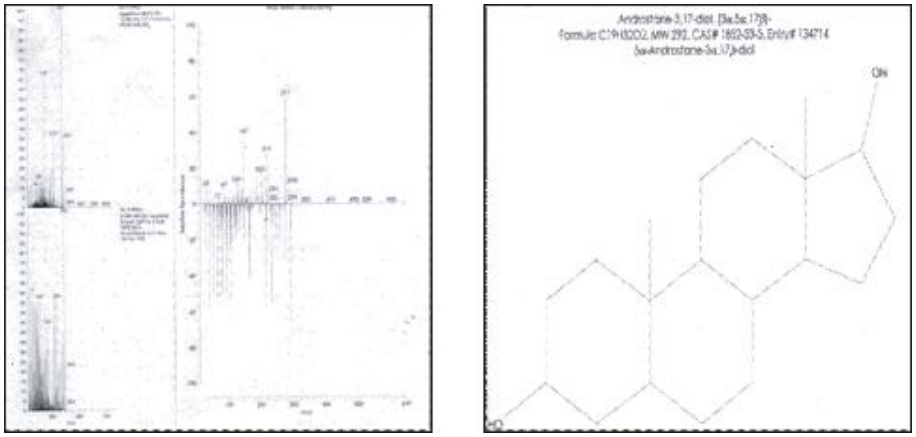

Fig. 2j: MS analysis and probable presence of Androstan.

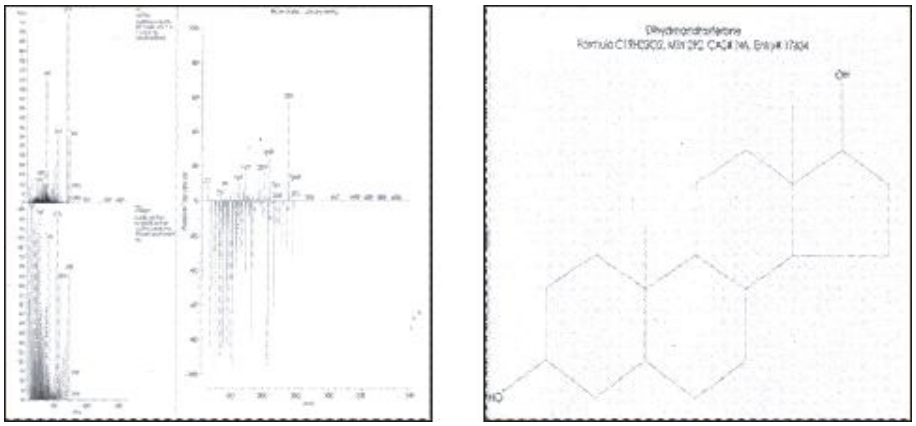

Fig. 2k: MS analysis and probable presence of Dihydroandosteron derivatives.

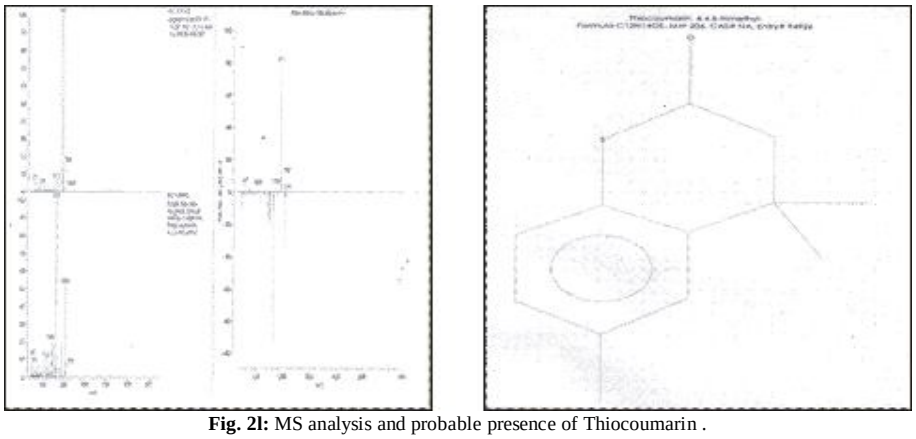

Fig. 2l: MS analysis and probable presence of Thiocoumarin .

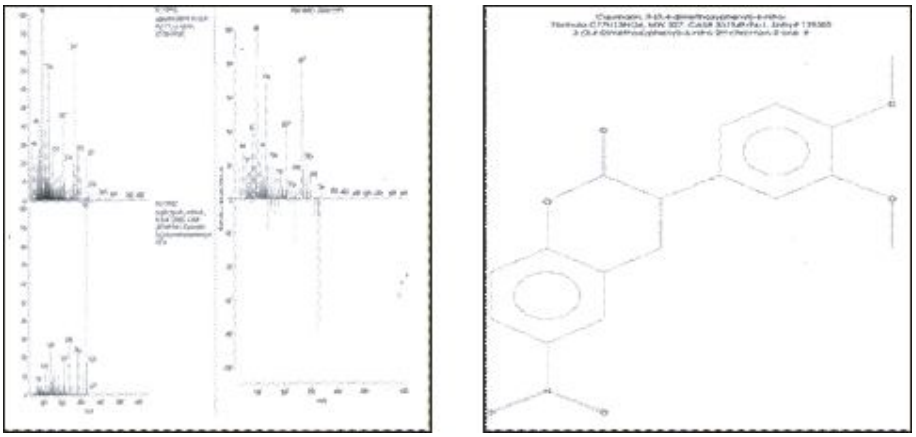

Fig. 2m: MS analysis and probable presence of cinnamon derivatives.

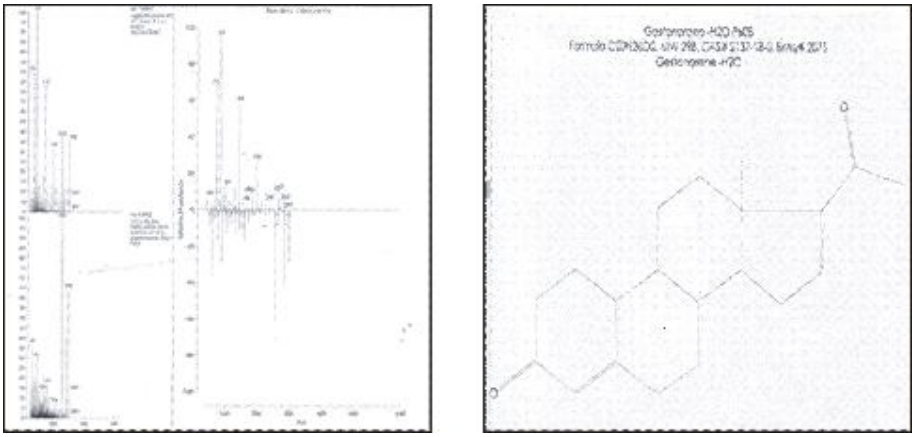

Fig. 2n: MS analysis and probable presence of Gestonorone.

Table. 3: GC MS scan of plant and its fractions.

Fraction	Phytochemical showing maximum probablity of occurance in molecular mass comparison
Total methanolic extract	Oliec acid, tricosene, erucic acid, tetra decanoic acid, pyrrolidinone, piperidine, eicosanoic acid,
Alkaloid fraction	Eicosane, Pieprizine, pyridine, Androsta-trione, pregnene, thio coumarin, tetrahydroisoquinoline, crinamine, Androstane,

Table 4: subfractions of alkaloids.

A	Dichloro acetyl phenyl piperazine, cinnamolaurine, Androsta 1, 4 diene trione, 3 α acetyl 20 keto 11 pregnene, tetrahydroisoquinoline, flourocinnamic acid, crinamine, Androstane 3 17 diol, dihydroandrostarone,
B	4, 4, 6 triemethyl Thiocoumarin, coumarin, gestonorone,
C	Eicosane, Oleic acid, Palmityl ester, dimethoxy tetrahydroisoquinoline, aminophenyl flouro cinnamic acid,
D	Eicosine, Dodecane, Hepta decane.
E	Trimethyl Dodecane, Penta Decane.
F	Tricosane, Hepta decane,
G	Benzene dicarboxylic acid diundecyl ester, tridecanoic acid, diacyl ester.
H	Hepta decanoic acid hydroxyl propanediyl ester, Heptacosane.

DISCUSSION

Our study showed that *Litsea glutinosa* bark contains various phytochemicals including alkaloids, steroids, triterpenoids, saponins, and tannins. The dried bark powder yielded 6.66% yield with methanol. Alkaloids gave 1.12% yield, present in most abundant quantity. These results supported the study of Mandal *et al.,* (2000), who observed similar results. Our study is the first to report antifungal analysis of *Litsea glutinosa* bark extract and its alkaloid content. When absorption maxima was calculated on autoanalyzer, it was found to be co incident with the results of Yang *et al*., (2005). GC MS analysis revealed the presence of various phytochemicals, including quinoline derivatives. Yang *et al.,* (2005) has reported two aporphine alkaloids from this plant, and we observed similar structured compound in GC MS analysis. This plant was found to contain Oleic acid which is reported to have variety of biological effects, including hypotensive effect (Teres *et al.,* 2008). Eicosanoids are signaling molecules formed during intracellular signaling of inflammation, suggesting that this plant may have a role in inflammation. Apart from these, we also observed various phytoestrogens in our plant like Pregene derivative and Androsta-triones. Phytoestrogens are proven osteoprotective agents by so many workers (Cassidy *et al*,. 1993; Fang *et al.,* 2003; Yang *et al.,* 2006). Thus justifying our previous study where it was observed that this plant acts as a potent osteoprotective agent, without having substantial effect on the uterus (Parikh *et al.,* 2009).This plant is also reported to have aphrodisiac effect in male rats by increasing the ejaculation latency (Sukh Dev, 2006). Our analysis showed the presence of various testosterone derivatives in it, thereby supporting its aphrodisiac effect. This plant was also found to contain coumarin and cinnamic acid like flavonoids dervatives and, cinnamolaurine and crinamine

like alkaloids which are considered to be having pharmaceutical property (Hahlbrock and Sheel, 1989). Many piperizine derivatives are established pharmacological drugs, and GC MS revealed various piperizine derivatives. Piperizine and piperidine derivatives are used in synthesis of various pharmaceutical drugs. Piperizine citrate is a standard drug used in the treatment of helminth infection. Presence of various piperizine derivatives justifies the recent study which showed that this plant is having antihelmintic property (Lohita *et al.,* 2010). Piperidine derivatives have been isolated from numerous natural alkaloids and they are ubiquitous building blocks in the synthesis of pharmaceuticals. Presence of these many important phytochemicals appeal for further analysis of this plant in various pathological conditions. Our study suggested that *Litsea glutinosa* is rich in alkaloids, and various other important phytochemicals, justifying its use in treatment of Diarrohea and dysentery as well as variety of other diseases. Presence various phytoestrogens and other alkaloids suggest that consumption of this plant can be helpful in treating osteoporosis and it can be worth exploring this plant for other pharmacological interventions.

CONCLUSION

Our study proved that *Litsea glutinosa* is an amalgam of variety of important phytochemicals which contributes towards its multirole pharmacological properties like antibacterial, anti-inflammatory, anti-rheumatic, osteoprotective, aphorodisiac etc., and justifies its wide ethnomedical usage.

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