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Screening and isolation of bioactive factors from *Commiphora myrrha* and evaluation of their antimicrobial activity.

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Abstract

The medicinal plants represent an enormous reservoir of potential microbial compounds that could be useful as an alternative to synthetic microbicides and are being used to develop drugs. In the present study, preliminary phytochemical analysis and antibacterial activity of *Commiphora myrrha* (Burceracea) the resin collects from bark and stem of the plant by the process incision. The resin powder extracts of *Commiphora myrrha* were tested against different gram negative bacteria by disc diffusion method. It was found that ethyl acetate and hexane extract possess bacterial growth inhibition where as methanol extract having antibacterial activity only on higher concentration and the extract is separated by TLC and silica gel preparative chromatography to fractionate bioactive constituents. Thereafter, the fraction purified by HPTLC and active fraction was analyzed by FTIR.

Key words: *Commiphora myrrha*, Antibacterial activity, Minimum Inhibitory Concentration, Zone Of Inhibition.

1. Introduction:

The crude preparations of medicinal plant extracts are exhibiting a great potential in clinical pathology. Scientists are combing the earth for phytoalexins which could be developed to battle various infectious diseases. A lot of studies have found that, the magnitude of synthetic antibiotic can be enhanced by combining it with extracts of antibiotics obtained from natural plant products (1). Bacterial pathogens have evolved numerous defense mechanisms against antimicrobial agents. The phenomenon of antibiotic resistance exhibited by different microbes is different to different antibiotics (3). The indiscriminate use of various antibiotics has led to the emergence of multi drug-resistant pathogens. Although several novel antibiotics are being developed, the efforts become futile once the microbe develops the resistance to the drug. The present study is concentrated on the medicinal plants *Commiphora myrrha* which is very effective in the treatment of urinary tract infection (2). With this background, the present study was carried out to evaluate the antibacterial potential of different solvent extracts of resin powder of bark and stem of the plant by the process incision of *Commiphora myrrha*.

2. Materials and methods

2.1. Plant Materials:

The resin powder of bark and stem of the plant by the process incision of *Commiphora myrrha* collected from Visakhapatnam hills (Eastern Ghats) andharapradesh. Plant material was then cut into smaller pieces and then first washed with tap water followed by washing with distilled water. It was than dried under sharing sunlight until water droplets got completely evaporated. Peel and plant were then kept in hot air oven for two days so that it could get dried. Dried resin was then taken for grinding by the help of mixer grinder. The coarse powder of plant sample was then used throughout the study (4-5).

2.2. Test Organisms:

Microbial strains of urinary track resistant pathogens *Klebsiella, Enterococci Escherichia coli, Pseudomonas aeruginosa* were provided by different diagnostic centeres they were subcultured and used throughout the studies (15).

2.3. Extraction procedure:

The coarse powder of plant material was dissolved in different solvents. The solvents used were non polar as well as polar (methanol, ethanol and water).50 gm of ground peel and 50 gms of whole plant powder were added 500 ml of water contain soxhlet apparatus so that secondary metabolites got completely extracted. The extracts were then filtrate obtained was evaporated to dryness at 50-65^0 c in a rotary vacuum evaporator to obtain dark color molten mass (14).

2.4. Phytochemical Analysis of Extract:

The method described by Harborne with slight modifications were used to screen the bioactive compounds present in the extracts (6).

Test for steroids:

10 ml of the plant extract was evaporated to dry mass and dissolved in 0.5 ml of solvent. Acetic anhydride (0.5 ml) and 2ml of concentrated sulphuric acid were added. A green color or blue

color or a mixture of these two colors was indicated as positive for in the presence of steroid compounds.

Test for Tannins:

1 cm^3 of freshly prepared 10% KOH was added to 1 cm^3 of the extract. A dirty white precipitate indicated the presence of tannins. Powdered coarse powder of test plant (1.0) was weighed into a beaker and 10 ml of distilled water added. The mixture was boiled for five mintues. Two drops of 5% $FeCl_3$ were then added. Production of greenish precipitate indicated the presence of tannins.

Test for Flavanoids:

A small piece of magnesium ribbon was added to extract of the plant material,this was followed by the drop wise addition of concentrated hydrochloric acid. Colors varying from orange to red crimson to magneta indicated flavonones.

Test for Alkaloids:

The extract of plant sample (0.5g) was stirred with 5ml of HCL on a steam bath. The solution obtained filtrate was treated with two drops of mayer's reagent. The two solutions were mixed and made up to 100 ml with distilled water. Turbidity of the extract filtrate on addition of Mayer's reagent was regarded as evidence for the presence of alkaloids.

Test for saponins:

The extract of plant sample (0.5g) was introduced in to a tube containing 5.0 ml of distilled water and shake vigorously for 2 minutes formation of froth indicates the presence of saponins.

Test for glycosides:

The extract of plant sample 1g added in to separate beakers .to one of the beakers was added 5ml dilute sulphuric acid while 5ml sulphuricacid is added to other beaker. The two beakers were heated for 3-5 minutes and the contents Filtered in to labeled test tubes. The filtrate was made alkaline with 5%sodium hydroxide and heated with fehlings Solution for 3 mins. The presence of reddish precipitate in the acid filtrate and the absence of such precipitate in the aqueous filtrate were regarded as positive for glycosides.

3. Antimicrobial activity:

Antibacterial activity was assessed by Agar well diffusion method of Kirby Bauer where in Muller hinton agar plates were prepared and were spreaded with 30ul of the available pathogenic cultures. Wells of 6mm diameter were bored using sterile borer. Wells were loaded with antimicrobial, tetracycline as standard and distilled water as Control and were incubated at 37^oC for 24 hours (9).

3.1.Minimum Inhibitory Concentration

MIC of the antimicrobial extracts was also determined using broth serial dilution technique wherein the Antimicrobial was diluted serially in a series of test tubes containing nutrient broth and they were loaded with the respective pathogen against which MIC was to be calculated. The tubes were incubated and then growth of the pathogen was detected using spectrophotometer at 600 nm. Concentration in the tube where growth increased drastically was stated as Minimum inhibitory concentration (8).

4. Thin layer chromatography:

The crude extracts were analyzed by TLC. The plates were developed by silica gel and optimized mobile phase. The developed plates were exposed to uv chamber at 254 nm in order to detect the spots. The different ratios of mobile phases like Ethyl acetate & n-Hexane (2:3) Ethyl acetate & n-Hexane (3:2) Ethyl acetate & Toluene (1:9) Ethyl acetate & toluene (95:5) Ethyl acetate & Toluene (93.7).(10).

5. Fourier-transform infrared spectroscopy (FTIR)

FTIR has proven to be a valuable tool for the characterization and identification of compounds or Functional groups (chemical bonds) present in an unknown mixture of plants extract. In addition, FTIR spectra of pure compounds are usually so unique that they are like a molecular"fingerprint". For most common plant compounds, the spectrum of an unknown compound can be identified by comparison to a library of known compounds. Samples for FTIR can be prepared in a number of ways. For liquid samples, the easiest is to place one drop of sample between two plates of sodium chloride. The drop forms a thin film between the plate and analyse the functional groups (11).

6. Results & Discussion:

The development of drug resistance in human pathogens against commonly used antibiotics necessitated a search for new antimicrobials of mainly plant origin. The Preliminary phytochemical screening and antibacterial screening of various extract of resin powder extracts of *Commiphora myrrha* showed good results as illustrated in the below Table-1.

6.1. Phytochemical analysis:

Preliminary phytochemical screening of the whole plant resin extract of *Commiphora myrrha* positive results for the presence of secondry metabolites like flavonoids, alkaloids, tannins, glycosides, steroids, saponins, tannins and terpenoids. Bio active compounds like flavonoids, glycosides are rich in methanolic extract (12).

Table-1

Methanol extract Contain chemical compounds	**Color test result *commyphera myrrah***
Flavonoids	++
Alkaloids	-
Tannins	+
Glycosides	++
Steroids	++
Saponins	++
Terpenoids	+

Table-1 Preliminary phytochemical screening Methanolic extract resin of *commiphora myrrah.*

6.2. Antimicrobial activity:

Nearly 80% of the world populations depend on the traditional medicine for primary health care, mainly includingthe use of natural products . Researchers have extensively studied the biological properties of and resin of *Commiphora myrrha* extracts their results showed that this plant is ethno medically valuable . *Commiphora myrrha* resin extracts are currently used for treatment of UTI diseases and in the preparation of therapeutic formulae. Flavonoids rich of *Commiphora myrrha* have antibacterial, antifungal and antiprotozoal activity. In the current study the methanolic and extract of *Commiphora myrrha* extracts showed Zone ofinhibition of atleast 12 mm against *P.aeruginosa* which was greater than that of cefotaxime 24 , 17mm against *E.coli* which was a little lesser than that of Standard (16mm) and 14mm against *E.coli* which was greater than that of standard cefotaxime (16mm) respectively. The antibacterial activity of peels of *Commiphora myrrha* resin extract may bei ndicative of presence of metabolic toxins or broad spectrum antimicrobial compounds that act against gram – ve bacteria. Ethanolic extracts exhibited higher degree of antibacterial activity as compared to that of other extracts tested against bacteria that cause gut infection, stomachache, diarrhea. Reported that *Commiphora myrrha* contains large amount of alkaloids (25%) and antibacterial activity may be indicative of presence of secondary metabolites. The ethanolic extract of *Commiphora myrrha* extracts showed some extent of antibacterial activity against microorganisms.

Test Organisms	Antimicrobial activity		Zone of inhibition		Zone of inhibition	
	MIC	MBC	Extract (200 µg/ml)	Antibiotic (100 µg /ml)	100 µg /ml	50 µg /ml	25 µg /ml
Pseudomonas	6.25 mg	12.5 mg	12 mm	40 mm	11mm	10mm	--
E.coli	12.5 mg	25 mg	17 mm	24 mm	10mm	6 mm	--
Enterococci	3.12 mg	12.5 mg	22 mm	30 mm	17mm	12 mm	10 mm
Klebsiella	12.5 mg	25 mg	15 mm	26 mm	10mm	--	--

Table-2
Antimicrobial activity of *commiphora myrrha* in various concentrations.

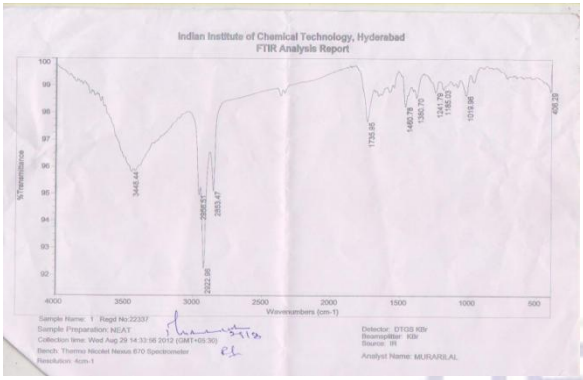
6.3. Thin layer chromatography:

The extracted compound is separated by using different polar and non polar solvents like Ethyl acetate & n-Hexane (2:3) Ethyl acetate & n-Hexane (3:2) Ethyl acetate & Toluene (1:9) Ethyl acetate & toluene (95:5) Ethyl acetate & Toluene (93.7) and observed five spots under uv chamber at 254 nm in order to detect the spots.

6.4. **Fourier-transform-infrared spectroscopy (FTIR)**

FTIR has proven to be a valuable tool for the characterization and identification of compounds or Functional groups (chemical bonds) present in an unknown mixture of plants extract. The below figures shows the different functional groups in bioactive fraction isolated from *commiphora myrrha*.

Activefraction-1



Active fraction-2

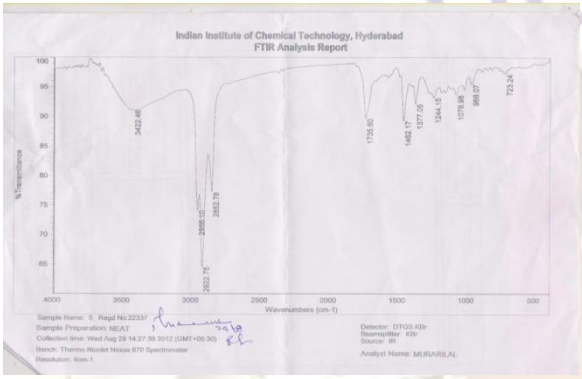


Figure 1&2: Different functional groups isolated bioactive fractions.

7. Conclusion:

The present study Agar well diffusion method of cefataxime sodium, a cephalosporin antibiotic shown the highest zone of inhibition against pseudomonas aeruginosa at 100mg/ml (40mm) where as commiphora myrrh extract shows the highest zone of inhibition against Enterococci at 200mg/ml (22mm). Five bacterial strains were used to evaluate the effect of plant extract by agar well diffusion method. In all the organisms the highest zone of inhibition was observed for Enterococci at 100mg/ml (17mm). And no inhibition zone found to Klebsiella at 50mg and 25mg finally the plant extract shows the inhibition zones at 100mg, 50mg, 25mg, against the organism Enterococci. For remaining organisms less or no inhibition zones were found. So Enterococci organism is more sensitive to *commiphora myrrha*. The results obtained in the present study showed that the MIC against Enterococci is high i.e. 3.12mg/ml.

References

1. El Ashry ES, Rashed N, Salama OM, Saleh A. Components, therapeutic value and uses of myrrh. *Pharmazie* 2003; 58-3: 163.
2. Drago I, Mombelli B, De Vecchi E, Fassina MC, Tocalli L, Gismondo MR. *In vitro* antimicrobial activity of propolis dry extract. *J Chemotherapy* 2002; 12: 390–395.
3. Bonajar Shahibi GH. Evaluation of antibacterial properties of iranian medical plants against micro coccus aureus, serratia marcescens, Klebsiella Pneuomiae and bodella branchoseptica. Asian. J. Sci. 2004; 3-1: 82-86.
4. Oyaizu AB, Andrea L, Klara S, Agnes K. Reducing power of the natural polyphenol of Sempervivum tectorum Invitro and Invivo. 2003; 47(1-4):99-102.
5. Shen T, Lou HX. Chemical constituents from resin of Commiphora species and their biological activities. Nat. 2008.
6. Shen T, Lou HX Chemical constituents from resin of Commiphora species and their biological activities. Nat. Prod. Res. Dev. 2008; 20-2: 360-366.
7. Ding AW. Isolation and biological activities of neomyrrhaol and other terpenes from the resin of *Commiphora myrrha*. Plata Med. 2009b 75-1: 351-355.
8. Harborne, J.B., Phytochemical methods. In A guide to modern techniques of plant analysis 3rd ed.,pp:40-137. 1998.
9. Kroschwitz, J.I. and M. Howe-Grant, Kirk-Kandha tribe of Orissa, India. J. Ethnopharmacol. 1992.
10. Baur AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standard single disk method. Am J Clin Path 1966; 45: 493-496.
11. Prashanth D, Asha M, Amit A Antibacterial activity of Punica granatum. Fitoterapia. 2001-72:171–173.
12. Sahuvinod.K, Irchhaiyaraghuveer, Shashialok, Gurjar himanshu. Phytochemical investigation and chromatographic evolution of the ethanol extract of whole plant extract. Issue 1, Vol. 1 2010.
13. Shibumon G and Benny P J A review on the medicinal significance of common fruits. Int. J. Biomed. Res. Analysis. 2010; 1-2: 60-64.
14. Stockwell, C., 1988. Nature's pharmacy. London United Kingdom. Century Hutchinson Ltd.
15. Supayang PV, Treechada S, Surasak L, Thanomjit S, Tetsuya I, Takeshi H Inhibitory effect of active compounds from Punica granatum pericarp on verocytotoxin production by enterohaemorrhagic Escherichia coli O 157: H 7: J. Health Science. 2005; 51: 590-596.