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In vitro antibacterial activity of certain folk medicinal plants from Darjeeling Himalayas used to treat microbial infections

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ABSTRACT

Antibacterial activity of ethanolic extracts of 33 folk medicinal plants used for the treatment of parasitic diseases were examined with four bacteria (two Gram positive-*Bacillus subtilis* and *Staphylococcus aureus* and two Gram negative - *Escherichia coli* and *Salmonella typhi*) following disc diffusion method. Among 33 plants examined 21 (63 %) plants showed antibacterial property with *B. subtilis*, 16 (48 %) plants with *S. aureus*, 18 (54 %) plants with *E. coli* and 8 (24 %) with *S. typhi*. Five of the medicinal plants (*Asparagus racemosus, Prunus cerasoides, Rhododendron arboreum, Terminalia bellerica and Woodfordia fruticosa*) were found inhibitory against all the bacteria. Inability of six plants (*Acer thomsonii, Alnus nepalensis, Castanopsis hystrix, Evodia fraxinifolia, Symplocos theifolia* and *Zanthoxylum nitidum*) to inhibit the growth of any of the bacteria tested indicates that these are less utilized plants to treat skin diseases where fungi are common pathogens. Plants utilized to combat enteric and urinary tract infections *viz.*, *A. racemosus, Cyperus rotundus, Docynia indica, Ficus cunea, Rheum australe, Rhododendron arboreum, Rhus semialata, Terminalia bellerica* and *Woodfordia fruticosa* showed antibacterial activity preferably to Gram-negative organisms. Careful phytochemical, pharmacological and toxicological standardisation for these medicinal plants must be instituted for their development as safe, efficacious, cheap antibacterials of plant origin vis-a-vis conservation of traditional knowledge.

Keywords: Darjeeling, antibacterial, medicinal, plants,

1. Introduction

The history of medicinal plants can be traced back to Vedic periods about 4500-1600 BC. Ayurveda has an extensive medical formulations based on products of plant, animal and mineral kingdoms. Medicinal plants constitute the dominant part of drug substances. There are some 1250 Ayurvedic medicinal plants [1] which go into formulating therapeutic preparation as per Ayurvedic and other traditional systems. If folklore medicinal herbs are added to these the total number of plants with medicinal applications used throughout India will exceed 2000. Therefore India has often been referred to as the Medicinal Garden of the World.

The folk medicines of Himalayas has gifted many new plant drugs to modern medicine like *Rauwolfia serpentina* for high blood pressure, *Adhatoda zeylanica* for bronchitis, *Tylophora indica* for asthma and whooping cough. Considerable research works have been done not only on the pharmacological and phytochemical aspects, but also on the antimicrobial properties of higher plants.

Osborn [2] carried out first large scale systematic search for the plants having antibacterial properties and screened 2300 species of plants of which 63 were found antimicrobial. Similarly, Okazaki and KawaGvchi [3] extensively studied the antimicrobial activity of over 1300 species of higher plants in Japan including herbal drugs and 50 kinds of essential oils. Petershova [4] studied fungicidal activities of 1281 species from 184 families against number of pathogens, 25 species were fungicidal to varying degree, remarkable antifungal activities were observed in the species of Anacardiaceae, Compositae, Cruciferae (Brassicaceae), Labiatae (Lamiaceae), Liliaceae, Ranunculaceae, Rosaceae and Solanaceae.

To date, around 3000 plants have been officially documented as having medicinal potential but more than 6000 plants are used by traditional herbal practitioners [5]. For medicinal plants to be used alongside modern medicine careful phytochemical, pharmacological and toxicological standardisation for the chosen plants must be instituted so that dosage levels can be described in an informed way. With so much pharmaceutical research going in drug companies in the Western world the choice of which disease condition needs to be focused on by third world researchers is a vital question. The focus indeed should be on parasitic diseases rather than problems of physiological disorder such as cancer and viral type that are concentrated on by large pharmaceutical companies of the West [6].

Darjeeling Himalayas, a part of Eastern Himalayas belt and specifically the Singalila range is known to be the abode of vast number of medicinal plants which is not only used by local people but also has been attracting plant hunters for the last three centuries [7]. Many reports on the documentation of traditional medicinal plants from this area are available [8, 9, 10, 11] but laboratory work to verify antimicrobial property of these plants is scanty. Even tens of thousands of antimicrobial compounds exist, the ability of microbes to develop resistance to even the most powerful antimicrobial compounds is amazingly rapid [12]. Extraction of bioactive molecules from medicinal plants facilitates pharmacological studies leading to a synthesis of a more potent drug with a reduced toxicity [13, 14]. Plant based extracts can be extracted from any part of plant like barks, leaves, fruits, seeds and fruit rinds etc. [15]. The mass screening of plants in the search for new drugs is vastly expensive and inefficient. It would be cheaper and perhaps more productive to re-examine plant remedies described in ancient and medieval texts [16]. The basis of traditional medicine is in its use for a number of years and therefore its clinical existence comes as a presumption. However, for bringing more objectivity and also to confirm traditional claims, systematic clinical trials are necessary. Therefore this work aims to examine the *in vitro* antibacterial activity of few selected folk medicinal plants of Darjeeling Himalayas.

2. Materials and Methods

2.1 Plant materials

Medicinal plants materials were collected from profusely grown sites of Darjeeling Hills with the help of local herbalists and experienced women in the field of traditional health care. Voucher specimens of the samples are stored at the Herbarium of Department of Botany, Darjeeling Govt. College.

2.2 Microorganisms

Four bacteria, two from Gram-positive group (*Bacillus subtilis* and *Staphylococcus aureus*) and rest two from Gram-negative group (*Escherichia coli* and *Salmonella typhi*) were obtained from the stock culture of Microbiology Research Laboratory, Postgraduate Department of Botany, and Darjeeling Government College.

2.3 Preparation of plant extracts

Each plant sample was washed to remove debris, dried and ground to powder and was stored in a sterile glass bottle in the refrigerator. The 5 g portions of sieved powder was added to 50 ml of solvent (ethanol), sonicated for 30 min and left overnight at room temperature. The crude extract was prepared by decanting, followed by filtration through muslin cloth, and further filtered with Whatman No. 1 filter paper to obtain a clear filtrate. The filtrate was evaporated to obtain 10 ml of concentrated extract and sterilized by membrane filtration using 450 nm bacteriological filters. Such sterilized filtrate was stored in screwcapped airtight containers in the refrigerator.

2.4 Screening of antibacterial activity

This procedure is based on disc diffusion method of Baur *et al.*, [17]. Overnight grown bacterial cultures (1.5 x 10^8 CFU/ml) were spreadplated on nutrient agar plates to achieve semiconfluent growth. Sterile filter paper discs were soaked in extracts, allowed to dry between the applications and placed on plates which were then incubated at 37 °C for 24 hrs. Streptomycin (10 µg/ml) and sterile distilled water were taken as positive and negative control respectively. Growth was evaluated and presence of inhibition zone around the disc was considered positive for antibacterial activity.

3. Results and Discussion

In the present study, a total of 33 plant species were selected and their ethanol extracts were tested for antibacterial activity against four bacteria – two from Gram positive group (*Bacillus subtilis* and *Staphylococcus aureus*) and rest two from Gram negative group (*Escherichia coli* and *Salmonella typhi*). Ethnomedicinal use and antibacterial activities of medicinal plants is presented in Table 1. Among 33 plants examined 21 (63 %) plants showed antibacterial property with *B. subtilis*, 16 (48 %) plants with *S. aureus*, 18 (54 %) plants with *E. coli* and 8 (24 %) with *S. typhi*. Five of the medicinal plants (*Asparagus racemosus, Prunus cerasoides, Rhododendron arboreum, Terminalia bellerica and Woodfordia fruticosa*) were found inhibitory against all the bacteria. This broad spectrum activity may be attributed to the presence of novel secondary metabolites. It is expected that the plants demonstrating broad spectra of activity may help to discover new chemical antibacterial metabolites that could serve as selective agents for the maintenance of human health [18].

Inability of six plants (*Acer thomsonii, Alnus nepalensis, Castanopsis hystrix, Evodia fraxinifolia, Symplocos theifolia* and *Zanthoxylum nitidum*) to inhibit the growth of any of the bacteria tested indicates that these are less utilized plants to treat skin diseases where fungi are common pathogens. Thus further study taking fungi as test organism is warranted. Plants utilized to combat enteric and urinary tract infections *viz*., *A. racemosus, Cyperus rotundus, Docynia indica, Ficus cunea, Rheum australe,*

Rhododendron arboreum, Rhus semialata, Terminalia bellerica and *Woodfordia fruticosa* showed antibacterial activity preferably to Gram-negative organisms. Generally Gram negative bacteria are more resistant than Gram positive bacteria [20, 21]. Ethanolic extracts are preferred for the antimicrobial screening because principle antibacterial phytochemicals like tannins, polyphenols, polyacetylenes, flavonoids, terpenoids, sterols and alkaloids are ethanol soluble [22].

Table 1: Ethnomedicinal use and antibacterial activities of certain medicinal plants from Darjeeling Himalayas

S. No	Botanical name [Family]	Parts used	Ethnic use for treatment of	Bs	Sa	Ec	St
1	*Acer thomsonii* Miq [Aceraceae]	Bark	Skin diseases	-	-	-	-
2	*Achyranthes bidentata* Blume [Amaranthaceae]	Root, stem	Skin disease	+	-	+	-
3	*Alnus nepalensis* D Don [Betulaceae]	Bark	Skin disease	-	-	-	-
4	*Artemisia indica* Willd [Asteraceae]	Leaf	Cut, bleeding	+	-	+	-
5	*Asparagus racemosus* Willd [Liliaceae]	Root	Diarrhea, UTI	+	+	+	+
6	*Bergenia ciliata (Howarth) Sternberg [Saxifragaceae]*	Root	Cuts, wounds, diarrhoea	+	+	-	-
7	*Betula cylinder stachys Wall [Betulaceae]*	Bark	Dental problems	+	+	-	-
8	*Bombax malabaricum* DC [Bombacaceae]	Spine	Skin diseases	+	-	+	+
9	*Castanopsis hystrix A DC [Fagaceae]*	Bark	Skin diseases	-	-	-	-
10	*Cinnamomum tamala Nees [Lauraceae]*	Bark	Skin diseases	+	+	+	-
11	*Cyperus rotundus* L. [Cyperaceae]	Root	Skin diseases, UTI	-	+	-	+
12	*Cuscuta reflexa* Roxb. [Convolvulaceae]	Whole plant	Liver problem, pimples	-	+	+	-
13	*Docynia indica* [Rosaceae]	Fruit	Diarrhoea, Dysentery	-	-	+	-
14	*Equisetum* sp. [Equisetaceae]	Plant body	Skin diseases	+	-	-	-
15	*Erythrina stricta* Roxb. [Fabaceae]	Bark	Skin diseases	+	-	+	-
16	*Eupatorium adenophorum* Spreng [Asteraceae]	Leaf	Cut, wound	+	-	-	-
17	*Euphorbia royleana* Boiss [Euphorbiaceae]	Latex	Skin diseases	+	-	+	-
18	*Evodia fraxinifolia* Hk f [Rutaceae]	Fruit	Indigestion, skin disease	-	-	-	-
19	*Ficus cunia* Ham. [Moraceae]	Bark	Child diarrhoea	+	+	-	+
20	*Heracleum wallichii* DC [Apiaceae]	Fruit	RTI, Cold, Body ache	-	-	+	-
21	*Nardostachys jatamansi* DC [Valerianaceae]	Root	RTI	+	+	-	-
22	*Ocimum sanctum* L [Lamiaceae]	Twig	RTI, Skin diseases	-	+	+	-
23	*Prunus cerasoides* D Don [Rosaceae]	Twig	Toothache	+	+	+	+
24	*Rheum acuminatum* Hk f [Polygonaceae]	Young twig	Diarrhoea, Dysentery	+	+	+	-
25	*Rhododendron arboreum* Smith [Ericaceae]	Petals	Diarrhoea, Dysentery	+	+	+	+
26	*Rhus semialata* Muer [Anacardiaceae]	Fruit	Diarrhoea, Dysentery	+	-	+	-
27	*Rubia cordifolia* L [Rubiaceae]	Whole plant	Skin diseases	+	+	-	-
28	*Sida acuta* Burn [Malvaceae]	Root	Dental problems	+	-	+	-
29	*Symplocos theifolia* Don [Symplocaceae]	Bark	Skin diseases	-	-	-	-
30	*Terminalia bellirica* (Gaertn) Roxb [Combretaceae]	Fruit rind	Diarrhoea, Dysentery	+	+	+	+
31	*Urtica dioica* L [Urticaceae]	Roots	Skin diseases	-	+	-	-
32	*Woodfordia fruticosa* (L) Kurz [Woodfordiaceae]	Petals	Diarrhoea, Dysentery	+	+	+	+
33	*Zanthoxylum nitidum* (Roxb.) DC	Fruit	Ringworm, Flatulence	-	-	-	-

+ = presence of inhibition zone, - = absence of inhibition zone, Bs **=** *Bacillus subtilis,* Sa= *Staphylococcus aureus,* Ec= *Escherichia coli,* St = *Salmonella typhi,* RTI = respiratory tract infections, UTI = urinary tract infections

Lag phase for botanical medicine is now rapidly changing for a number of reasons. Problems with drug-resistant microorganisms, side effects of modern drugs, and emerging diseases where no medicines are available, have stimulated renewed interest in plants

as a significant source of new medicines.

The US National Cancer Institute regularly earmarks large appropriations to screen 50,000 natural substances for activity against cancer cell lines and the AIDS virus. China, Germany, India and Japan, among others, are also screening wild species for new drugs. The WHO long recognised that traditional medicinal plants could be useful in an integrated health care delivery system of a country. However such plants must not be dangerous, be effective and that preparations are not adulterated or made harmful by parasites and microorganisms [23]. Proven agro-industrial technologies need to be applied to the cultivation and processing of medicinal plants and the manufacture of herbal medicines [24].

4. Conclusion

It is evident from the study, most of the selected plants appeared to contain antibacterial substances. It is hoped that careful phytochemical, pharmacological and toxicological standardisation for these medicinal plants must be instituted for their development as safe, efficacious, cheap antibacterials of plant origin and may create an opportunity for conservation of medicinal plants vis-a-vis diverse traditional knowledge.

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