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HR-LCMS of Phytoconstituents and Antimicrobial and Anti-oxidant Activity of Leaf and Callus of *Guaiacum officinale* L.

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Abstract

Guaiacum officinale (Zygophyllaceae) commonly known as Lignum Vitae, its native to Central America, introduced to India. Plant has been enlisted in IUCN endangered category because of over exploitation. The objectives of the present study were to analyze qualitative and quantitative phytochemical constituents in *G. officinale* leaf and callus using standard procedures. And also, extracts were subjected to antibacterial, antifungal and antioxidant activities. The results show the presence of all phytoconstituents in all the tested extracts. The antibacterial potential was tested against *E. coli* and *B. subtilis* bacteria by well and disc diffusion method, chloroform extract showed the highest inhibition in both the pathogens. The chloroform leaf and callus extract demonstrated the most potent antioxidant activity, as free radical scavenging activity, with an IC50 value of 11.06 and 13.35 µg/ml respectively. The reducing power assay revealed that the chloroform leaf and callus extracts exhibited the highest antioxidant activity with 1.56 µg/ml and 1.952 µg/ml respectively. We analyzed the major chemical constituents present in the chloroform callus extract, a total of 9 compounds were identified. The results demonstrated that both the leaf and callus extracts exhibited significant antifungal activity against *F. oxysporum*. The extracts of *G. officinale* demonstrated significant antimicrobial and anti-oxidant activities, suggesting their potential utility in development of therapeutic agents and dietary supplements.

***Key words*:** *Guaiacum officinale*, Secondary metabolites, total phenolic content, antibacterial, antifungal, antioxidant, HR-LCMS, Endangered

Medicinal plants play a vital role in maintaining individual and community health. These plants contain chemically active compounds that exert specific physiological effects on the human body providing therapeutic benefits [15]. Development of synthetic based drugs has become very expensive. In contrast, many plants-based medicines have been safely used for centuries without any adverse effect [16]. India has a rich heritage of traditional medicine systems, including Siddha, Ayurveda and Unani. These ancient practices involve preparing medicines from individual plant parts or combining multiple parts of various plants. *Guaiacum officinale* commonly known as Lignum vitae, is a slow growing, broad leaved, evergreen tree of Zygophyllaceae family. This species produces a diverse array of chemical compounds, categorized into primary and secondary metabolites. Phytochemical analysis of *Guaiacum officinale* has identified numerous bioactive compounds in its bark, flower and leaves. The primary constituents include saponins, notably guaianin and guaiacin [19].

Lignum vitae's highly prized wood and medicinal properties have led to severe overexploitation, and listed as an endangered species by the International Union for Conservation of Nature (IUCN) in 2019 [4]. *Guaiacum officinale*, a traditional medicinal herb has been used for centuries to treat various ailments, including uterine problems, angina, tonsillitis, rheumatism, fish poisoning, HIV and as abortifacient [2], [9], [26]. Despite of various usage in ailments and their diverse medicinal value are because of bioactive compounds, including alkaloids, steroids, terpenoids, flavonoids, saponins, proteins and amino acids [24]. An antimicrobial activity was conducted to evaluate the efficacy of *Guaiacum officinale* against specific pathogens, focusing on its inhibitory effects and potential pharmaceutical applications. LCMS analysis was employed to identify the bioactive compounds present in the plant. LCMS enabled the detection and characterization of specific phytocompounds and unknown organic compounds, facilitating their identification through spectral interpretation [14]. The findings of this study provide prospects for the development of a wide range of therapeutic agents.

MATERIALS AND METHODS

Preparation of the plant extracts

Guaiacum officinale L. plants were collected from Rajmandry, Andra Pradesh, India; another plant has been located in JSS Suttur matt, Mysore, India [23]. Leaves and leaf callus are selected for the present work and collected, leaves are washed under running tap water & dried in shade for complete dry, and completely ground for fine powder. Leaf callus was washed using dis. Water & dried in hot air oven @ 30ºC for

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complete dry, and ground for fine powder. 250mL of different solvents (hexane, chloroform, ethyl acetate, ethanol, methanol and water) were added to 20g of leaf and leaves callus. The plant powder were subjected to Soxhlet extraction at 60° for 5 hours. The resulting extracts were then concentrated at room temperature, and the residues were stored at 4°C for subsequent analysis.

Phytochemical screening of extracts

The different solvents extracts were used for the preliminary and quantitative phytochemical analysis using standard procedures. The qualitative tests are as follows:

a) *Test for alkaloids*

To 2 mL of test solutions few drops of Dragendroff's and Mayer's reagents were added separately. Formation of bright orange ppt and white ppt respectively indicates the presence of alkaloids.

b) *Test for carbohydrates*

To 2mL of test solution few drops of Barfoed's and Fehling's reagent were added separately. Formation of brick red ppt and formation of green to yellow to red ppt respectively indicates the presence of carbohydrates.

c) *Test for proteins*

To 2mL of test solution few drops of Ninhydrin solution were added and boiled in water bath, formation of purple colour; and 2mL of test solution mixed with 4% NaOH is added with few drops of 1% copper sulphate, formation of violet colour confirms the presence of proteins.

d) *Test for phenols*

Iodine solution and ferric chlore solutions were added to 2mL of test solution separately, formation of transient red colour and deep blue colour confirms the presence of phenols.

e) *Test for tannins*

Ferric chloride solution and 1% gelatine solution with 10% sodium chloride solution were added to 2mL of plant extract separately, formation of blackish blue and white ppt separately indicates the presence of tannins.

f) *Test for saponins*

To 2mL of test solution, 2mL of water were added and shaken, persist foam for 10 min., indicates the presence of saponins.

g) *Test for flavonoids*

Shinoda and ferric chloride test were conducted, magnesium ribbons along with conc. HCl; and ferric chloride solution added to 2mL of test solutions separately. Formation of red to pink colour and blackish green respectively indicates the presence of flavonoids.

h) *Test for sterols*

Salkowski test: few drops of concentrated sulphuric acid were added to extract, appearance of red colour in lower layer indicates the presence.

Liebermann Burchard test

few drops of acetic anhydride added to extract, 1mL of conc. Sulphuric acid was added from the sides of test tubes, appearance of reddish-brown ring indicates the presence.

i) Test for quinones

To 2mL of plant extract, 1mL of alcoholic KOH and conc. HCl is added separately. Formation of red to blue dolour and yellow ppt respectively indicates the presence of quinones.

j) Test for gums

To 2mL of extract, 100 mL of dis. water and 25mL of alcohol were added. Formation of white ppt indicates the presence of gums.

Quantitative analysis

Protein estimation: Total protein estimation of *G. officinale* extract was done by Biuret method described by Plummer [8], with slight modifications. Bovine serum albumin is taken as standard. The crude extract is dissolved in Ethyl acetate solvent. 3mL of Biuret reagent is added to all test tubes. Incubated for 10 minutes at room temperature. Absorbance measured at 540nm.

Carbohydrate estimation: The total sugar concentration of extract was estimated using Antrone method. Different aliquots of extract along with glucose (0-100µg) where made up to 1mL using distilled water. 5mL of antrone reagent is added. The blue green solution measured colorimetrically at 620 nm. The total sugar concentration was calculated according to standard glucose calibration curve [8].

Phenolic estimation: Total phenolic content was determined using Folin-Ciocalteu colorimetric method prescribed by Malick and Singh [7]. Gallic acid is used as standard. The crude extracts were dissolved in ethyl acetate, 1mL of Folin-Ciocalteu reagent was added and left for 5 minutes. 2mL of 10% Na_2CO_3 was added to the solution. Incubated at 90°C for 10 minutes. Absorbance recorded at 660 nm.

Total flavonoid estimation: Determined using aluminium chloride assay. 0.5mL of extract is taken, 2mL of distilled water was added followed by 0.1mL of sodium nitrite and allowed to stand for 6 min, 2mL of sodium hydroxide is added, volume made up to 5mL using distilled water. Incubated for 15 min. mixture turns to pink and absorbance recorded at 510nm. The total flavonoid content was expressed in mg of Quercetin equivalents per gram of extract [20].

Total Saponin estimation: Standard saponin solution is prepared by dissolving 10mg of diosgenin in 4mL of distilled water. 1mL of extract is added, 0.25mL of vanillin reagent was added and 2.5mL of sulphuric acid added slowly on the inner side of the wall. The solution was mixed and tubes were kept in 60°C water bath. Absorbance was measured at 544nm [18].

Thin layer chromatography

Thin layer chromatography (TLC) of herbal plant extracts was done according to standard methods [28]. TLC plates were prepared using 10g of Silica gel dissolved in 20mL of distilled water, distributed over plates and allowed to dry in Hot air oven at 110°C for 30 min. Extracts were dissolved in ethanol, spots were made using capillary tube and run in different solvent systems like Ethyl acetate: Methanol: Water (10:1:1); Toluene: Ethyl acetate: Formic acid (4:1:0.5); Toluene: Acetic acid (9:2); Chloroform: Water (6:4); Toluene: Ethyl Acetate (4:1); Hexane : Ethyl acetate (3:1); Hexane: Methanol and Methanol : Water (8:2). The movement of active compound was expressed by its retention factor (Rf).

$$Rf = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

Antibacterial assay: The antibacterial assay of sample was carried out by well and disc diffusion method with slight modification. Antibacterial activity was performed by Nutrient agar disc and well methods. Stock solution and agar medium were prepared and solidified. Different dilution of plant extracts, (25mg, 50mg and 100mg/mL) and standard antibiotics i.e., chloramphenicol (1mg/mL) were prepared and tested against *Escherichia coli* and *Bacillus subtilis* stains. Zone of

inhibition formed around well and disc were recorded after an incubation of 24 hours. The formation of inhibition zone was considered positive for antibacterial activity of extract [6].

Antifungal assay: The antifungal activity was carried out by Potato dextrose agar well diffusion method. Different dilution of plant extracts (25, 50, and 100mg/mL) were prepared and tested against *Fusarium oxysporum* and *Aspergillus niger*. Zone of inhibition formed around the wells was recorded after an incubation of 72 hours. Nystatin (1mg/mL) is used as standard [6].

Antioxidant activity

DPPH free radical scavenging activity: The scavenging efficacy of various concentrations (25, 50, 100, 200, 300 µg/mL of extracts) of stem extracts of *G. officinale* was investigated on the basis of scavenging effect against DPPH radicals [4]. Ascorbic acid was used as standard. Scavenging potential of stem extracts were determined using the formula:

$$\text{Scavenging activity (\%)} = \frac{(\text{Ac-At})}{\text{Ac}} \times 100$$

Ferric reducing power assay: The ferric reducing efficacy of various concentrations (25, 50, 100, 200, 300 µg/mL of extracts) of stem extracts of *G. officianale* was investigated [4]. Ascorbic acid was used as reference antioxidant. An increased in absorbance with increase of concentration indicates reducing ability of extract/ ascorbic acid.

Liquid chromatography-mass spectroscopy

The extracts of leaf and callus were prepared in chloroform and then subjected to LCMS analysis. LCMS analysis was performed at the sophisticated analytical instrument facility, IOE, Vijnana Bhavan, University of Mysore. The analysis was performed on Water LCMS (Water Acquity system). Chromatographic separation was performed on an Egilent Eclipse C-18 column (5µm, 15cm, 4.6 mm id) using a binary mobile phase system consisting of 0.1% formic acid in water (A) and 90% acetonitrile in water (B) a flow rate of 500µL/min. the LC gradient program started 50 to 1000 m/z [22].

Statistical analysis

Experimental analyses were conducted in triplets (n=3). The results are represented as Mean± Standard Error. The data were analyzed statistically by one-way analysis of variance followed by Turkey's mean range test using SPSS software ver. 14. Probability values $p<0.05$ were considered significant.

RESULTS AND DISCUSSION

The dry weight and percentage of yield

The dry weight and percentage of yield of leaves and leaf callus extracted from different solvents is represented in (Table 1). The highest extraction yields were achieved with ethanol, yielding 7.35% from leaves and 11.75% from leaf callus; the next highest yields were obtained with methanol (3.81% from leaves) and chloroform (9.9% from leaf callus) [19].

Table 1 The dry weight and percentage yield of *Guaiacum officinale* extracts

Solvent	Dry weight (g)		% of yield	
	Leaves	Leaf callus	Leaves	Leaf callus
Hexane	0.656	0.325	1.64^{e}	1.625^{f}
Chloroform	1.255	1.98	3.14^{c}	9.9^{b}
Ethyl Acetate	0.493	0.563	1.23^{f}	2.82^{d}
Ethanol	2.94	2.35	7.35^{a}	11.75^{a}
Methanol	1.522	1.355	3.81^{b}	6.78^{c}
Water	0.91	0.34	2.28^{d}	1.7^{e}

Table 2 Phytochemical screening of *Guaiacum officinale* bark extract

Phytochemicals	Tests	Hexane		Chloroform		Ethyl acetate		Ethanol		Methanol		Water	
		Leaf	Leaf callus	Leaf	Leaf callus	Leaf	Leaf callus	Leaf	Leaf callus	Leaf	Leaf callus	Leaf	Leaf callus
Alkaloids	Dragendorff's test	+	+	+	+	+	+	-	+	+	+	+	+
	Mayer's test	+	+	+	+	-	-	-	-	-	+	+	+
Carbohydrates	Fehling's test	+ Green	+ Green	+	+	+ Red	+	+ Red	+	+	+	+	+
	Benedict's test	-	-	-	+	+	-	+	+	+	+	-	-
Amino acid/	Ninhydrin test	-	+	+	+	+	+	-	+	+	+	+	+
Protein	Biuret test	-	+	+	+	+	-	-	+	-	-	+	-
Tannins	Ferric chloride test	-	+	+	+	+	+	-	+	-	-	+	+
	Gelatin test	-	+	+	+	+	+	-	+	-	-	+	+
Saponins	Foam test	-	-	-	+	+	+	-	-	-	+	+	+
Flavonoids	Shinoda	+	+	+	+	+	+	+	+	+	+	+	+
Sterols	Salkowski	+	+	+	+	+	+	+	+	+	+	+	+
	Liebermann Burchard	+	+	+	+	+	+	+	+	+	+	+	+
Phenols	Iodine test	+	+	+	+	+	+	+	+	+	+	+	+
	Ferric chloride	-	+	+	+	-	+	-	+	+	-	-	+
Quinones	Alcoholic KOH	+	+	+	+	+	+	+	+	+	+	+	+
	Conc. HCl	+	+	+	+	+	+	+	+	+	+	+	+
Gums	Alcohol test	+	+	+	+	+	+	+	+	+	+	+	+

Preliminary phytochemical screening of leaf and leaf callus extracts

Secondary metabolites play a crucial role in mediating biological activities, including antibacterial and antifungal properties. Various phytoconstituents exhibit a multitude of biological activities. Phytochemical screening of both leaf and leaf callus revealed the presence of several promising compounds, including tannins, saponins, carbohydrates, alkaloids, terpenoids, flavonoids, phenols and quinones. Specifically, carbohydrates, alkaloids and saponins were detected in Chloroform, ethyl acetate and methanolic extracts, while terpenoids, steroids, phenols, quinones and flavonoids

were present in extracts obtained with all solvents refer (Table 2).

Quantitative analysis of secondary metabolites

The quantitative analysis of secondary metabolites in leaves and leaf callus of *Guaiacum officinale* was performed using standardized procedures, and the results are presented in (Table 3).

Table 3 Quantitative analysis of different extracts of *Guaiacum officinale* bark

Solvents	Protein		Carbohydrates		Phenols		Flavonoids		Saponins	
	Leaf	Leaf callus	Leaf	Leaf callus	Leaf	Leaf callus	Leaf	Leaf callus	Leaf	Leaf callus
H	4.60±0.17^{c}	4.01±0.25^{c}	6.34±0.51^{d}	5.43±0.15^{d}	3.66±0.36^{e}	2.76±0.63^{e}	1.02±0.00^{f}	0.92±0.01^{f}	1.80±0.04^{e}	1.22±0.14^{f}
Cl	5.00±0.12^{b}	6.00±0.21^{a}	30.89±2.78^{a}	29.09±1.08^{a}	4.40±0.25^{c}	3.70±0.51^{b}	1.35±0.07^{c}	2.53±0.20^{a}	4.99±0.21^{d}	5.67±0.12^{a}
EA	1.92±0.3^{d}	1.82±0.5^{d}	8.16±0.53^{c}	7.61±0.35^{c}	3.14±0.38^{f}	2.15±0.18^{f}	1.21±0.01^{d}	1.52±0.10^{d}	1.33±0.01^{f}	1.23±0.10^{e}
Eth	0.99±0.04^{e}	0.79±0.40^{e}	5.56±0.22^{e}	4.63±0.32^{e}	4.43±0.21^{b}	3.23±0.12^{c}	1.66±0.02^{b}	1.68±0.02^{c}	5.44±0.02^{a}	5.58±0.12^{b}
Mt	0.45±0.03^{f}	0.51±0.05^{f}	10.04±0.52^{b}	9.40±0.22^{b}	4.55±0.07^{a}	5.45±0.17^{a}	1.11±0.02^{e}	1.00±0.02^{e}	3.56±0.08^{c}	3.25±0.18^{d}
Aq	5.08±0.08^{a}	5.00±0.00^{b}	3.46±0.71^{f}	3.64±0.17^{f}	3.77±0.07^{d}	3.07±0.00^{d}	2.55±0.01^{a}	2.53±0.01^{b}	5.36±0.00^{b}	4.84±0.00^{c}

H- Hexane; Cl- Chloroform; EA- Ethyl Acetate; Eth- Ethanol; Mt- Methanol and Aq- Aqueous

Total protein content

Leaf extract showed highest phenolic content in aqueous (5.08mg GAE/100g) followed by chloroform (5.00mg GAE/100g). In contrast, leaf callus extract exhibited highest content in chloroform (6.00mg GAE/100g) surpassing the other solvent extracts.

Determination of carbohydrates:

The total carbohydrate content was determined to be significantly higher in chloroform extract both in leaves (30.89mg/100g) and leaf callus (29.09mg/100g).

Total phenolic content

The total phenol content was quantified using a standard curve generated from gallic acid. Leaf extract showed highest phenols in methanol (4.55 µg GAE/1µg) followed by ethanol (4.43 µg/1µg). Whereas, leaf callus extract exhibited highest phenolic content in methanol (5.45 µg GAE/1 µg) followed by chloroform (3.70 µg GAE/1 µg).

Total flavonoid content

The total flavonoid content was high in aqueous extract (2.55 mg/100g) of leaf and chloroform extract (2.53 mg/100g) of leaf callus.

Estimation of saponin content: The total saponin content was found to be highest in ethanolic extract (5.44 mg DE/100g) of leaf and chloroform extract (5.67 mg DE/100g)

Thin layer chromatography

The results of the thin layer chromatography (TLC) profiling are presented in (Table 4). Chloroform extract of both leaf and callus leaf showed the presence of alkaloid (0.9); flavonoid (0.5 and 0.9 respectively); steroid (0.75 and 0.6 respectively); tannins (0.5 and 0.9). The Rf value of various phytoconstituents present in different extracts, using diverse mobile phases, are presented in (Table 4, Fig 1-2).

TLC is a valuable technique for identifying bioactive compounds. In this study, TLC profiling of leaf and lead callus chloroform extracts revealed the presence of alkaloids, flavonoids, phenols, steroids and tannins. The chloroform extract demonstrated superior efficacy in extracting a maximum number of secondary metabolites compared to other solvents (hexane, ethyl acetate, ethanol and methanol). The Rf value serves as a polarity indicator, enabling the optimization of solvent systems for the isolation and purification of compounds from complex plant extracts [5]. A high Rf value in less polar systems indicates non polar compounds, while low Rf values suggests a polar compounds [29].

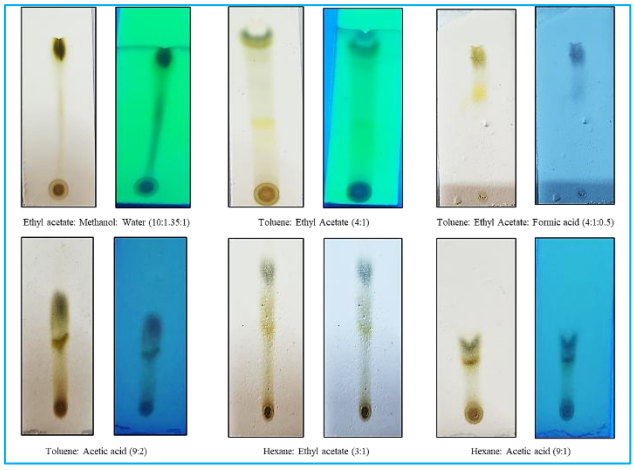

Fig 1 TLC plates of chloroform leaf extracts on different solvent mixture

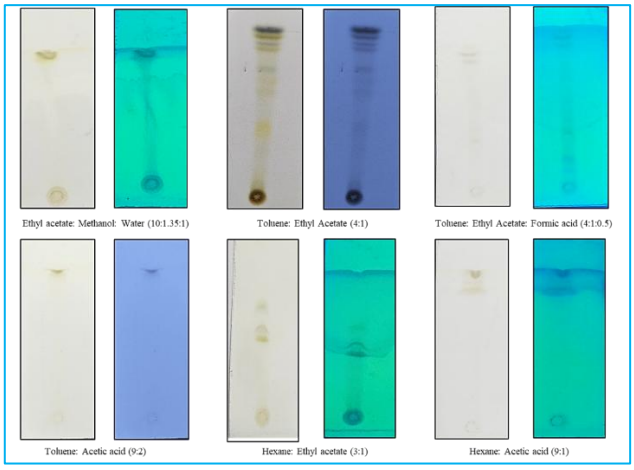

Fig 2 TLC plates of chloroform leaf callus extracts on different solvent mixture

Antibacterial activity

The antibacterial properties of plant extracts have been well documented, with numerous studies demonstrating their effectiveness against a range of pathogenic bacteria, including antibiotic resistant strains. This study focused on the antibacterial activity of leaf and leaf callus chloroform extracts against *Escherichia coli* and *Bacillus subtilis*, finding that *Escherichia coli* was more susceptible to the both extracts. The antibacterial properties of the extracts were evaluated using both well and disc diffusion method shown in (Fig 3-4). Hayet *et al*. [12] reported methanolic extract of *Peganum harmala* leaves showed strong antibacterial property against Gram

positive than Gram negative bacteria. In the present study the Gram-negative bacteria *Escherichia coli* is showing highest inhibitory activity than Gram positive bacteria *Bacillus subtilis* (Fig 3-4).

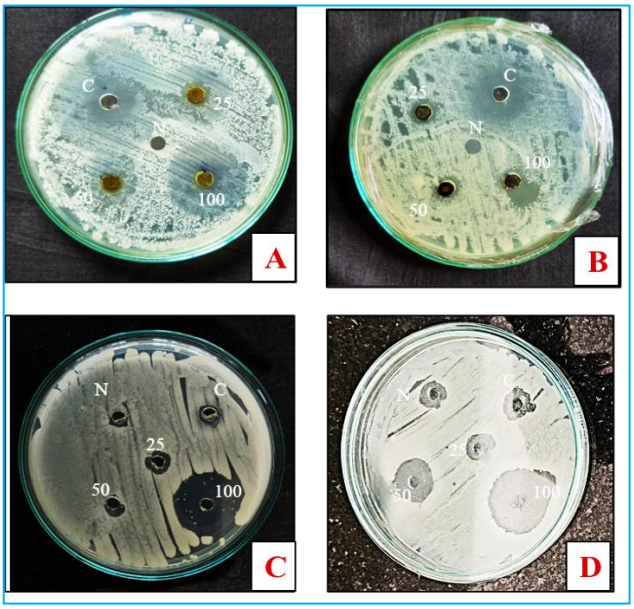

Fig 3 Antibacterial activity- well method of chloroform extracts of *G. officinale* leaf and leaf callus, (A-B) *E. coli* and *B. subtilis* on leaf extract; (C-D) *E. coli* and *B. subtilis* on leaf callus extract

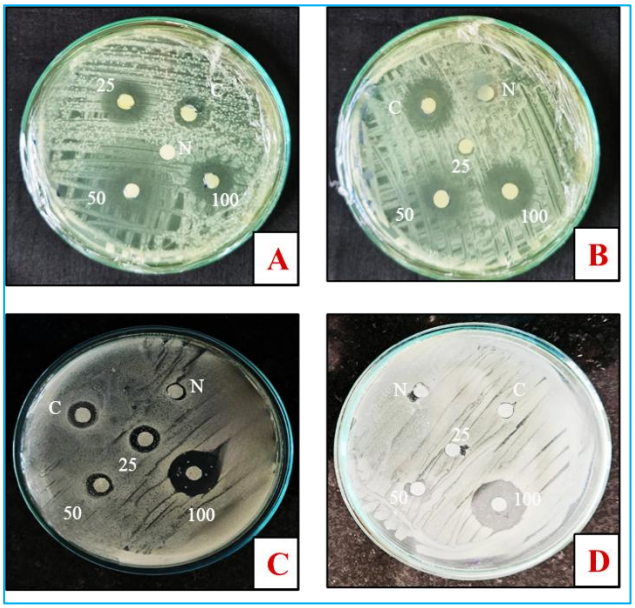

Fig 4 Antibacterial activity- disk method of chloroform extracts of *G. officinale* leaf and leaf callus, (A-B) *E. coli* and *B. subtilis* on leaf extract; (C-D) *E. coli* and *B. subtilis* on leaf callus extract

Table 4 TLC of *G. officinale* leaf and leaf callus chloroform extract

S. No.	Phytochemical	Solvent system	Leaf		Leaf callus	
			Fluorescence spot at 224nm	Rf value	Fluorescence spot at 224nm	Rf value
1.	Alkaloids	Ethyl acetate: Methanol: Water (10:1.35:1)	Green	0.8	Green	0.8
			Yellow	0.75	Yellow	0.75
			Light green	0.5		
		Toluene: Ethyl Acetate (4:1)	Purple	0.9	Purple	0.9
			Light green	0.825	Light green	0.85
			Blue	0.82	Blue	0.825
			Light green	0.8	Light green	0.8
			Blue	0.75	Blue	0.7
			Orange	0.625	Orange	0.6
			Green	0.4	Light Green	0.5
			Yellow	0.325	Yellow	0.4
					Light green	0.2
		Toluene: Ethyl Acetate: Formic acid (4:1:0.5)	Light green	0.9	Purple	0.9
			Green	0.64	Light green	0.85
			Yellow	0.6	Yellow	0.6
					Light green	0.4
					Green	0.2
					Light green	0.15
2.	Flavonoid	Toluene: Acetic acid (9:2)	Green	0.5	Green	0.9
			Light green	0.36	Light green	0.875
3.	Steroids	Hexane: Ethyl acetate (3:1)	Green	0.75	Yellow	0.6
			Light green	0.625	Green	0.5
			Yellow	0.55	Light green	0.45
4.	Terpenoids	Hexane: Acetic acid (9:1)	Green	0.5	Green	0.9
			Yellow	0.375	Light green	0.85
					Yellow	0.8

Antifungal activity

The antifungal assay results (Fig 5) indicate that the plant extract exhibited a maximum inhibitory effect against both selected plant pathogenic fungi, surpassing that of the commercial fungicide Nystatin. Notably, the extract demonstrated exceptional efficacy against *G. officinale* extracts

and significantly better result against *Fusarium oxysporum* compared to the fungicide.

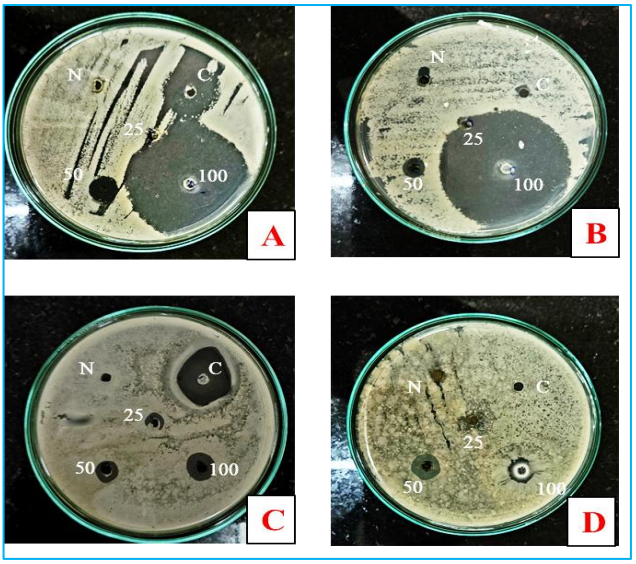

Fig 5 Antifungal activity- well method of chloroform extracts of *G. officinale* leaf and leaf callus, (A-B) *F. oxysporum* and *A. niger* on leaf extract; (C-D) *F. oxysporum* and *A. niger* on leaf callus extract

Determination of antioxidant activities

Antioxidant activity of *G. officinale* leaf and callus extracts showed promising antioxidant activity in DPPH and ferric reducing power assay.

Determination of DPPH radical scavenging activity

The DPPH of bark extracts revealed significant results of inhibition ($p \geq 0.05$). the decrease in absorbance indicates an increase in free radical scavenging activity regarding the colour changes from deep purple to colourless solution. The result showed the highest percentage of activity in chloroform leaf and callus extract with IC50 of 11.06 µg/ml and 13.35 µg/ml respectively followed by methanol leaf and ethanol callus extract with IC50 of 10.99 µg/ml and 11.75 µg/ml respectively radical scavenging activity (Fig 6-7).

Determination of ferric reducing antioxidant power assay

The reducing power assay of *G. officinale* leaf and callus was measured at 700nm. An increase in absorbance indicates an increasing reducing activity with the colour changes to light blue to dark blue colour. The chloroform leaf and callus extracts show the highest reducing power compared to other extracts. Chloroform leaf and callus extracts shows 1.56 µg/ml and 1.952 µg/ml respectively, followed by methanol leaf and callus extract 1.53 µg/ml and 1.78 µg/ml respectively (Fig 8-9).

Table 5 Bioactive compounds in chloroform callus extract of *G. officinale*

Name of the compound	RT (min)	Mass	Formula	DB Diff (ppm)
Quercetin-3-O-pentoside	2.573	459.21	$C_{21}H_{20}O_{11}$	2.2
Isorhapontigenin-3-O-glucoside	2.675	549.24	$C_{26}H_{28}O_{12}$	5.46
Quercetin-3-O-glycoside	2.742	583.22	$C_{21}H_{20}O_{12}$	5.32
Asiaticoside	2.878	622.22	$C_{48}H_{78}O_{19}$	3.53
Kaempferol	3.284	274.22	$C_{15}H_{10}O_6$	0.73
Quercetin-3-O-rutinoside	4.909	593.15	$C_{27}H_{30}O_{16}$	15.34
Bacopaside I	5.281	621.19	$C_{35}H_{56}O_{13}$	4.66

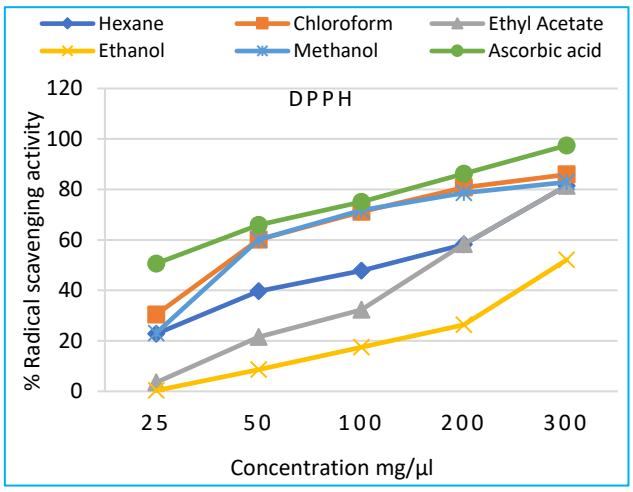

Fig 6 DPPH radical scavenging activity of various solvent extracts of *G. officinale* leaf

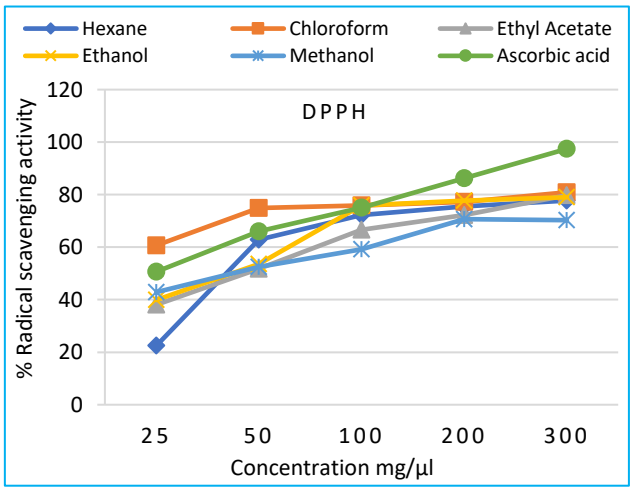

Fig 7 DPPH radical scavenging activity of various solvent extracts of *G. officinale* leaf callus

HR-LCMS analysis

HR-LCMS analysis of chloroform extracts of *G. officinale* callus extract revealed 5 major peaks, indicates the presence of diverse phytochemical constituents. Comparison of the HR-LCMS data with library databases enabled the characterization and tentative identification of these compounds. Identifies compounds are Quercetin-3-O-pentoside, Isorhapontigenin-3-O-glucoside, Quercetin-3-O-glycoside, Asiaticoside, Kaempferol, Quercetin-3-O-rutinoside and Bacopaside I. (Table 5, Fig 10).

Preliminary phytochemical and quantitative analysis done for the different solvent extracts of stem of *G. officinale*, shows the presence of all secondary constituents like saponins, tannins, flavonoids, phenols, alkaloids, steroids and carbohydrates. Ahmad *et al.* [2] confirm the presence of saponin as a major bioactive compound in *G. officinale* fruits.

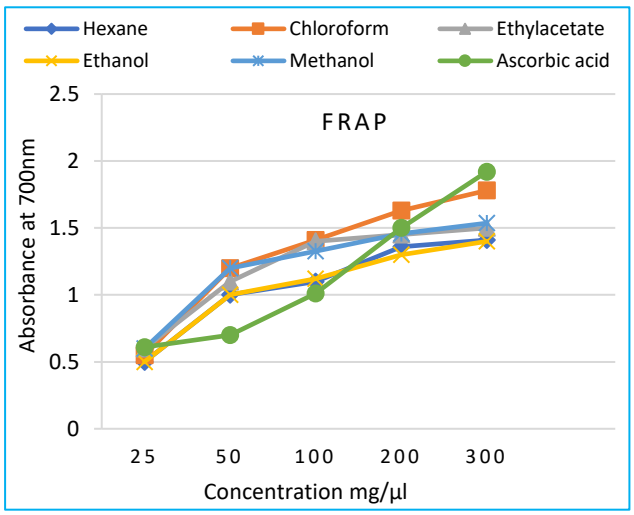

Fig 8 Ferric reducing antioxidant power assay of various solvent extracts of *G. officinale* leaf

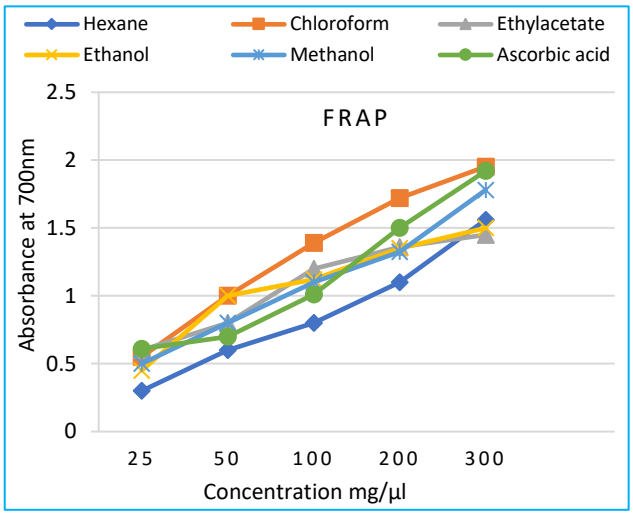

Fig 9 Ferric reducing antioxidant power assay of various solvent extracts of *G. officinale* leaf callus

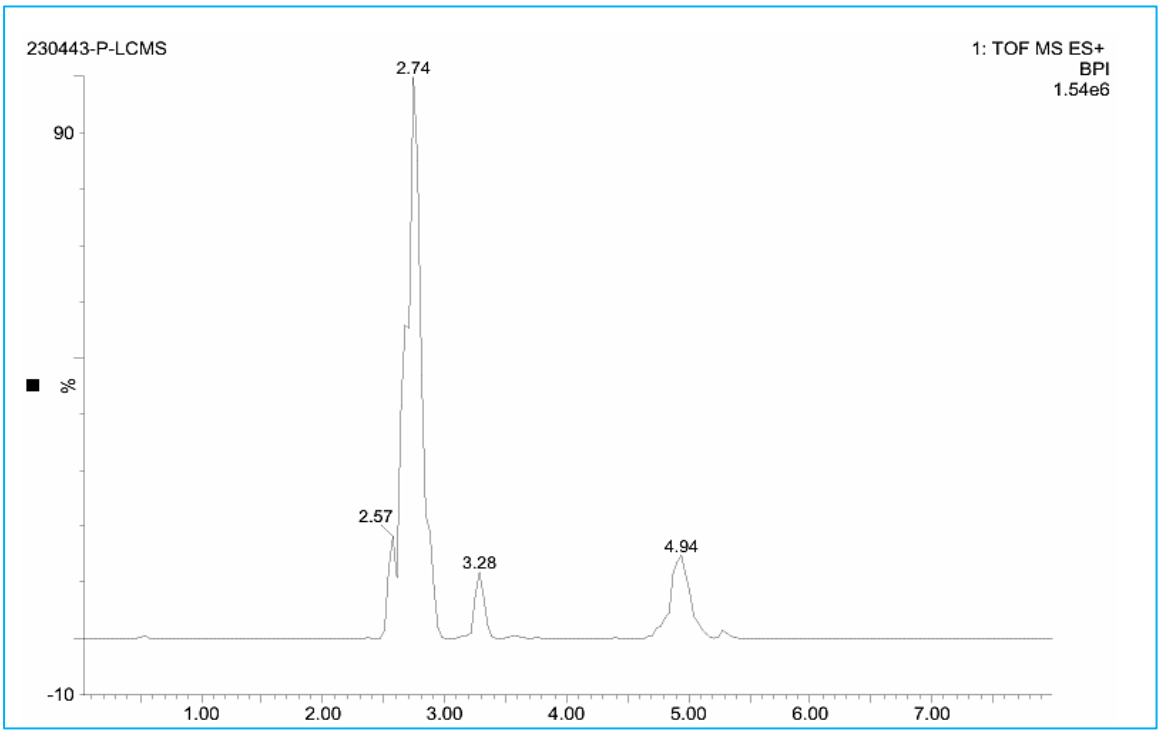

Fig 10 Chromatogram of chloroform callus extract of *G. officinale*

According to some other findings, Mabhiza *et al.* [17], Hanada *et al*. [11], the presence of alkaloids and polyphenols shows the highest bacterial inhibition activity which is similar to the present study where phenolic exudates were observed during *Escherichia coli* well diffusion method. The data reflects that all ethyl acetate extract shows significant zonation against both Gram positive and Gram negative [27]. There is much finding for antibacterial activity of *Guaiacum officinale*, some reports were on *Guaiacum coulteri* which exhibits anti-tuberculosis and anti-helicobacter activity [25], [30].

The DPPH (2,2-Diphenyl-1-picrylhydrazyl) assay was applied to access antioxidant potential of *Guaiacum officinale*. Mabhiza *et al.* [17] findings show the presence of antioxidant potential. Ethanolic twig extract of *Guaiacum officinale* showed the highest scavenging activity and compared with Vitamin C plant, which is used to cure cardio-vascular dieses and anti-cancerous agent [19]. Gan *et al.* [10] revealed the presence of phytoconstituents such as alkaloids and polyphenols which are responsible for strong antioxidant activity. This finding is similar to the present work.

CONCLUSION

From the present study we can conclude that plant can be excellent source of Phytoconstituents, HR-LCMS analysis of the chloroform callus extract of *Guaiacum officinale* revealed the presence of diverse secondary metabolites with potential medicinal properties. In particular chloroform leaf and callus extract possess highest phenolic content, antimicrobial activity against bacteria and fungi and exceptional antioxidant capability. This natural antioxidant holds great promise as a therapeutic agent. Future work includes the purification and manufacture of new drug.

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