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RESEARCH ARTICLE

EVALUATION OF ANTIBACTERIAL EFFICACY OF MEDICINAL PLANTS OBTAINED FROM THE HILLY AREAS OF NORTHEASTERN STATES OF TRIPURA

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ABSTRACT

This paper explores the antibacterial efficacy of certain Indian medicinal herbs obtained from the north-eastern states of Tripura. The employed method of screening the potency of herbs was disc diffusion method. The antibacterial activity of the herbs were compared with standard antibiotic ointment available in the market. Both gram positive and negative bacteria were used as test organisms. The activity index of the herbal extract against the pathogens were calculated. Among the five chosen herbs, two of them showed the highest antibacterial activity.

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INTRODUCTION

The hot climatic zone of Agartala imbibes the colonization of bacteria, especially the skin pathogens which proliferate in the skin utilising the components released in the sweat. Microbes degrade sweat and lead to bad odour in the body. Medicinal herbs with their potent antibacterial activity fight against microorganisms and help the people to lead a healthy life. Hence, they render a magical touch to mankind. Its inherent bioactive compounds play an important role in fighting against various dreaded diseases caused by pathogens. Agartala, the capital city of the north-eastern state of Tripura is known for its greeneries. Abundant numbers of medicinal plants are available in the hilly areas of Agartala. Most of the Tripurians prefer to use the crude extract of the herbs as medicines for curing wounds, cuts, rashes, skin allergy, cough, cold etc. During the hot summer the humidity is high and people over there perspire a lot. Microorganisms reside on sweat and degrade its components causing bad odour. A survey was done which revealed the utilization of some herbs with potent antibacterial activities. The herbs are *Calendula officinalis*, *Azadirachta indica*, *Terminalia chebula*, *Peristrophe bicalyculata* and *Gardenia jasminoides*. *Calendula officinalis*, commonly called as marigold has wound healing property and haemostatic activity. The phytochemical substances found in them are alkaloids, glycosides, triterpenoids, terpenoids, flavonoids, phenols, reducing sugars, saponins, steroids and tannins.

Calendula ointments are available in the markets which are often used for curing burns, inflammations, diaper rashes, skin ulcerations and abrasions etc (Mathur et al., 2011). *Azadirachta indica*, is commonly known as Neem. Usually it is believed that boiled neem leaf water makes an excellent antiseptic to clean wounds and eases skin problems. Phytochemical constituents are more or less similar to that of marigold. The percentages of cardiac glycosides are comparatively more in neem than other plants (Faiza Aslam et al., 2009; Saradhajothi koon and Subbarao budida, 2011). Neem is still considered to be one of the best chemotherapeutic agents for all the diseases. *Terminalia chebula* is known as Haritaki, with a tremendous healing power. It is reported to have various bioactive compounds such as tannins, chebulinic acid, ellagic acid, gallic acid, punicalagin, flavonoids. It has antioxidant, antidiabetic, antibacterial, antiviral, antifungal, anticancerous, antiulcer, antimutagenic, wound healing activities etc (Manoj Kumar et al., 2009). *Peristrophe bicalyculata* is known as Nasabhaga. The leaves of the plant were used traditionally as analgesic, antipyretic, anti-inflammatory, sedative, stomachic, anticancer, fertility, diuretics and diarrhoea. This plant is used by the traditional healers for curing many skin related problems; it is also used as an antidote for snake poison (Janakiraman et al., 2012). *Gardenia jasminoides* are known as Cape Jasmine. The inherent potential of this plant helps in cell-to-cell communication, cell adhesion, and molecular recognition in the immune system. It has also been used as an analgesic, an antipyretic and an anti-inflammatory medicine (Surya Prakash et al., 2012). The current investigation of the work reveals the antibacterial activity of these above mentioned plants. As the inhabitants of Tripura believed to have high potential healing

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power in these plants, necessary steps were taken to bring out the antimicrobial property in them.

MATERIALS AND METHODS

Collection of Herb and Study Area

The medicinal herbs were collected from the tribal belt area of kunjaban, Agartala (The capital city of Tripura), India. In discussion with the tribal belt localites, these medicinal herbs were selected for screening antimicrobial property restored in them. The selected herbs are as follows:

Table 1. List of selected medicinal plants for the study

Botanical Names	Common Names	Part selected for Extraction
<i>Calendula officinalis</i>	Marigold	Leaves
<i>Azadirachta indica</i>	Neem	Leaves
<i>Terminalia chebula</i>	Haritaki	Leaves
<i>Peristrophe bicalyculata</i>	Nasabhaga	Leaves
<i>Gardenia jasminoides</i>	Cape Jasmine	Leaves

Method of Extraction

Drying, garbling and grinding processes of the herbs were done before subjecting it to the solvent extraction. Each 20gm of the powdered plant material was reflexed in a soxhlet apparatus in 100ml of the solvent (Chloroform, Ethanol and Methanol).

After overnight incubation, the supernatant was filtered through Whatman no.1 filter paper and the filtrate was dried to evaporate the organic solvent at room temperature. The filtrate solution was used as an extraction solution (Natarajan, 2002).

Antibacterial assessment of the Extracts

The antibacterial activities of all the herbal extracts were tested against clinical pathogens by Disc diffusion assay¹⁴. The evaluations of the results were recorded by measuring the zone of inhibition in millimeter.

Culture medium used: Muller-Hinton Agar (MHA)

Composition of media:

Beef infusion	30g
Casamino acid	1.75g
Starch	0.15g
Agar	1.7g
Distilled water	100ml
pH	7.4

Procedure

Steriled MHA was dispensed in petri dishes. Broth culture adjusted to 0.5 McFarland standard were used as inoculum. Using sterile cotton swab the test organisms were coated over the surface of the agar plate. The Whatman no.1 filter paper

Table 2. Results of antibacterial assessment of the herbal extracts

Sl No.	Herbal Extract Utilized	Zone Of Inhibition (in mm)**			
		<i>E.coli</i>	<i>S.aureus</i>	<i>Bacillus sp.,</i>	<i>Pseudomonas sp.,</i>
1	<i>Calendula officinalis</i>	18	22	21	19
2	<i>Azadirachta indica</i>	18	21	20	18
3	<i>Terminalia chebula</i>	14	16	11	13
4	<i>Peristrophe bicalyculata</i>	15	16	16	14
5	<i>Gardenia jasminoides</i>	16	18	17	13
6	<i>Cosvate(skin ointment)</i>	26	28	26	27

** Values are mean of three replicates.

Table 3. Phytochemical Analysis (Based on colour reaction)

Sl No.	Phytochemical constituents	Presence or absence of the components in the herbs				
		Marigold	Neem	Haritaki	Nasabhaga	Cape jasmine
1	Alkaloids	+	++	+	++	++
2	Carbohydrates	+	+	+	+	+
3	Flavonoids	++	+	++	+	++
4	Glycosides	+	++	+	+	+
5	Phenols	+	+	+	+	+
6	Proteins	+	++	++	+	+
7	Resins	-	+	+	+	+
8	Saponins	++	+	+	++	+
9	Steroids	+	+	+	+	++
10	Tannins	-	+	++	+	+
11	Thiols	+	+	+	+	+
12	Triterpenoids	+	++	+	+	++

++ represents very high content , +represents the presence of the content.

Table 3. Evaluation of activity index of the herbal extract against pathogens

Sr. No	Microorganisms	Average activity index of marigold	Average activity index of neem	Average activity index of haritaki	Average activity index of nasabhaga	Average activity index of cape jasmine
1	<i>E.coli</i>	0.6923	0.6923	0.5384	0.5769	0.6153
2	<i>S.aureus</i>	0.7857	0.75	0.5714	0.5714	0.6428
3	<i>Bacillus sp.,</i>	0.8076	0.7692	0.4230	0.6153	0.6538
4	<i>Pseudomonas sp.,</i>	0.7037	0.6666	0.4814	0.5185	0.5909

disc loaded with 50µl of the medicinal plant extracts, were placed over the agar surface. Antibiotic ointment cosvate was used as positive control (20µl). The plates were incubated at 37°C for 18-24 hours. The incubated plates were examined for the interruption of growth over the inoculum. The size of the zone of inhibition was used to evaluate the inhibitory effect of the test sample.

Test organisms: Cultures used were clinical pathogens obtained from hospitals of Agartala. The microorganisms were tested biochemically for its axenic property.

Antibiotic ointment: Cosvate was used. The concentration of this antibiotic dilution range was made as 20µl.

Measuring the Activity Index

The activity index was measured using the following formula given below (Nandita Dasgupta et al., 2012; Nandita Dasgupta et al., 2012).

$$\text{Activity Index} = \frac{\text{Zone of inhibition of the herbal extract}}{\text{Zone of inhibition of the antibiotic used}}$$

Phytochemical Analysis of the Herbal Extracts (based on the colour change)

The presence of bioactive phytochemicals like flavonoids, alkaloids, saponins, proteins, glycosides, terpenoids etc were confirmed through the chemical reactions based on the colour change (Raman, 2006).

RESULTS AND DISCUSSION

The tabulated results illustrate the antibacterial activity of the selected medicinal herbs, against both gram positive and gram negative bacteria. The maximum activity was obtained from the methanolic extraction of the herbs. Hence, results are recorded from the methanolic extraction only. It was proved that petroleum ether extraction of *C.officinalis* gave the highest antibacterial activity which is contrasting to the current investigation (Mathur et al., 2011). Bacterial infections on skin may lead to rashes and irritations. Skin ointment cosvate are often been used in Agartala to get rid of such problems. Therefore, the potency of the herbs are compared with the chemically available ointment. The results of *A.indica* against *S.aureus* and *E.coli* were reported with the ethanolic extraction as the highest antimicrobial property. It is generally noted that gram positive bacteria are more susceptible than gram negative bacteria. The probable reason could be the structural complicity of gram negative bacteria. The phytochemical constituents showed the presence of bioactive compounds which fight against various pathogens in this current research. Similar results were noted in Aslam et al., 2009, Ioana-Raluca et al., 2011 article. The activity index of each herb was calculated⁶. The results showed that the maximum activity index was obtained from marigold and neem compared to the other three microorganisms, against various organisms mentioned in the table 3. Therefore it can be confirmed that

herbal extracts can be made to use over chemical substances as medicinal therapy.

Conclusion

Our nature has given us medicinal substances in the form of herbs of different types. Instead of using chemically synthesized substances as medicine for ailments, we can explore the efficacy of these herbs and make use of it as therapeutic agents. Moreover herbal medicines have no or negligible side affects which is a boon aspect for mankind.

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