

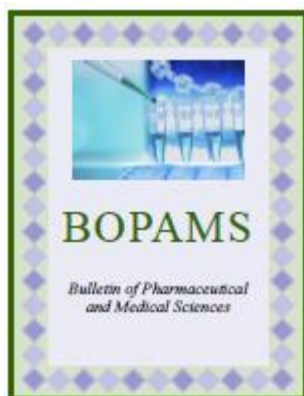


ANTI-MICROBIAL EVALUATION OF SOME ANTI-DIARRHOEAL PLANTS OF MEDICINAL PLANTS OCCURRING IN LOCAL AREA OF NAGARAM TALUK, ANDHRA PRADESH, INDIA

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ABSTRACT

Based on an ethnobotanical survey in different villages of Nagaram mandal related to microbial and inflammatory diseases, 10 plant species belonging to nine botanical families were inventoried. Selected plant species used in the treatment of rheumatism, skin diseases, Scabies, itches, boils, abscess, eczema, leucoderma, eye diseases, pains, bruises and sprains; internally for cough, cold, fever and microbial infections were selected and submitted to a biological investigation including antimicrobial and anti-inflammatory activities. Phytochemicals as secondary constituents contain terpenoids flavonoids and tannins and alkaloids. Medicinal plants have antibacterial and anti-inflammation activities. The present study involves ten different medicinal plants namely *Cannabis sativa*L., *Celosia cristata*, *Cinnamomum verum*, *Curcuma longa*, *Datura metel*, *Ipomoea aquatica*, *Ipomoea batatas*, *Ixora coccinia*, *Litsea monopetala* and *Psidium guajava* respectively. Based on personal interview with senior public in the study area selected plant parts of selected medicinal plants were washed, air dried and then powdered. The ethanolic extract of plant samples were used for the phytochemical analysis to find out the phytochemical constituents in the plants. The result suggests its anti-inflammatory and antibacterial potential of the herbs, which could be due to the bio-active principles which are anti-inflammatory and antibacterial in nature. The present study therefore emphasizes the use of selected ten plant materials as an anti-inflammatory and antibacterial drug against macrophage cells and bacteria respectively.

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INTRODUCTION

The medicinal plants are useful for healing as well as for curing of human diseases because of the presence of phytochemical constituents [1]. Phytochemicals are naturally occurring in the medicinal plants, leaves, vegetables and roots that have defense mechanism and protect from various diseases. Phytochemicals are primary and secondary compounds. Chlorophyll, proteins and common sugars are included in primary constituents and secondary compounds have terpenoid, alkaloids and phenolic compounds [2]. Terpenoids exhibit various important pharmacological activities i.e., anti-inflammatory, anticancer, anti-malarial, inhibition of cholesterol synthesis, anti-viral and anti-bacterial activities [3]. Terpenoids are very important in attracting useful mites and consume the herbivorous insects [4]. Alkaloids are used as anaesthetic agents and are found in medicinal plants [5].

Diarrhoea is a killer disease worldwide and unfortunately, it happens to be amongst the symptoms of many other diseases. In most rural communities of developing countries like India, diarrhoea poses serious problems particularly to children due to amongst other reasons, lack of adequate sanitation and pipe borne water. The disease burden worldwide from water, sanitation and hygiene together has been calculated to be 4 % of all deaths and 5.7 % of the total disease burden. Amongst the many known water borne diseases, diarrhoeal diseases (including cholera) kill more than 1.8 million people every year, mostly children from developing countries (WHO, 2004). Diarrhoea has long been recognized as one of the most important health problems in developing countries). It is defined as an increase in the frequency, fluidity or volume of bowel movements and is characterized by increased frequency of bowel sound and movement, wet stool, and abdominal pain. In clinical terms it is used to describe increased liquidity of stool, usually associated with increased stool weight and frequency.

Treatment of diarrhoea is generally non-specific and is usually aimed at reducing the discomfort and inconvenience of frequent bowel movements. To overcome the menace of diarrhoeal diseases in developing countries, the World Health Organization (WHO) has included a programme for the control of diarrhoea, which involves the use of traditional herbal medicine [10].

Medicinal plants are of important therapeutic aid for various ailments including the diarrhoeal diseases. It is estimated that over 20,000 species from several families are useful for these purposes. However, there have been numerous reports on the use of traditional plants for the treatment of diarrhoeal diseases In recent years, secondary plant metabolites (phytochemicals) with unknown pharmacological activities have been extensively investigated as a source of medicinal compounds [11].

In this study, we aimed to determine the *in vitro* antibacterial activity of extracts from some selected medicinal plants from Nagaram Mandal of Andhra Pradesh against the most common bacterial pathogens. The ten selected plants in this study have remained as integral part of traditional medicine in Nagaram mandal to treat different types of infectious diseases, including diarrhea, respiratory tract infection, cholera, and skin and wound infections.

There are high rates of occurrence of stomach ailments among the Nagaram mandal people. The diseases gets magnified given the fact that they lack proper medication because of high poverty rates hence prefers the use of local treatment by use of medicinal plants instead of hospitalization. The community believes in medicinal plants first before the patient is hospitalized and in most cases, hospitalization is because of intoxication due to overdoses. In addition, hospitalization comes in when most patients become more serious with the ailments, which may be because of inefficiency of the medicinal plants used probably due to under dosing. It becomes necessary therefore to carry out an evaluation of the most common plants the Nagaram Mandal public use for the treatment of stomach ailments to validate their efficacy.

Materials and Methods

Plant Materials and Extract Preparation

Popular plants used in traditional medicine by the ethinc people across nagaram mandal, based upon previous ethnobotanical literatures and potential medicinal values as judged by local healers, were screened and selected to include in the present study. The collected plants were identified at the National Herbarium and Plant Laboratories, Nagarjuna University.. The most potential parts of the plants that could exhibit antimicrobial activity as judged by the traditional trend for the parts to be used for the treatment of diseases were selected for the study (Table 1). All collected plant materials were air-dried at room temperature under shade, and pulverized into fine powder and processed for extract isolation. The elaborated steps for the isolation and processing of the plant materials are covered in supplementary information

Study Area Description

The study area is the part of the Guntur district, Nagaram mandal consist of 73 Villages and 25 Panchayats. Thotapalle is the smallest Village and Allaparru is the biggest Village. It is in the 10 m elevation(altitude). Geographical coordinates are 16°0'16"N/80°43'28"E (Long/Latitude) (Figure 1). It is located 53 KM towards South from District headquarters Guntur. 331 KM from State capital Hyderabad

towards west. Telugu is the Local Language here. Also People Speaks Urdu . Total population of Nagaram Mandal is 51,388 living in 14,546 Houses, Spread across total 73 villages and 25 panchayats . This area is also located in Coastal line of the state and 20 KM far way from bay of bengal. Telugu is the Local Language here. Also People Speaks Urdu . Total population of Nagaram Mandal is 51,388 living in 14,546 Houses, Spread across total 73 villages and 25 panchayats. Weather and Climate of Nagaram Mandal is Hot in summer. Nagaram summer highest day temperature is in between 32 ° C to 42° C . Average temperatures of January is 24 ° C , February is 26 ° C , March is 28 ° C , April is 31 ° C , May is 33 °C. The sub district is home to about 50 thousand people, among them about 25 thousand (50%) are male and about 25 thousand (50%) are female. 75% of the whole population are from general caste, 23% are from schedule caste and 3% are schedule tribes. Child (aged under 6 years) population of Nagaram mandal is 9%, among them 51% are boys and 49% are girls. There are about 15 thousand households in the sub district and an average 3 persons live in every family. 100% population of Nagaram mandal live in the Nagaram Sub District rural part. Total about 30 thousand people in the sub district are literate, among them about 16 thousand are male and about 14 thousand are female. Literacy rate (children under 6 are excluded) of Nagaram is 65%. 71% of male and 58% of female population are literate here. Overall literacy rate in the sub district has increased by 4%. Male literacy has gone up by 2% and female literacy rate has gone up by 5%.



Figure 1: Location of Study area

Reasons for undertaking the present work and its significance

The importance of survey of plant resources have been emphasized by Jain (1978) who says After independence our planners realized that in agricultural country like India where the flora' is so varied and rich, a proper consensus of the flora of the country aid its evaluation for economic exploitation is very important". In this regard, it is essential that we should have full knowledge regarding the occurrence, frequency, distribution and phenology of various plants for their proper utilization.

Material and Methods

Survey on the Use of Medicinal Plants

The information regarding the traditional knowledge, local uses of plants within the study area, the local names, and parts used, purposes, modes of administration, and curative properties, and so forth was recorded through intensive interviews and discussions with elderly people (men/women), herbal healers, local vaidas, using a well- structured questionnaire (Annexure-1).

The ethnobotanical surveys were carried out from March 2013 to July 2014 using semi structured questionnaire (Martin 1995) and interview was conducted with the senior residents of the study area. Prior to the administration of the questionnaire, conversations with the informants were held with the assistance of local Farmers' Association representative to elaborate the objective of the study and to build on trust with the common goal to document and preserve the knowledge on medicinal plants. 350 informants were interviewed and the female informants' age ranges from 30 to 85 years and the mean age is 51 years, and the male informants' age ranges from 30 to 93 years and the mean age is 64 years (Figure 2). The informants, except the

healers, were selected randomly and no appointment was made prior to the visits. They were asked to give their knowledge about the plants they use against a disease, plant parts harvested, method of preparation of the remedy, details of administration and the dosage. Specimens of the reported medicinal plants were collected during regular systematic walk in the fields.

Chemicals: All chemicals and reagents had analytical grade. Silver nitrate, n-hexane with high purity purchased from Sd Fine Chemicals, India.

Apparatus and Instruments: The conventional Soxhlet extraction apparatus was used, which consists of a condenser, a Soxhlet chamber, and an extraction flask. The extractor thimble was permeable one with 44 mm internal diameter and 200 mm external length. The rotary evaporator was used for evaporation of solvent of extracted material.

Collection of plant materials: Materials of 10 plant species (Table 1) were harvested in March 2013 from Nagaram mandal, Andhra Pradesh, India. They were carefully washed, oven-dried for 1 h at 160°C and put in the shade in an aerated place till complete drying, then were ground into a fine powder.

Sampling and extraction: The fresh sample of various plant materials was collected at the end of May in local Area agricultural fields. The samples were ground in grinding mill with particle size of less than 2 mm (Figure 1). The raw grinded sample was sealed and stored in desiccators for further usage. 25gm homogenized plant powder sample was extracted with 100 ml ethanol for 1hour. The extraction was repeated for 3 times and then the extracts were filtered through whatman filter paper no 42.

Phytochemical screening: The plant extracts were screened for their qualitative chemical composition, using standard methods described in the literature [4-6]. The identification of the following groups was considered: alkaloids, anthocyanins, coumarins, flavonoids, reducing sugars, saponosides, sterols-triterpenes and tannins.

Alkaloids: 0.5 g of each extract was agitated with 5 ml of hydrochloric acid in a steam bath, then 1 ml aliquots of filtrate were treated with a few drops of Mayer's reagent or Dragendorff's reagent. The presence of a precipitate after treatment with either reagent was a preliminary indicator of the presence of alkaloids. To remove nonalkaloid compounds that could lead to false-positive reactions, part of the extract was alkalinized with 40% ammonia solution then treated twice with chloroform. The second chloroform extract was concentrated and then retested with the Mayer and Dragendorff reagents.

Flavonoids: Flavonoids were detected by using the Shibata reaction or cyanide test. Briefly, 3 ml of extract was evaporated and the residue was dissolved in 2 ml of 50% methanol, then a few magnesium shavings and a few drops of concentrated hydrochloric acid were added. The development of a red-orange or purplish color indicates the presence of flavones aglycones.

Reducing sugars: One milliliter of extract was dissolved in 2 ml of distilled water and 1 ml of Fehling liquor and boiled for 30 min. The formation of a brick-red precipitate indicates the presence of reducing sugars. **Saponosides:** 1% of each sample decoction was returned gradually in 10 ml test tubes for a final volume of 10 ml. After two vigorous shakes, the tubes were left to stand for 15 min and the height of foam was measured. The tube in which the height of the foam was at least 1 cm, showed the presence of saponosides. However, the height of the foam indicated the value of the foam index.

Sterols and terpenes: These families of compounds were identified by using the Lieberman-Burchard reaction. Briefly, 0.5 g of extract was dissolved in 0.5 ml of chloroform with 0.5 ml of acetic anhydride, and cooled on ice before carefully adding sulfuric acid. A change in color from purple to blue indicates the presence of sterols, while a green or purple-red color indicates the presence of triterpenes.

Tannins: Initially, the Styasny reagent was used to detect the presence of tannins: A drop of the extract was placed on a slab of silica gel and eluted in an atmosphere saturated with chloroform/acetic acid/ formic acid (5:4:1), thereafter, the plates were sprayed with 10 ml of a methanol solution at 5% nitrous acid and heated in an oven at 80°C for 10 min. The presence of tannins was revealed by the appearance of blue spots. For the classes of tannins, boiled aqueous extract (1 ml) was mixed with 1% ferric chloride. A black-blue color indicated the presence of gallic tannins and a dark green color, condensed tannins.

For all the samples tested, according to the precipitation or color intensity of each tube, following evaluations were given: (+);(-).

Selection of Bacterial Strains: Microorganisms *Escherichia coli*, *Pseudomonas aeruginosa* *Staphylococcus aureus*, pure slant cultures were obtained from National Collection of Industrial Microorganism Pune, India.

Antimicrobial susceptibility test

The disc diffusion method was used to screen the antibacterial activity [7]. Muller Hinton agar (MHA) plates were prepared by pouring 15 ml of molten media into sterile petriplates [8]. The fresh grown bacteria were suspended in sterile saline to achieve concentration of 10^7 CFU/ml. This suspension was spread on the surface of MHA agar plates. The plates were allowed to dry for 5 min. The concentrations of extracts (200 mg/ml) were put on 6 mm, sterile disc of Whatman filter paper No.1. The disc was then placed on the surface of the medium and the compound was allowed to diffuse for 5 min and the plates were kept in incubation at 37°C for 24 hours for bacteria and 48 hours at 25°C for fungal agents. Inhibition zones were examined around the disc appeared at the end of incubation, which if present, were measured with a transparent ruler in millimetres. This study was performed in triplicate.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

MIC was determined by micro-dilution method [9] using serially diluted (2 folds) plant extracts according to National Committee for Clinical Laboratory Standards, 2000. The MIC of the extracts was determined by dilution of the polyherbal drug of various concentrations. Equal volume of each extract and nutrient broth were mixed in wells of Microtiter plate. Specifically 0.1 ml of standardized inoculums ($1-2 \times 10^7$ CFU/ml) was added in each tube. The plates were incubated aerobically at 37°C for 18-24 h for bacteria and 48h at 25°C for fungal growth. Two control wells were maintained for each test batch. These included antibiotic control (containing extracts and growth media without inoculum) and organism control (a tube containing the growth medium, saline and the inoculum). The lowest concentration (highest dilution) of the extract that produced no visible bacterial growth (no turbidity) when compared with the control were regarded as MIC. However, the MIB was determined by sub-culturing the test dilution on to a fresh drug free solid medium and incubated further. The highest dilution that yielded no bacterial colony was taken as MBC.

Media used

Muller-Hinton agar and broth (Hi-media, Mumbai, India), Sabouraud dextrose agar pH 7.3 ± 0.2 (Hi-media), were used for antibacterial and antifungal activity respectively.

Table 1: List of plants and their data available in the Nagaram mandal used in the present Phytochemical study

Sample No	Botanical name	Family	Used part as medicine	Medicinal practice in the study area	Place of Occurrence in Mandalam
S1	<i>Cannabis sativa</i> L.	<i>Cannabaceae</i>	Seed	Seeds powder mixed with oil for typhoid, jaundice, malaria, and fever.	Eletipalem, Edupalle, Pedapalle,
S2	<i>Celosia cristata</i>	<i>Amaranthaceae</i>	Whole Plant/Flower	Dysentery and strangury, diarrhoea and excessive menstrual discharges.	Pedamatlapudi, Siripudi, Peddavaram
S3	<i>Cinnamomum verum</i>	<i>Lauraceae</i>	Bark	Parched mouth, bronchitis, hiccup, piles, diarrhoea and heart trouble.	Pudiwada, Siripudi, Peddavaram
S4	<i>Curcuma longa</i>	<i>Zingiberaceae</i>	Rhizome	Scabies, itches, boils, abscess, eczema, leucoderma, eye diseases, pains,	Nagaram, Edupalle, Pedapalle,

				bruises and sprains; internally for cough, cold, fever.	
S5	<i>Datura metel</i>	<i>Solanaceae</i>	Seed, Leaves, Root	Insanity, fever with catarrh, diarrhoea, skin diseases and cerebral complications.	Pedamatlapudi, Siripudi, Peddavaram
S6	<i>Ipomoea aquatica</i>	<i>Convolvulaceae</i>	Whole Plant	Leucoderma, leprosy, fever, jaundice, biliousness, bronchitis and liver complaints	Pedamatlapudi, Siripudi, Peddavaram
S7	<i>Ipomoea batatas</i>	<i>Convolvulaceae</i>	Whole Plant, Root	Low fever and skin disease, strangury and diarrhoea.	Nagaram, Edupalle, Pedapalle,
S8	<i>Ixora coccinia</i>	<i>Rubiaceae</i>	Root, Flower	Hiccup, fever, gonorrhoea, diarrhoea, dysentery, leucorrhoea	Pudiwada, Siripudi, Peddavaram
S9	<i>Litsea monopetala</i>	<i>Lauraceae</i>	Bark	Diarrhoea and dysentery.	Allaparru, Dhulipudi,
S10	<i>Psidium guajava</i>	<i>Myrtaceae</i>	Root bark, Root	Diarrhoea, dysentery.	Eletipalem, Siripudi, Peddavaram

Results and discussion

Ancient Indian system of medicine (Ayurveda) was mainly based on herbal treatment. These classes (alkaloids, tannins, flavonoids etc.,) of compound were known to have activity against several pathogens and therefore aid the antimicrobial activity and suggested their traditional use for the treatment of various illnesses [9].

Phytochemical screening: Phytochemical screening using qualitative analysis on aqueous alcoholic extracts from selected plant extracts showed the presence of following constituents given in the below Table 2.

Table 2: phytochemical constituents analyzed by qualitative analysis

Functional Group	Strain Extract									
	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
Alkaloids	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Flavonoids	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Reducing sugars	-	-	-	-	+++	-	-	-	-	+++
terpenoids	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Tannins	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++

Anti-microbial Activity

The result showed that the zone of inhibition was highest in the extract of S1 to S4 and some of bacterial effect in S5 listed in Table 3 and in Figure 2 (i.e. 12.45 ± 0.56 , and minimum in S6-S10 against Three microorganisms was used Ciprofloxacin as positive control and its zone of inhibition was 25 ± 2.52 and in the case of *S. aureus* maximum zone of inhibition was obtained in S1 i.e. 12.5 ± 0.20 and minimum zone of inhibition S10 i.e. 2.9 ± 0.22 and ampicilin were used as the positive control and its zone of inhibition was 25 ± 1.65 .

Table 3: Determination of MIC, MBC (mg/ml) of Ten selected samples

Strain Extract	P. aeruginosa		E. coli		S. aureus	
	MIC	MBC	MIC	MBC	MIC	MBC
S1	6.25	12.5	12.5	25	12.5	12.5
S2	12.5	25	25	5.0	25	25
S3	12.1	10.2	14.25	11.24	15.39	12.13
S4	10.24	10.27	10.25	10.24	11.07	11.05
S5	1.35	11.22	10	10.8	10.8	11.64
S6	5.35	10.38	14.2	8.9	15.33	9.612
S7	6.17	5.34	11.2	6.7	12.09	7.23
S8	5.47	6.14	10	2.4	10.8	2.59
S9	11.2	2.1	14.2	1.8	15.336	1.94
S10	14.2	2.9	2.1	11.27	2.268	12.1716

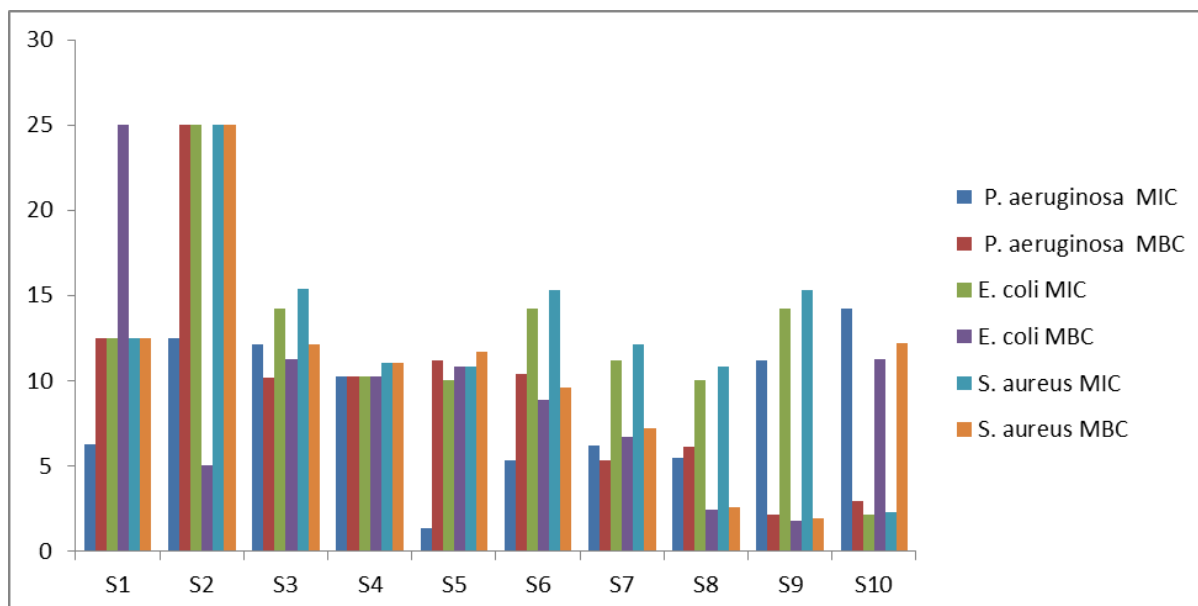


Figure 2: Graphical representation of antimicrobial activity of plant extract against three microbial cultures

Conclusion

In this study we evaluated the antibacterial activity of 10 commonly used traditional medicinal plants from Nagaram mandal. Some plant extracts displayed a potent antibacterial activity with MIC <math>< 25 \mu\text{g}/\text{mL}</math>, indicating that these plants could be a good source for the antibacterial to combat bacterial infections. Further studies are necessary for these potent plant extracts to evaluate the other parameters of antimicrobial efficacy.

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Annexure-1. Questionnaire used to collect information on plant use.

Informant Details

Name:

Sex:

Age:

Panchayat:

Village:

Mandal:

District:

Subsidiary occupation:

Main occupation:

Education:

Ethnobotanical uses of plants.

- 1) Local/vernacular name of plant:
 - 2) Scientific name of plant:
 - 3) Part used of plant:
 - 4) Name of ailment/other purposes in which plant part is used:
 - 5) Mode of preparation:
 - 6) Use (externally/internally):
 - 7) Availability in natural habitat:
 - 8) Cause of declining of ethnobotanical plants if any (overgrazing, encroachments, forest fire, mining activities, climatic change, and others):
 - 9) Who knows best about plant and uses: vaids, shepherds, old people/new generation, and others:
 - 10) Any ethnobotanical plant species under cultivation:
 - 11) Any awareness camps /trainings /exposure visits organized for ethnobotanical plants:
 - 12) Any conservation practices on ethnobotanical plants:
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