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Phytochemical analysis and screening of antibacterial activity of some selected Indian medicinal plants

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Abstract

Phytochemical screening and evaluation of antibacterial activity of ten plant species namely Aloe barbadensis, Aegel marmelos, Azadirachta indica, Bahunia variegate, Cannabis sativa, Emblica officinalis, Eugenia jambolana, Gmelina arborea, Nerium oleander and Vitex negundo have been carried out against six bacterial strains namely Escherichia coli 101, Escherichia coli 119, Micrococcus luteus, Bacillus subtilus, Bacillus cereus and Pseudomonas fluorescence. All the plants species taken in present study have shown the presence of tannin, flavonoids, terpenoids and alkaloids. Pholobatanins have found to be present in Aegel marmelos, Bahunia variegate and Eugenia jambolana while cardiac glycosides have been shown positive test on Aloe barbadensis, Azadirachta indica, Cannabis sativa, Nerium oleander and Vitex negundo. Methanol extract of Emblica officinalis have been shown significance potential against all test pathogens except Pseudomonas fluorescence followed by Nerium oleander, Azadirachta indica and Bahunia variegate.

Keywords: : Medicinal Plants, bacterial strain, phytochemical screening.

Introduction

The use of medicinal plants as a source for relief from illness can be traced back over five millennia to written documents of the early civilization in China, India and the Near east, but it is doubtless an art as old as mankind. Neanderthals living 60,000 years ago in present day Iraq used plants such as hollyback, these plants are still widely used in ethnomedicine around the world [1]. Countries like India have been using crude plants as medicine since Vedic period. A major part of the total population in developing countries still uses traditional folk medicine obtained from plant resources [2]. Biologically active compounds present in the medicinal plants have always been of great interest to scientists working in this field. In recent years this interest to evaluate plants possessing antibacterial activity for various diseases is growing [25].

Plant materials remain an important resource to combat serious diseases in the world. The traditional medicinal methods, especially the use of medicinal plants, still play a vital role to cover the basic health needs in the developing countries [4]. The medicinal value of these plants lies in some chemical active substances that produce a definite physiological action on the human body [5]. Medicinal plants as a pesticides are superior over synthetic pesticides due to low concentration of active ingredient, target specificity, biocidal activity against pest and pathogens, low mammalian toxicity and biodegradability [6]. Plants produce a diverse range of bioactive molecules, making them rich source of different types of medicines. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds [7]. Phytomedicines derived from plants have shown great promise in the treatment of various diseases including bacterial and viral infections [8, 9].

Antibacterial activity of leaf extract of 10 plant species collected from different locations in India has been tested against 6 bacterial species namely *Escherichia coli* 101, *Escherichia coli* 119, *Pseudomonas fluorescence*, *Micrococcus luteus*, *Bacillus subtilus,* and *Bacillus cereus* reported in this communications. These plants spp. suspected to possess biocidal chemicals have been selected based on the information available in literature and field observation on plant that remain relatively free from the disease symptoms [10,11].They grow in different altitude and location in India.

Materials and methods

Collection of bacterial culture:

Bacterial culture of *E. coli* 101, *E. coli* 119, *P. fluorescence*, *M. luteus*, *B. subtilus,* and *B. cereus* were collected from Department of Biotechnology and Bioscience, Banasthali University, Rajasthan. The details have been reported in table-1. They were cultured in petri plates containing nutrient agar media and incubated at 28±1˚C (table-1).

Table1- Bacterial cultures screened in present study

Name of bacterial pathogens	Disease caused	Place of collection	Optimum temperature ° C
Escherichia coli 101	Non pathogenic	Department of Biotechnology and Bioscience, Banasthali University, Rajasthan, India	28±1
Escherichia coli 119	Non pathogenic	Department of Biotechnology and Bioscience, Banasthali University, Rajasthan, India.	28±1
Micrococcus luteus	Asthma	Department of Biotechnology and Bioscience, Banasthali University, Rajasthan, India	28±1
Bacillus subtilus	Food poisoning	Department of Biotechnology and Bioscience, Banasthali University, Rajasthan,India	28±1
Bacillus cereus	Food poisoning	Department of Biotechnology and Bioscience, Banasthali University, Rajasthan,India	28±1
Pseudomonas fluorescence	Non pathogenic	Department of Biotechnology and Bioscience, Banasthali University, Rajasthan, India	28±1

Table-2 Plant species, family, part used and place of collection

Name of plant species	Family	Plant part used	Location	Altitude (ft)
Aloe barbadensis[12]	Liliaceae	Leaf Gel	Medicinal Garden, Banasthali University, Rajasthan, India	1010
Aegel marmelos[13]	Rutaceae	Leaves	Medicinal Garden, Banasthali University, Rajasthan, India	1010
Azadirachta indica[14]	Meliaceae	Leaves	Medicinal Garden, Banasthali University, Rajasthan, India	1010
Bahunia variegate[15]	Caesalpiniaceae	Leaves	Nursery of O.C.F Factory, Shahjahanpur, Uttar Pradesh, India	525
Cannabis sativa[16]	Cannabaceae	Leaves	Nursery of O.C.F Factory, Shahjahanpur, Uttar Pradesh, India	525
Emblica officinalis[17]	Euphorbiaceae	Leaves	Medicinal Garden, Banasthali University, Rajasthan, India	1010
Eugenia jambolana[18]	Myrtaceae	Leaves	Medicinal Garden, Banasthali University, Rajasthan, India	1010
Gmelina arborea[19]	Verbenaceae.	Bark	Botanical Garden, Patanjalai Yogpeeth Haridwar, Uttrakhand, India.	919
Nerium oleander[20]	Apocynaceae	Leaves	Medicinal Garden, Banasthali University, Rajasthan, India	1011
Vitex negundo[21]	Verbenaceae.	Leaves	I.D.P.L Rishikesh, Uttrakhand, India	1142

Latitude range = 26˚21'29.41" N to 30˚04'45" N
Longitude range = 75˚13'37.15" E to 76˚35'10.72" E

Collection of plant species:

Medicinal plant species collected from different locations in India have been reported in table-2. For preparation of methanol extract 10gm of dry power of plant leaves were extracted 4 times with 5ml methanol/gm of plant material for 48hrs at room temperature. All these extract concentrated by flash evaporation at 45˚C.

Phytochemical screening of crude extracts:

Plant materials collected were screened for presence of flavonoids, alkaloids, terpenoids, saponins, flavanoids, pholobatannins. Chemical tests were conducted on aqueous extract of plant sample and also of the powered specimens using standard procedures to identify the constituents [22].

Test for tannins:

Approximately 5g of each portion of the herbal extract was stirred with 10ml of distilled water on a magnetic stirrer, filtered, and ferric chloride reagent added to the filtrate. A blueblack green, or blue-green precipitate was taken as evidence for the presence of tannins.

Test for phlobatannins:

About 2 ml of aqueous extract was added to 2 ml of 1% HCl and the mixture was boiled. Deposition of a red precipitate was taken as an evidence for the presence of phlobatannins.

Test for flavonoids: Shinoda test was performed to test the presence of flavonoids. To alcoholic solution of the extracts, magnesium powder and few drops of concentrated HCl were added. Formation of orange, pink, red to purple colours indicated the presence of flavonoids.

Test for terpenoids:

5 ml of aqueous extract of each plant sample is mixed with 2 ml of $CHCl_3$ in a test tube. 3 ml of concentrated H_2SO_4 is carefully added to the mixture to form a layer. An interface with a reddish brown coloration is formed if terpenoids constituent is present

Test of alkaloids (Hager's test)

2 ml of aqueous extract was added to Hager reagent (Saturated solution of picric acid) yellow coloured precipitate indicates the presence of alkaloids.

Test for cardiac glycosides: Salkoski test was used to identify cardiac glycosides. 0.5g of the extract was dissolved in 2ml of chloroform and sulphuric acid carefully added to form a lower layer. Formation of reddish-brown colour at the interface indicated the presence of steroidal ring (i.e. aglycone portion of the cardiac glycoside).

Screening of plant extracts and metal complexes for bacterial activity:

Disc diffusion method:

Screening of plant extract for antibacterial activity was done by the disc diffusion method. The antibacterial activity was assessed using the simple disc diffusion method [23] where plant extract impregnated filter paper disc are placed on nutrient agar media incubated with the test bacteria so as to get a lawn culture on incubation. The drug diffuses in to the medium and inhibits bacterial growth around the disc, if it is effective this indicates this indicates the antibacterial activity of the drug tested. Larger the inhibition zone higher will be the antibacterial activity. If the plant extract is not effective or is not having antibacterial activity then the zone of inhibition will not be observed.

It was performed using a 37 hrs culture at 28˚ C in 20ml of nutrient broth. Fifty micro leteres of the test bacterial suspensions were spread over the plates containing Nutrient Agar using a sterile cotton swab in order to get a uniform microbial growth on both control and test plates. Sterile wathman filter paper discs of 0.5 cm diameter were loaded in condensed methanolic solvent for plant extract. Dried and placed on solid media incubated with test bacteria. Two discs loaded with (20µl extract being equivalent to 100mg of plant material) compound and other two loaded with similar amount of solvent for control. The plates were left for 30min. at room temperature to allow the diffusion of the extract, and then they were incubated at 37˚C for 18 hrs.(18hrs. was fixed as the optimum since there was no change in the inhibition upto 24hrs.) After the incubation period, the zone of inhibition was measured with a ruler.

Studies were performed in duplicate, the mean value of inhibition zone was calculated and reported in table-4.

Results and Discussion

Results on phytochemical screening have been summarized in table-3. All the plant species taken in the present investigation have shown presence of tannins, flavanoids, terpenoids and alkaloids. Phlobatannins have been found to be present only in *Aegel marmelos, Bahunia variegate* and *Eugenia jambolana. Aloe barbedensis, Azadirachta indica, Cannabis sativum, Nerium oleander* and *Vitex negundo* have shown the presence of cardiac glycosides.

Antibacterial susceptibility has been reported in Table-4. Results on screening of antibacterial activity reveals that *Bahunia variegate* and *Nerium oleander* possess significant antibacterial activity ranging inhibition zone from 9 to 25mm against all the test pathogens used in present study. These plant species possess broad spectrum antibacterial constituents.

A. marmelos has inhibited both the strains of *E. coli, B. subtilus* and *B. cereus*. *A. indica* have shown significant activity against all the strains except *E.coli*119. All the plant species explored in

Table-3 Phytochemical screening of medicinal plants

Name of the plant species	Chemical Constituents Screened					
	Tannins	Phlobatannins	Flavanoids	Terpenoids	Alkaloids	Cardiac glycosides
Aloe barbadensis	+	-	+	+	+	+
Aegel marmelos	+	+	+	+	+	-
Azadirachta indica	+	-	+	+	+	+
Bahunia variegate	+	+	+	+	+	-
Cannabis sativa	+	-	+	+	+	+
Emblica officinalis	+	-	+	+	+	-
Eugenia jambolana	+	+	+	+	+	-
Gmelina arborea	+	-	+	+	+	-
Nerium oleander	+	-	+	+	+	+
Vitex negundo	+	-	+	+	+	+

Table-4 Antibacterial activity of plant extract:

Name of Plant species	Inhibition zone in mm					
	*E. coli*119	*E. coli*101	*P. florescence*	*B. subtilus*	*B. cerus*	*M. luteous*
Aloe barbedensis	-	-	-	-	-	-
Aegel marmelos	9	9	-	30	70	-
Azadirachta indica	-	29	16	18	13	17
Bahunia variegate	16	20	25	12	14	9
Cannabis sativum	6	5	6	-	7	-
Emblica officinalis	20	25	-	21	20	13
Eugenia jambolana	15	11	-	-	-	8
Gmelina arboria	6	-	-	-	-	7
Nerium oleander	20	20	25	19	17	20
Vitex negundo	23	11	9	19	8	-

present investigation have exhibited activity against three or more bacterial species except *G. arborea* possess activity only against *E. coli* 119 and *M. luteus.* It may possess specific antibacterial constituents while rest of the plant species may possess broad spectrum antibacterial constituents. Both the strains of *E. coli* have been found to be more susceptible in comparison to other microbial strains taken in present study.

Pseudomonas fluorescence has been found to be most resistant among all the microbes screened against the present investigation, followed by *B. subtilus, M. luteus* and *B. cereus.*

The result lends credence to the folkloric use of these medicinal plants in treating microbial infection and shows that in all extract of plants *Aegel marmelos, Azadirachta indica, Bahunia variegate, Nerium oleander* and *Vitex negundo* an could be exploited for new potent antibacterial agents. Extract of these plants could be subjected to further isolation and purification of active compound to discover novel lead antibacterial agents.

The effective plant extracts are expected to contain wide spectrum antimicrobial chemicals. Therefore, the results described above may be helpful in developing/ synthesizing the plant based natural

antibacterial agents that may be used for preventing the incidence of many diseases caused by the bacteria involved in the present investigations.

These results will be helpful in developing herbal antibiotic remedies for curing of bacterial diseases in human beings.

Conclusion

Many medicinal plants have been found to be effective in the cure of bacterial diseases. Due to increasing antibiotic resistance in microorganisms and side effects of synthetic antibiotics medicinal plants are now gaining popularity in the treatment of bacterial infections. Medicinal plants are considered as clinically effective and safer alternatives to the synthetic antibiotics. Extensive research in the area of isolation and characterization of the active

principles of these plants are required so that better, safer and cost effective drugs for treating bacterial infections can be developed.

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