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PHYTOCHEMICAL SCREENING OF *GARUGA PINNATA* ROXB ROOT USING GC-MS ANALYSIS AND POTENTIAL OF ANTIBACTERIAL ACTIVITY

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ABSTRACT

Garuga pinnata Roxb commonly known as kondavepa is the deciduous tree belongs to family burseraceae with a unique medicinal properties including as astringent, bronchodilator, stomachic, expectorant, pulmonary infection, antidiabetic etc. The present study explore the primary phytochemical study using gas chromatography-mass spectroscopy (GC-MS) and in vitro anti microbial study (against gram positive bacteria *Staphylococcus aureus* and gram negative bacteria *Escherichia coli*) was performed on n-hexane root extract of *Garuga pinnata Roxb.* After preliminary phytochemical investigation done by GC-MS analysis shows the presence of various phytoconstituent in n-hexane extract. Gaschromatograph of n-hexane extract shows the presence of 20 phytoconstituent which are subjected to mass spectroscopy. The mass spectroscopy of these peaks does not give any prominence result it shows only presence of different functional groups. The list of possible phytoconstituent is given by checking compound in NIST and NBS library. Further results also indicate that n-hexane extract of *Garuga pinnata* have marked amount of total phenols which could be responsible for the anti microbial activity. The mechanism and chemicals remains unclear and could be further investigated by detailed phytochemical investigation.

Introduction

Plant produces these chemicals to protect themselves but the research have shown that they have the capacity to treat human diseases in an effective way[1]. Medicinal plants are the richest bio-resources of folk medicines and traditional systems of medicine; and food supplements, nutraceuticals, pharmaceutical industries and chemical entities for synthetic drugs[2]. Comparing to modern medicine the herbal medicine was the lifesaving drug. Among 4,00,000 plant species only 6% of the plants are studied for their biological activity and only few have been phytochemically investigated[3]. This shows that the investigation is needed for many medicinal plants for its activity and pharmacological properties. The present study explore the n-hexane extract of dried root of *Garuga pinnata* for phytochemical analysis followed by qualitative and quantitative determination of the compounds. The non-nutritive plant chemicals are called as phytochemicals which have the properties to protect or prevent diseases. Phytochemical constituents are the basic source for the establishment of several pharmaceutical industries. The constituents are playing a significant role in the identification of crude drugs.

Garuga pinnata, (Burseraceae) (Figure1/table 1) commonly called as golika or kakad is one such medicinal plant possesses several pharmacological properties, where, few are already reported and few are yet to be investigated. The leaves of this plant are found to be having noticeable amount of phenolic compounds, which may involve in controlling various oxidative and reductive processes[4]. Leaves contain amentoflavone. Stem bark extract gave positive tests for steroids, terpenes, alkaloids, flavonoids and saponins. An euphane triterpene alcohol has been isolated from this plant[5]. Two diarylheptanoids, 6'-Hydroxygaruganin and Garuganin were isolated from *G. pinnata*[6]. Pheophorbide-α and-β methyl esters which are isolated from methanol crude extracts of this plant are reported paramount cytotoxic activity against KB and its drug resistant human cancer cell lines[7]. Garuganins I and II compounds isolated stem bark hot petrol and methanol extracts exhibits analogous mechanisms of antibacterial action[8]. The fruits are stomachic and expectorant; given in diarrhea whereas, the stem juice is commonly used as eye drops to cure opacities of the conjunctiva[9]. The stem bark of this plant in the combination of pepper is used to treat the diabetes[10]. But it there is no study found in the study about the root composition and its biochemical activity. With his aim, the present study carried out to extract the phytochemical constituents in the root extract of *Garuga pinnata* and tested for anti microbial activity of the extract against gram positive and gram negative bacteria.

Figure 1: Garuga pinnata

Table 1: Botanical Classification

Kingdom	Plantae
Order	Sapindales
Family	Burseraceae
Genus	*Garuga*
Species	*Pinnata*

Experimental

MATERIALS AND METHODS

***Chemicals*:** All Chemicals used in the entire study were AR grade obtained from SD fine, Merck chemicals, India, Pvt Ltd.,

***Plant Material*:** Dried roots of *Garuga pinnata* Roxb were collected in the month of April 2012 from Local (Elur) agricultural fields area and plant is botanically identified by the department of Botany, CRR College and a voucher specimen for this collection has been maintained in dept of botany, CRR College. Dried root powder collected in bulk (500grams) and completely dried for one month in sunlight to eliminate surface moisture. Then dried powder packed into envelops and kept in oven at 55^{o}C temperature for further dryness. Dried mass later grinded separately in a mortar obtained fine powder and sieved; which was then kept in plastic bags for further use.

Preparation of plant extract: The dry root powder of *Garuga pinnata* Roxb passed through sieve (100μ). The coarse powdered drug (200grams) was extracted in Soxhlet apparatus for 48 h with n-hexane (60-75°C, 2L) extract obtained was concentrated under reduced pressure in rotatory evaporator below 60°C temperature to get semisolid sticky residue (20gm)

GC-MS Analysis: GC-MS analysis of n-hexane extract sample was performed using a Perkin-Elmer GC Clarus 500 system and Gas chromatograph interfaced to a Mass spectrometer (GC-MS) equipped with Elite-I, fused silica capillary column (30mm x 0.25mm 1D x 1 µMdf, composed of 100% Dimethyl poly siloxane). For GC-MS detection, an electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.99%) was used as the carrier gas at constant flow rate 1mL/min and an injection volume of 2µl was employed (split ratio of 10:1); Injector temperature 250°C; Ion-source temperature 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5seconds and fragments from 45 to 450 Da. Total GC running time was 72 minutes. The relative % amount of each component was calculated by comparing its average peak area to the total areas, software adopted to handle mass spectra and chromatograms was a Turbomass. Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained (Table 2 & Figure 2).

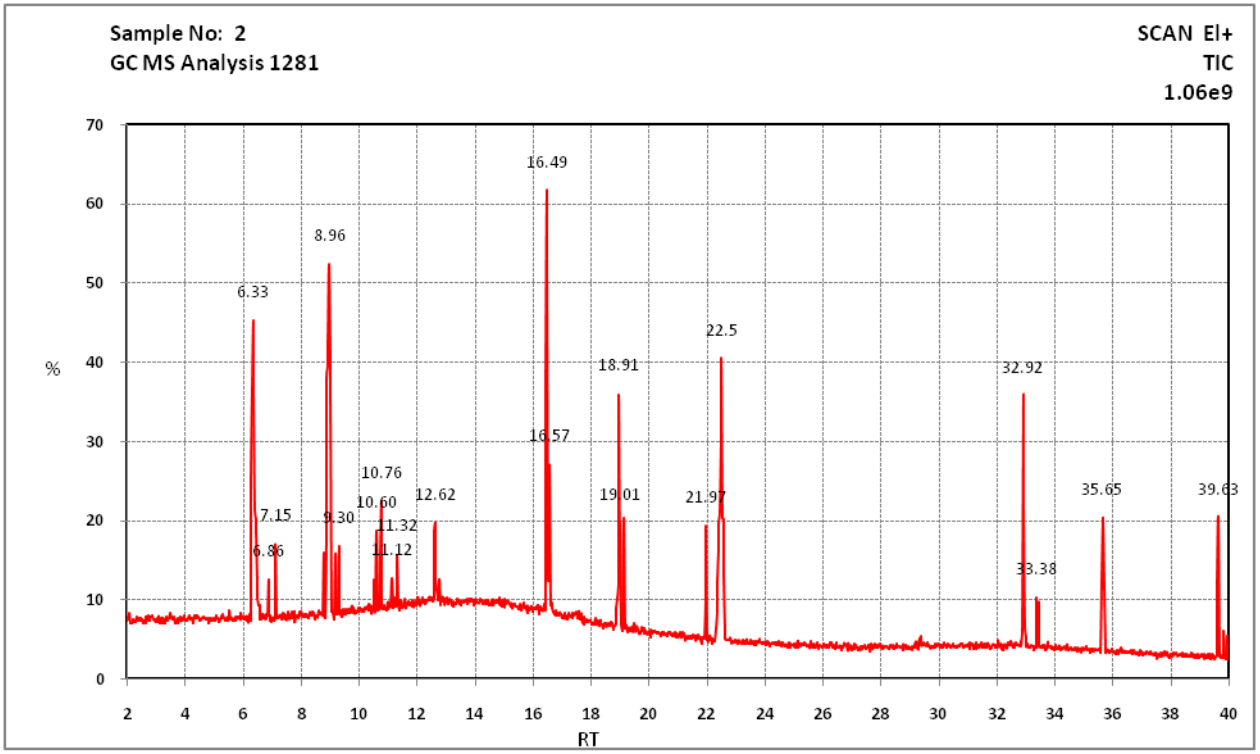

Figure 2: GC MS Phytochemical screening of *Garuga pinnata* Roxb root n-hexane extract

Table 2: Components detected in n-hexane extract of *Garuga pinnata* Roxb root extract

S.NO	RT	n-hexane root extract	Molecular Formula	Molecular Weight	Peak Area (%)	Activity
1.	6.33	β –caryophyllene	$C_{15}H_{24}$	204.36	46.5	Anti inflammatory Anticancer, Antioxidant, Antimicrobial
2.	6.86	α- humulene	$C_{15}H_{24}$	204.35	10.2	Anti-inflammatory,Flavor
3.	7.15	Terpineol	$C_{10}H_{18}O$	154.25	14.8	Flavor and aroma,Antioxidant,Antimicrobial.
4.	9.96	Lavandulol	$C_{10}H_{18}O$	154,25	52.2	Antioxidant,Antimicrobial.
5.	9.30	2,6-Dimethyl-1,5,7-Octa trien-3-ol	$C_{10}H_{16}O$	152.23	12.4	Flavoring agents

6.	10.6	Estazolam	$C_{16}H_{11}ClN_4$	294.7	11.8	Anxiolytic, anticonvulsant, sedative and skeletal muscle relaxant properties.
7.	10.76	2-hexane	C_6H_{14}	86.18	12.1	Industrial uses manufacturing. Toxic.
8.	11.12	3-Heptanone	$C_7H_{14}O$	114.18	10.8	Flavor and fragrance agents
9.	11.32	α-amorphene	$C_{15}C_{24}$	204.35	11.4	Antimicrobial.Antioxidant
10.	12.62	Aristoloshene	$C_{15}H_{24}$	204.35	18.6	Industrial use,
11.	16.49	Caryophyllene	$C_{15}H_{24}$	204.36	64.7	Anti-inflammatory Antioxidant ,Antimicrobial, antitumor, Anticancer
12.	16.57	Trans-nerolidol	$C_{15}H_{26}O$	222.37	30.2	Flavoring agent and in perfumery
13.	18.91	Propofol	$C_{12}H_{18}O$	178.27	34.7	Pharmacokinetics, induction and maintenance of anesthesia,
14.	19.01	Pentadecenol	$CH_3(CH_2)_{14}OH$	228.41	20.5	Antimicrobial,antitumor,Anticancer
15.	21.97	n-Amylmercaptan	$C_5H_{12}S$	104.21	18.61	No activity reported
16.	22.5	Methyl cyclopentane	C_6H_{12}	84.16	40.3	Antioxidant
17.	32.92	Camphor	$C_{10}H_{16}O$	152.23	35.8	Moth repellent, Antimicrobial , flavoring, anti-odor element, Anticancer. Antioxidant
18.	33.39	Humulen-6,7-epoxide	$C_{15}H_{24}$	204.35	9.6	Anti-inflammatory
19.	35.65	Pinocarvone	$C_{10}H_{14}O$	150.22	21.4	Flavor and fragrance agents, Antimicrobial. Antioxidant.
20.	39.63	L-linalool	$C_{10}H_{18}O$	154.25	20.9	Industrial use, Insecticide

#Source: Dr. Duke's phytochemical and ethnobotanical databases [Online database].

Anti-Bacterial Activity by Disc Diffusion Method[11]

Preparation of Inoculum: *Escherichia coli* and *Staphylococcus aureus* strains were used. 50ml of nutrient broth was prepared in 100ml conical flask. It was sterilized & then inoculated with inoculum with the help of sterile loop in laminar air flow from preserved slants. They were then kept in incubator at 37°C for 24 hours for organism to grow.

Preparation of Media: 200ml of nutrient agar media (NAM) was prepared and the pH was maintained at 7.0 to 7.2.

Pour Plate Method: 1ml of prepared inoculum was poured in sterile Petri dish & then 15 ml of NAM was poured in it & allowed to solidify.

Disc Diffusion Method: After solidification the disc of whatman 42 filter paper imbibed with 20 µl plant extracts diluted with ethanol in aliquots 10%, 15% and 25% were carefully placed with the help of forceps at the centre of the Petri dish and then kept in incubator for 24hrs.

Measurement of Zones: With the help of antibiotic zone scale the zone of inhibition (ZOI) were measured

Phytochemical Qualitative Analysis

Phytochemical screening of root extract as been analysed, which revealed at the presence of constituents which are known to exhibit medicinal active compounds as well as physiological activities. Analysis of the plant extract gives a positive test in the qualitative analysis for alkaloids, Terpenoids, carbohydrates, saponin, tannin, glycosides, steroidal glycosides; phenolic compounds etc., identified by standard tests[12] and the functional groups are shown in the table.2 & 3

RESULTS & DISCUSSION

Metabolite profiling in plant species was done by gas chromatography mass-spectrometry method for last few years. But only a limited number of plant research laboratories have gas chromatography mass-spectrometry. The identified compounds occupy many biological properties. GC-MS analysis of phytoconstituents in plants gives a clear picture of the pharmaceutical value of that plant. Thus, this type of GC-MS analysis is the first step towards understanding the nature of medicinal properties in this medicinal plant and this type of study will be helpful for further detailed study. Further investigation is needed to identify the pharmacological importance and phyto-chemistry of *Garuga pinnata* Roxb.

GC-MS is the best techniques to identify the constituents of volatile matter, long chain, branched chain hydrocarbons, alcohols acids, esters, etc. Peak area, retention time and molecular formula were used for the confirmation of phytochemical compounds. The active principles with their Retention time (RT), Molecular formula, Molecular weight (MW) and peak area in percentage are presented in table 2. GC/MS analysis of n-hexane extract of the phytochemical components has been analyzed qualitatively in our lab by the common methods using chemicals. Analysis of the plant root extract gives a positive test in the qualitative analysis for alkaloids, Terpenoids, carbohydrates, saponin, tannin, glycosides, steroidal glycosides; phenolic compounds etc., identified by standard tests[10] and the functional groups are shown in the table 3. Major compound identified in the GC-MS analaysis is Caryophyllene (64.7%), later concentration is Lavandulol in third position in a row is Methyl cyclopentane, Trans-nerolidol, Terpineol, Propofol, Pentadecenol, n-Amylmercaptan, Camphor, Humulen-6,7-epoxide, Pinocarvone,L-linalool, and β –caryophyllene, α- humulene

Table 3: Display the presence/ absence of different phytochemicals in the root extract of *Garuga pinnata* Roxb

Phytoconstituents	**Test**	n-Hexane extract
Alkaloids	Wagner's test	++
Amino acids	Ninhydrin Test	--
Carbohydrates	Molish test	++
Cardiac glycosides	Keller-Killani test	--
Flavonoids	Shinoda's test	++
Phenolics	phenol test	++
Polysterols	Salkowski's Test	++
Proteins	Biuret test	--
Saponins	Frothing test	++
Steroids	Libermann-Buchard's test	++
Tannins	Ferric chloride test	--
Terpenes	Salkwaski's test	++

FT IR Analysis of root extract

Fourier Transform Infrared Spectroscopic Analysis the FTIR spectrum of n-hexane extract of *Garuga pinnata* Roxb given in figure 3. The region of IR radiation helps to identify the functional groups of the active components present in extract based on the peaks values of the FTIR spectrum. When the extract was passed into the FTIR, the functional groups of the components were separated based on its peaks ratio. The results of FTIR analysis confirmed the presence of Alcohol, Aldehyde, Alkyne, Alkene, Amines and Ester. The absorbance bands analyses in bioreduction process are observed in the region between 400–4000 cm^{-1} are 1018, 1109, 1690, 2924, 2838 and 3253.2 cm^{-1}. Major peaks were observed at 3253.2 cm^{-1} that could be assigned to the –OH stretching vibrations of alcohol. So the present study results indicate that the primary functional group

present in *Garuga pinnata* is O-H alcohol. Other functional groups present in the hexane extract of *Garuga pinnata* are groups of aldehyde/ketones, alkyne, alkene and amines, except ester. The results of the present study are in accordance with the study of Starlin T, Raj CA, Ragavendran P and Gopalakrishnan VK [13].

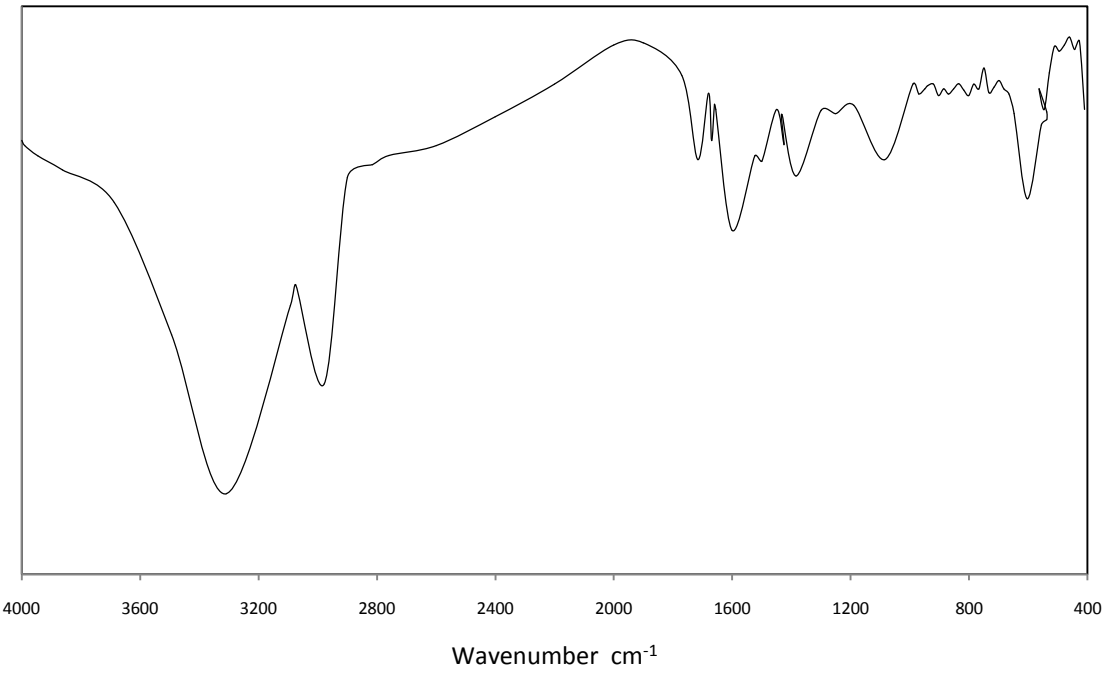

Figure 3: FT IR Spectra of n-hexane *G.Pinnata* root extract

The source of many plants (herbs and spices) can be often identified from the peak pattern of the chromatograms obtained directly from headspace analysis. Similarly, particular qualitative and quantitative patterns from a GC analysis will show all the compounds in the leaf extract. *Garuga pinnata* have many biological properties which can be used in various purposed to treat many diseases. The compounds identified by the initial qualitative analysis and GCMS analysis have many uses in medical field. Each compounds identified have their unique character to treat various diseases. Further studies needed to reveal its importance in specific field to treat the diseases properly

Anti microbial activity

The inhibitory action of identified compounds had been historically recognized and applied as a useful therapeutic agent for preventing wound infections. The antibacterial activities of extracted material were investigated against two different types of bacterial strains like *Escherichia coli* and *Staphylococcus aureus.* (Figure 4) showed excellent antibacterial activity against tested bacterial strains at the volumes of 50 µL/well in 10, 15, 25% dilutions. The zone of inhibition (in mm) ranges identified for *Escherichia coli* (15.2, 19.6 and 22.3 mm) and for *Staphylococcus aureus*(16.2,17.0 & 20.7 mm) (Table 4). The diameters of the inhibition zones for the two tested pathogens are listed in Table 3. Thus, our results show that root n-hexane extract sample has potential bacterial activity against *S.typhi* and *S.aureus* more precisely against *S.coli*

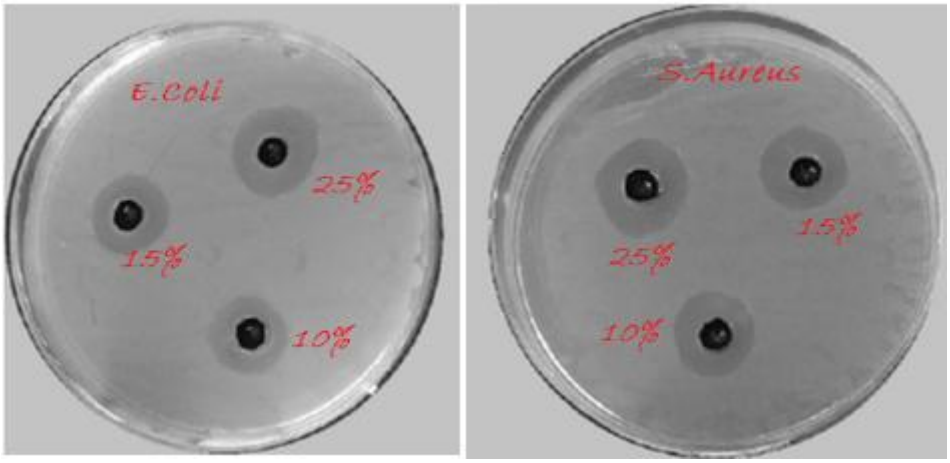

Figure 4: Determination of Anti bacterial activity by Disc method against *E.Coli & S.Aureus* bacterial cultural

Table 4 : Zone of inhibition (mm) obtained by disc diffusion method.

Components	Zone of inhibition(mm)	
	Staphylococcus aureus	*E.Coli*
10% extract	16.2	15.2
15% extract	17.0	19.6
25% extract	20.7	22.3

Conclusion

In conclusion, the present study has shown that extract of root extract of *Garuga pinnata* Roxb has significant anti-bacterial activity, but further studies are required to identify & isolate the actual phytoconstituents present in this plant which are responsible for other medicinal activities. The presence of phytochemicals such as total phenolics, alkaloids, phenolsetc., (Table 2) in *Garuga pinnata* Roxb provides some scientific evidence for the biological activities and also accounts for the multi pharmacological use of this plant in traditional medicine. The results of this study offer a base of using *Garuga pinnata* Roxb as herbal alternative for the synthesis of antimicrobial agents. It would be worthwhile to further isolate the compounds and determine their specific activity and also to understand the synergistic effect of compounds for therapeutic roles. The work is in progress to ascertain its biological activity and brighten the pharmacological profile of it in the arena of traditional medicine.

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