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Phytochemical Analysis and Antimicrobial Activity of *Commiphora wightii* Plant (Guggul) Extract.

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ABSTRACT

In traditional medicine and Ayurveda plants have been known to relieve various diseases. The medicinal activity of plants mainly secondary metabolites are playing key role. In the present study ethyl acetate and methanol extract of *Commophora wightii* were screened for the presence of phytochemicals by standard procedures. Phytochemical analysis revealed the presence or absence of alkaloids, flavonoids, proteins, quinons, reducing sugars, saponin, and phenolics. The FTIR analysis confirmed the major fragments of the extracts like aldehydes, amine, acid, carbohydrates, and halides functional groups. Antimicrobial effects of phytochemical extracts evaluated against *E. Coli, Bacillus subtillus and Enterobacter aerogenes.*
Keywords: *Commiphora wightii,* Secondary metabolites, antimicrobial activity, FTIR.

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INTRODUCTION

Herbal drug is most demanded now days have negligible side effects and enhances immunity. The present study describes the ingredients derived from *Commiphora wightii* and their novel use in addressing the disease and health problems as potential role as green drug. *Commiphora* is widely distributed in tropical region of Africa, Madagascar and Asia. The distribution further extends to Australia and the Indian Ocean and Pacific islands [1].

Guggul, an oleo gum resin which is extracted from the plant of C. wightii, belongs to the family *Burseraseae*. *Guggul* comprises of 61% resin, 29.3% gum, 0.6% volatile oils, 6.1% moisture, and 3.2% foreign matter [2].

Commiphora wightii plants have antioxidant activity because of presence of *guggulosterone* [3].

Traditionally oleo gum resin is collected by the tapping of oleo gum-resin from erect type *guggul* plants in summer and the yield is about 200-800 g per plant. The plant contains essential oils, mainly myresene, dimyrecene and polymyrecene, Z-*guggulosterone*, E-*guggulosterone*[4]. Commiphora wightii plant is one of the most exploited plant for gum and resin production due to this the plant is under Red data list of IUCN and various approaches have been made by various organizations to conserve this species of plant by means of plant tissue culture methods [5].

Medicinal plants are the richest source of drug for traditional system of medicines, modern medicines, food supplements, and chemical entities for synthetic drugs. Nearly 80% of the world's population relies on traditional medicines for primary health care, most of which involves the use of plant extracts [6]**.** Medicinal plants contain some organic compounds, which provide physiological action on the human body, which includes alkaloids, flavonoids, tannins, steroids [5]. These bioactive compounds are generally derived from leave, root, stem, barks [7]. Prsence of guggul is mainly confirmed by tapping the bark of *Commiphora wightii* where 200-300 g of dry resin is present which is used in treatment of various diseases such as obesity, hypercholesteremia and other disorders related to lipid metabolism[8]. *Commiphra wightii* plant produces *guggulosterone* that have the capability to stimulate Low density lipid (LDL) receptor binding activity in hepatocytes and enhances its catabolism, and also inhibits oxidative modification of LDL [9].

By the study of green drugs/bioactive compounds of plants researchers have been proved that these compounds act as a safer way than synthetic drugs just like statins to treat hyperlipidemia[10]. Plant of *Commiphora wightii contains flavonoids, E- guggulosterone and Z- guggulosterone.* These flavonoids can be described as pharmocotherapeutics as they can help in the treatment of diseases such as hypercholesterolemia, hypertension, obesity and diabetes [11].

MATERIAL AND METHODS

Preparation of plant extract

Figure 1: *Commiphorawightii (Burseraseae)*

Commiphora wightii plants were collected from CIMAP, Lucknow (Figure -1). Whole plant was washed and air dried at room temperature under shade. Dried plants were powdered with the help of pestle and mortar. 15g of the plant powder was weighed and subjected to extraction with 100ml of methanol and ethyl acetate solvent separately for 8h (60-90^{0}C) using soxhlet apparatus. The extract obtained was concentrated by distillation and dried by evaporation at 40-50^{0}C.

Fourier Transformed Infra-red Spectroscopy (FTIR)

Principle

In time domain spectroscopy which is generally achieved by fourier transform, radiant power data is recorded as a function of time. Radiant power (v) is plotted against the time [12].

FTIR consist of

- Moving mirror
- Fixed mirror
- Beam splitter
- IR radiation source & detector

Phytochemical analysis [13]:

Phytochemical tests were carried out on the methanol & ethyl acetate extracts of *Commiphora wightii*, using standard procedure to identify & to confirm the constituents present in these extracts.

Test for Alkaloids

Mayer's test: 50µl of both methanol and ethyl acetate extracts of *C.wightii* were treated with 1.36g of mercuric chloride and 5g of potassium iodide in 100ml distilled water/Mayer's reagent and studied for the formation of cream colour precipitate.
Wagner's reagent: 10-50µl of extract was treated with 1.27g of iodine and 2g of potassium iodide in 100ml distilled water /Wagner's reagent.

Test for flavonoids

NaOH and HCL test: Small amount of extract was treated with aqueous NaOH and HCL and observed for the formation of yellow orange colour.
H_2SO_4 test: Extract was treated with conc. H_2SO_4 and observed for the formation of orange colour.

Test for proteins

Millon's test: 2ml of Millon's reagent was mixed with plant extract and observed for the formation of white color which turns red on gentle boiling, confirms the presence of protein.
Ninhydrin test: The extract was treated with 2ml of 0.2% ninhydrin and observed for the formation of blue and purple colour indicating the presence of amino acid and protein respectively.

Test for tannins

Extract was treated with 10% lead acetate and observed for the formation of white color precipitate.

Test for reducing sugar and carbohydrate

Fehling's test: Fehling A and Fehling B reagents were mixed together in equal volume & 2ml of it was added to extract and boiled in water bath and cooled. The appearance of brick red precipitate at the bottom of the test tube indicated the presence of reducing sugar.
Benedict's test: *Commiphora wightii* plant extracts were mixed with 2ml of Benedict's reagent and boil, and observed for the formation of reddish brown precipitate, which indicates the presence of the carbohydrates.

Test for steroids

Libermannburchard's test: Extracts were treated with 2-3 ml of acetic anhydride and few drops of glacial acetic acid followed by drops of conc. H_2SO_4 and observed for the formation of bluish green colour that indicates the presence of steroids.

Test for phenolic compounds

By $FeCl_3$: The *guggul* extracts were treated with neutral ferric chloride solution and observed for the formation of violet colour, indicating the presence of phenolic compounds.
By 10% NaCl: The *guggul* extracts were treated with 10% sodium chloride solution and observed for the cream colour.

Test for saponins

1ml of each extracts of *guggul* were diluted with 20ml of distilled water and shaken well in a test tube and observed for the formation of foam on upper region of the test tube gives the information that the saponnins are present in the extracts of *Commiphora wightii*.

Antimicrobial activity testing of the extract against the selected bacterial strains

Agar well diffusion method was carried out for antimicrobial activity testing of extract against the selected microorganisms. A well of 10 mm diameter punched off with the help of sterile cork borer in the Muller Hinton agar plate and then 50µl of extract was carefully added to the well. Plates and tubes were incubated at 37°C in an incubator for 24 h [14]. The antibacterial activity was evaluated by measuring the diameter of zone of inhibition excluding the diameter of well. Streptomycin and penicillin were used as a positive control and methanol and ethyl acetate were used as negative control.

Micro-organisms selection and maintenance

Microorganisms used in this study: *Bacillus subtilis* (MTCC), *Escherichia coli* (MTCC), *Enterobacter aerogenes* (MTCC) were obtained from stock culture in the Department of Biotechnology, Lovely Professional University, Punjab. The organisms were stored on agar slant and kept in the refrigerator, prior to sub culturing.

Table 1: microorganisms selection

S. No.	Bacteria	MTCC No.
1	*E. coli*	MTCC 40
2	*Bacillus subtillis*	MTCC 121/441
3	*Enterobacter aerogenes*	MTCC 7325

RESULTS

Commiphora wightii

10 gm. of powdered plant of *Commiphora wightii* was taken and extraction was done with the help of methanol and ethyl acetate as solvents.

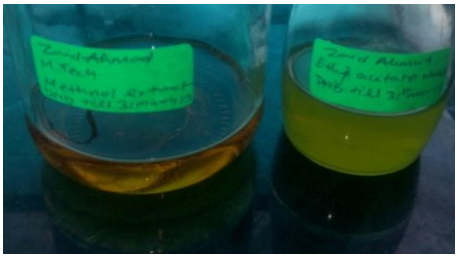

Figure 2: Methanol & Ethyl acetate extracts of *C. wightii*

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Fourier Transformed Infra-Red (FTIR)

A thin film of *guggul* active eluted fraction in methanol was applied on the glass and IR spectra were recorded by using SHIMADZU - 8400S spectrophotometer, Spectrum Instrument with FTIR paragon 1000 PC software at the Lovely Professional University, Punjab.

FTIR Spectra of *guggul*

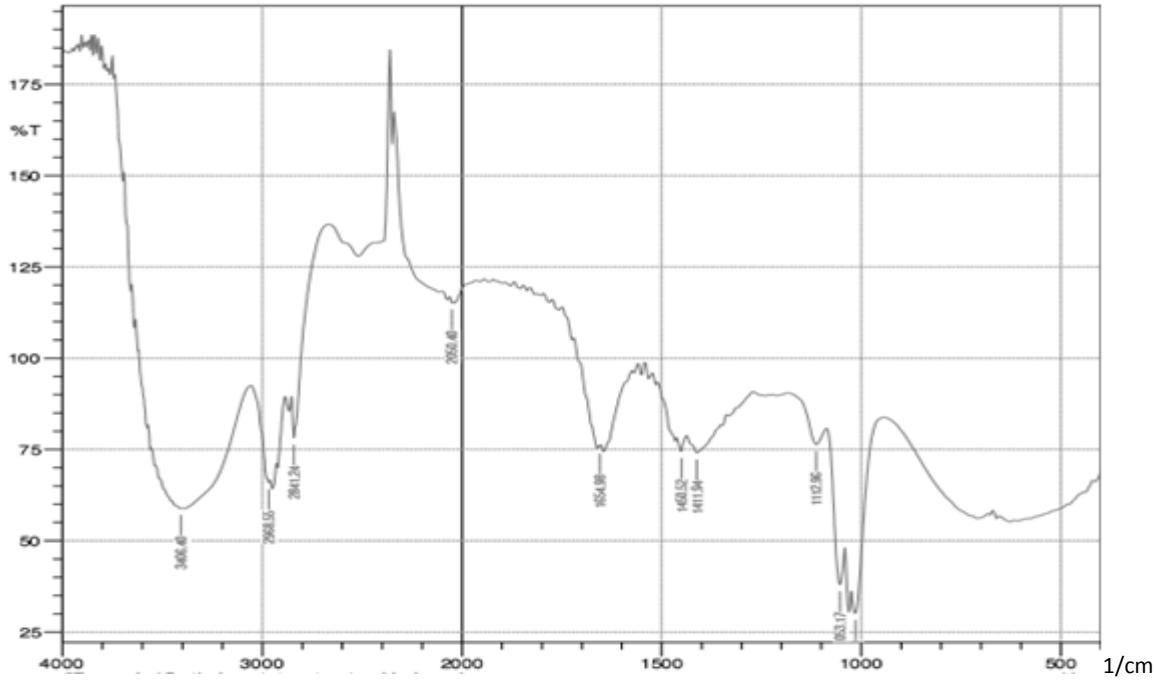

Figure 3: FTIR spectra of compound isolated from *Commiphora wightii* Methanol extract

Observations [15-21]

Table 2: IR spectra of compound isolated from *Commiphora wightii* Methanol extract Phytochemical test

Observed absorption (cm^{-1})	Reported frequencies (cm^{-1})	Functional group/Vibration Assignment	Reference
3406.40	3330-3500	-OH symmetric and asymmetric stretching	15, 16
2968.55	**Lipid** 2800-3000	$-CH_2$, $-CH_3$	17
2841.24	2800-3000	Lipid	17
2060.40	-	-	-
1654.98	Protein 1500-1700	α-helix	18
1450.52	Polyester 1440-1460	Various δ (C-H) modes	16
1411.94	1390-1640	Ionic Phosphate	19
1112.96	1000-1150	Polyester overlap carbohydrate, various C-O-C & C-C-O vibration	16
1063.17	1033-1164	Essential linear molecule (glucomannan)	20, 21

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Secondary metabolites have chemical properties i.e they react with chemicals to which they gives colour by which they shows the presence & absence of different compounds.

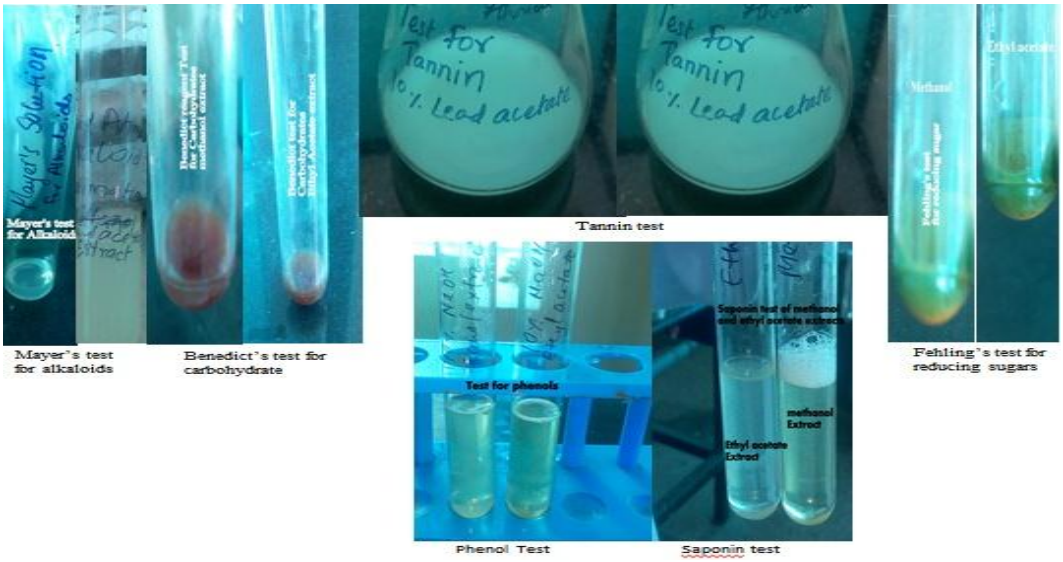

Figure 4: Phytochemical Screening test of *Commiphorawightii*methanol and ethyl acetate extracts

Test results: Phytochemical screening of *Commiphora wightii* extracts

Table 3: Phytochemical screening results

S.No.	Test	Observation	
		Methanol Extract	Ethyl Acetate Extract
1.	Mayer's test	+	+
2.	Dragandroff's test	+	+
3.	Protein	+	-
4.	Tannin	+	+
5.	Carbohydrate	-	-
6.	Reducing sugar	+	+
7.	Flavonoid	+	+
8.	Saponins	+	-
9.	Phenol	+	+

Antimicrobial Analysis

In the present study, the antibacterial activity assay of *Commiphora wightii* extracts against selected the one Gram- positive and two Gram-negative bacteria (Table 2). The extracts were extracted using ethyl acetate and methanol. The zone of inhibition of 13 mm was recorded against E. coli at 100 µl concentration of ethyl acetate extract, whereas 20.1mm zone of inhibition was shown by methanol extract at a concentration of 100µl (Table 2). *E. coli* and *B. subtillis* was found to be most susceptible organism (13 mm and 21mm zone of inhibition respectively) on the other hand gram negative bacteria, *Enterobacter aerogens* showed a maximum zone of inhibition of 14mm and 19mm by methanol and ethyl acetate extracts of *guggul*,

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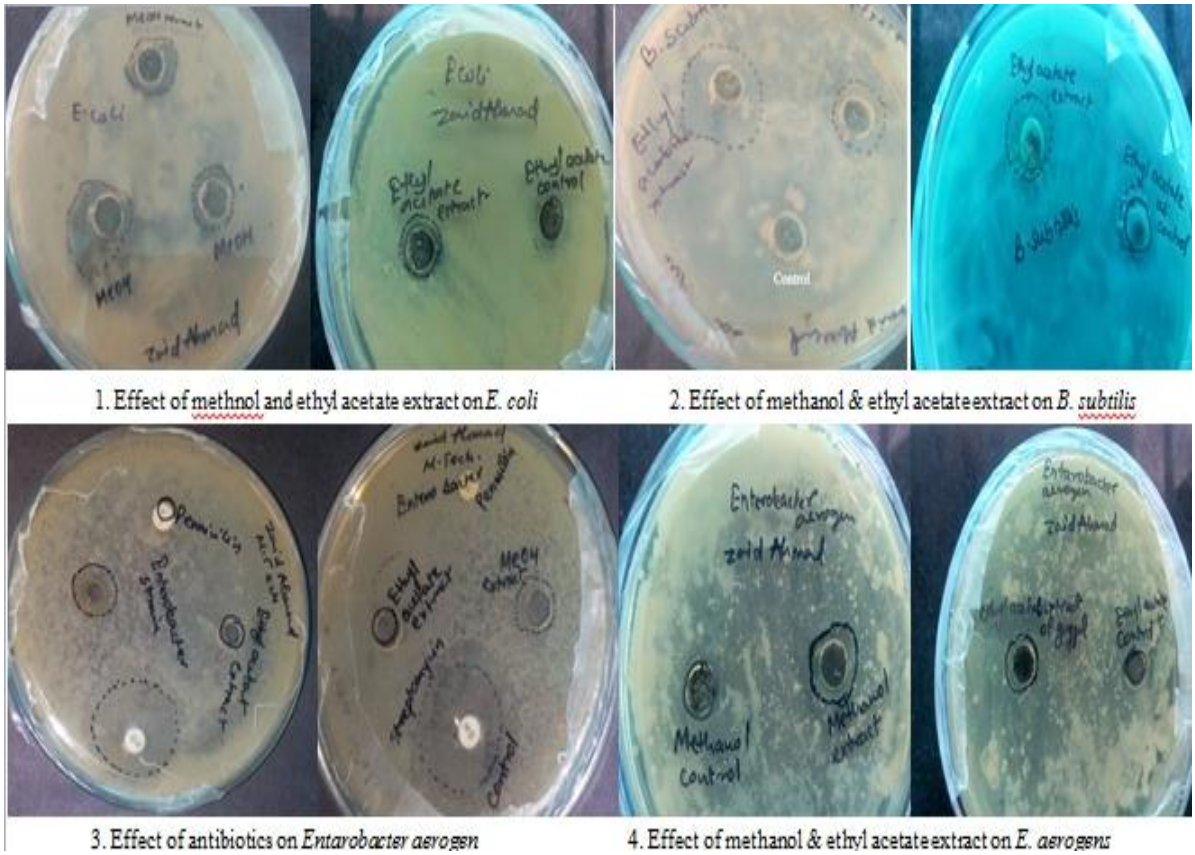

Figure 5: Zone of inhibition by *guggul* extracts against different micro-organisms

Table: 4 Zone of inhibition by *guggul* against microbes

Zone of inhibition of *Commiphora wightii* (in mm)								
Escherichia coli				*Bacillus subtilis*				*Enterobacter aerogenes*
Extract types	Zones of inhibition			Extract types	Zones of inhibition			Zone of inhibition
	Antibiotics		Sample		Antibiotics		Sample	Sample
Methanol	Tetracycline	29.5	15	Methanol	Tetracycline	34.1	12	17
	Streptomycin	19.7	22		Streptomycin	16.1	14	19
Ethyl acetate	Tetracycline	26.1	12	Ethyl acetate	Tetracycline	28.3	15	13
	Streptomycin	17.2	13		Streptomycin	18.8	21	14

DISCUSSION

By the study of IR spectra of *Commiphora wighii,* methanol and ethyl acetate extracts it was confirmed that the plant of *Commiphora wightii* possesses functional groups such as Amine, Alkanes. Lipid.Ionic Phosphate, polyester overlapping, α-helix, δ modes (-C-H), -C-O-C etc.

In the present study presence of different secondary metabolites like terpenoid, flavanoid, saponin, tannin, protein, alkaloid, glycosides and steroid was confirmed by various phytochemical tests for methanol & ethyl acetate extracts. These showed that the extracts are rich in most of the secondary metabolites. These bioactive compounds were reported to show medicinal activity as well as physiological activity. Some of *Commiphora wightii* plant metabolite were found to be absent in some of the extracts analysed. It has been

confirmed that phenolic compounds like tannins present in the cells of plants are potent inhibitors of many hydrolytic enzymes such as proteolytic macerating enzymes used by plant pathogens[22].

Antimicrobial activity was observed against *E.coli* and *Bacillus subtilis* which may be due to different bioactive compounds present in the plant of *Commiphora wightii*. Generally very less or no activity is observed against *Enterobacter aerogenes*.

CONCLUSION

From this study it can be concluded that various phytochemicals such as alkaloids, flavonoids, Saponins, & Tannin have been confirmed in all the *Commiphora wightii* plant. The evidence of antimicrobial interaction between various extract of these plants and their effects are associated with the presence of phytochemicals like steroid, saponins, tannins, flavanoids, and alkaloids which have been shown to possess antimicrobial properties. So, this plant can be used to discover bioactive compounds that may serve as lead for the development of new pharmaceutical agents for therapeutic needs & this plant can be explored as potential source of useful drugs.

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