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Comparative study on *in vitro* antibacterial and antifungal properties of five medicinal plants of west Bengal

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ABSTRACT

Medicinal plants are in rich source of antimicrobial agents. The present study was carried out to evaluate the antibacterial and antifungal effect of five important plants namely, Andrographis paniculata, Bacopa monnieri, Centella asiatica, Nardostachys jatamansi ,Saraca indica. The preliminary phytochemical analysis was tested by using different extract of these plants for the presence of various secondary metabolites like alkaloids, flavonoids, tannins, saponins, terpenoids, glycosides, steroids, carbohydrates and amino acids. The in vitro antimicrobial activity was screened against clinical isolates viz gram positive bacteria Staphylococcus aureus, Streptococcus pyogenes, gram negative bacteria Escherichia coli, Pseudomonas aeruginosa and fungal strain Aspergillus niger, Candida albicans. Extracts were found significant inhibition against all the pathogens.

Keywords: Plant extract, Phytochemicals, Antibacterial activity, Antifungal activity.

INTRODUCTION

Despite tremendous progress in human medicines, infectious diseases caused by bacteria, fungi, viruses and parasites are still a major threat to public health. Their impact is particularly large in developing countries due to relative unavailability of medicines and the emergence of widespread drug resistance [1]. Plants are the richest source of natural antimicrobial agents. Traditional healers claim that some medicinal plants are more efficient to treat infectious diseases than synthetic antibiotics [2]. From ancient times, different parts of medicinal plants have been used to cure specific ailments [3]. The medicinal plants are widely used because of its easy availability and cost effectiveness. The active principles of many drugs found in plants are secondary metabolites. The antimicrobial activities of plant extracts may reside in a variety of different components, including aldehyde and phenolic compounds [4]. India is well known for Ayurveda, which is one of important traditional medicine practiced. Herbs are widely exploited in the traditional medicine and their curative potentials are well documented [5].

Andrographis paniculata (Burm.f.) Nees (Acanthaceae), native to Taiwan, Mainland China and India, is a medicinal herb with an extremely bitter taste used to treat liver disorders, bowel complaints of children, colic pain, common cold and upper respiratory tract infection. It grows erect to a height of 30–110 cm in moist, shady places, locally it is known as Nilavembu, Sirunangai, Siriyanangai [6]. *Bacopa monnieri* L. (family Scrophulariaceae) is a traditional medicinal plant in India, commonly known as “brahmi”. It is a prostate, creeping, juicy, succulent, glabrous herb that branches profusely, found in wet places, damp or marshy areas near the border of the ponds, water cannels, wells, irrigated fieldsetc [7]. *Centella asiatica* (Linn.) (thankuni) Urban sys. synonym *Hydrocotyle asiatica* Linn. commonly known as Indian Pennywort, belongs to the family Apiaceae (previously known as Umbelliferae). *Centella asiatica* is one of the chief herbs for treating skin problems, to heal wounds, for revitalizing the nerves and brain cells, hence primarily known as a "Brain food" in India [8]. *Nardostachys jatamansi* is a flowering plant of the Valerian family that grows in the Himalayas of Nepal, China, and India. The plant grows to about 1 m in height and has pink, bell-shaped flowers. It is found in the altitude of about 3000–5000 meters. Nard oil is used as a perfume, an incense, a sedative, and an herbal medicine said to fight insomnia, birth difficulties, and other minor ailments [9]. *Saraca indica* L. (Family: Leguminosae) is a medium sized evergreen tree up to 9 m in height with

numerous spreading and drooping glabrous branches commonly known as Asoka. The bark of the plant is dark brown to grey or black; flowers are fragrant, numerous, dance and orange or red color; leaves are pinnate, 15-25 cm long having 4-6 pairs of oblong-lanceolate leaflets [10].

The aim of this study was the antibacterial and antifungal screening of five medicinal plants from West Bengal.

MATERIALS AND METHODS

Collection of plant material

The plants were collected from local market of West Bengal, India. The leaves were rinsed with distilled water and dried with paper towel; shade dried and powdered using electric grinder. The fine plant powder was transferred into sterile, air-tight container and stored for future use.

Preparation of plant extract

Five hundred gram plant powder was poured with double distilled water, and left for 72 hours at room temperature. Then, the flask was refluxed over hot water bath for 10 hours, and the mother liquor was filtered, process was repeated for four times to obtain the maximum yield. The filtrate obtained was evaporated to complete dryness under reduced pressure using a rotary evaporator. The aqueous extract thus obtained was kept in a closed bottle at low temperature until further use. Dried plant extract were stored in sterilized screw capped bottles at -20°C in a deep freezer [11].

Phytochemical analysis

The aqueous extract of the plant was subjected to qualitative chemical screening for the identification of the tannins, alkaloids, flavonoids and other phytoconstituents using standard procedures [12].

Test organism

In vitro antimicrobial activity was examined for aqueous extracts from the leaves of five medicinal plants. Amongst microorganisms investigated, two Gram-positive bacteria were *Staphylococcus aureus* ATCC25923, *Streptococcus pyogenes* ATCC21059, while two Gram-negative bacteria were Escherichia coli ATCC25922 and Pseudomonas aeruginosa ATCC27853, the fungal strains were *Aspergillus niger* ATCC9763 and *Candida albicans* ATCC7596. All the microorganisms were maintained at 4ºC on nutrient agar (for bacteria) and potato dextrose agar (for fungi) slants.

Inoculum preparation

Ten ml of distilled water was taken into the screw cap tube and pure colony of freshly cultured bacteria and fungi were added into the tube and vortex was done. The OD (optical density) was measured with the colorimeter and microbial population was confirmed to be within in 10^7 ml^{-1} to 10^8 ml^{-1} and then plated out as inoculums [13].

Antibacterial assay

Antibacterial activity of aqueous extract of plants was done by agar well diffusion method. Inoculum of test organisms was prepared by growing pure isolate in nutrient broth at 37^{o}C for overnight. The overnight broth cultures was sub-cultured in fresh nutrient broth and grown for 3hrs to obtain log phase culture. The agar plates were prepared by pour plate method using 20ml Muller-Hinton medium. The sterile M-H agar medium is cooled to 45^{o}C and mixed thoroughly with 1ml of growth culture of concerned test organism (1 x 10^8 cells) and then poured into the sterile petri dishes and allowed to solidify. Wells of 6 mm size were made with sterile cork borer and plant extracts were added. The agar plates were incubated at 37^{o}C for 24hrs. The diameter of zones of inhibition was measured in mm using Hi-Media zone reader [14].

Antifungal assay

The antifungal activity of the extract was tested by disc diffusion method [15, 16] against the two pathogenic fungi. Test fungal broths were prepared by using potato dextrose broth incubating at 37°C for 72 h. 20 ml quantities of potato dextrose with fungal culture were plated on PDA petri plates. Plant extract discs were kept on the plate. Blank disc impregnated with water followed by drying off was used as control. The activity was determined after 72 h of incubation at room temperature (37°C). The diameter of zone of inhibition produced by the extract was in mm using Hi-Media zone reader.

RESULTS AND DISCUSSION

Phytochemical analysis and *in vitro* antibacterial and antifungal activity were evaluated using selected medicinal plants in West Bengal namely *Andrographis paniculata, Bacopa monnieri, Centella asiatica, Nardostachys*

jatamansi, Saraca indica. The phytochemical analysis and antimicrobial activity were determined using the available literature methods.

Phytochemical analysis

The qualitative analysis of these five medicinal plants revealed that the presence of various secondary metabolites such as alkaloids, flavonoids, tannins, saponins, terpenoids, glycosides, phenol, steroids, carbohydrates and amino acids (Table 1).

Table 1.Qualitative phytochemical screening of crude extracts of medicinal plants

Phytoconstituents	*A. paniculata*	*B. monnieri*	*C. asiatica*	*N. jatamansi*	*S. indica*
Alkaloids	+	-	+	+	+
Tanins	+	-	+	+	+
Saponins	+	+	+	-	+
Flavonoids	+	-	+	-	-
Steroids	-	-	+	+	-
Glycosides	+	+	+	-	+
Terpenoids	+	+	+	-	-
Carbohydrates	+	+	+	+	+
Proteins	+	+	+	+	-
Phenol	+	-	+	+	-

(+) Presence of constituents, (-) Absence of constituents

Antibacterial activity

In vitro antibacterial activity of aqueous extract of five medicinal plants was screened individually by the presence or absence of zone of inhibition. Fig 1 represents the antibacterial activity of selected plants against gram-positive strain *S. aureus* and *S.pyogenes*. The figure showed that maximum of 18mm was recorded of *C. asiatica* against *S. aureus* whereas the lowest activity of 10mm was seen of *S. indica*. The antibacterial activity of other plant extract also significant, 16 mm for *A. paniculata* and 14mm for *B. monnieri*. But in case of *S. pyogenes*, highest activity of 15mm was found for *A. paniculata*, followed by 14mm, 13mm,11mm,10mm for *C. asiatica*, *B. monnieri*, *S. indica*, *N. jatamansi* respectively.

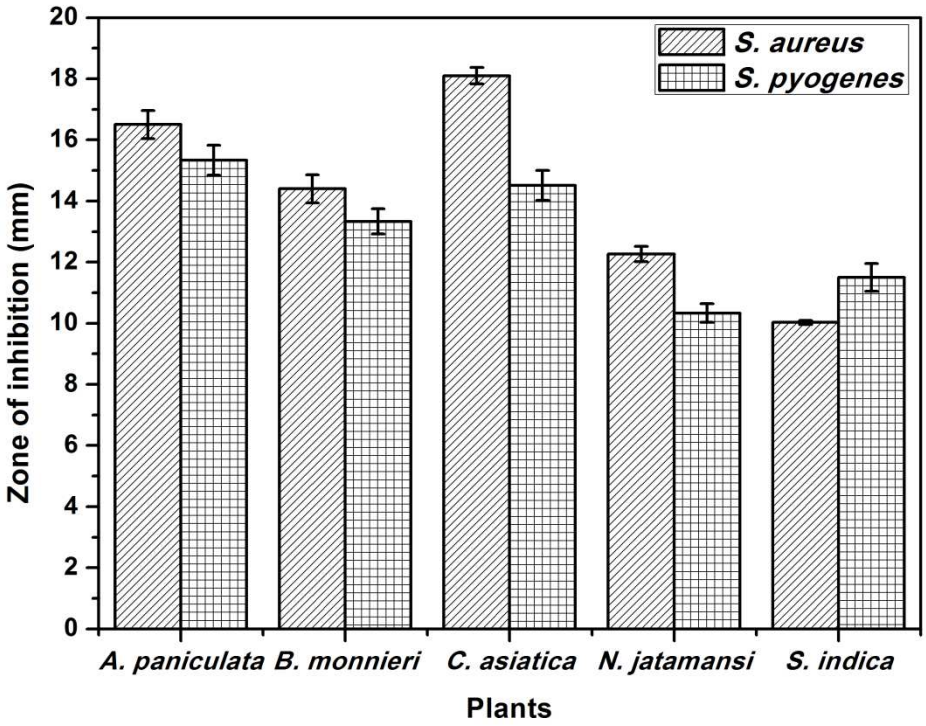

Fig 1. Antibacterial activity of aqueous plant extracts of selected plants against gram-positive bacteria *S. aureus* and *S. pyogenes*

As can be seen from fig 2, the antibacterial activity of five medicinal plants against gram-negative bacteria. The *in vitro* antibacterial activity of medicinal plants was evaluated against *E.coli* and *P.aeruginosa*. The crude extract of *A. paniculata* showed biggest zone of inhibition of 14mm against *E.coli* where *C. asiatica* and *B. monnieri* (12mm) showed almost same activity. But the lowest activity was found 8mm for *S. indica.* In case of *P. aeruginosa*, the

maximum activity was shown by *A. paniculata* of 11mm and minimum activity was exhibited by *N. jatamansi* of 5mm, followed by 9mm, 7mm, 6mm, for *C. asiatica*, *S. indica*, *B. monnieri* respectively.

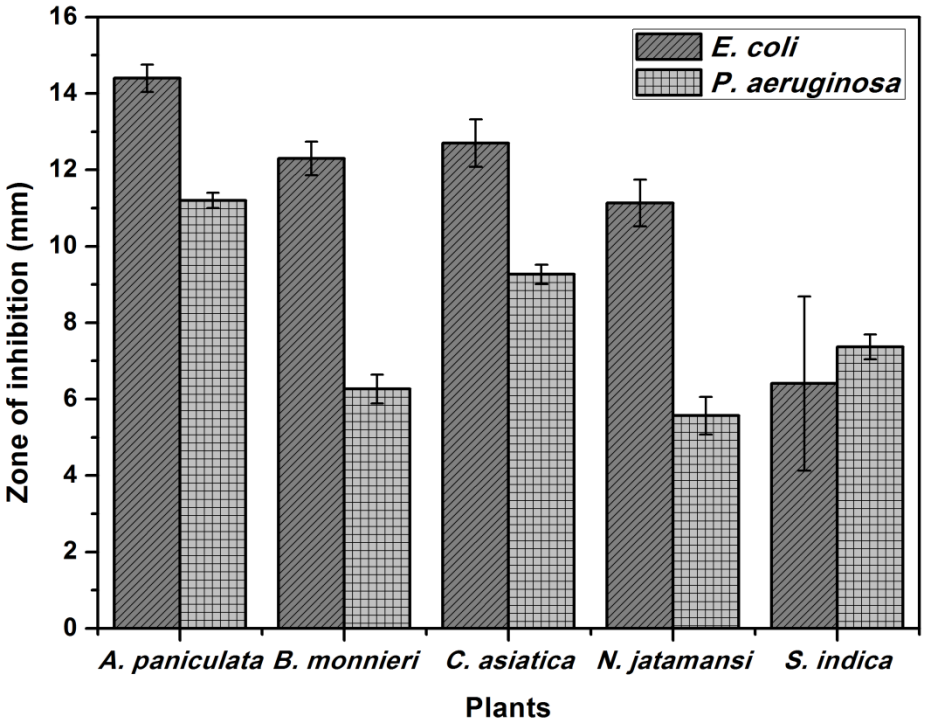

Fig 2. Antibacterial activity of aqueous plant extracts of selected plants against gram-negative bacteria *E.coli* and *P.aeruginosa*

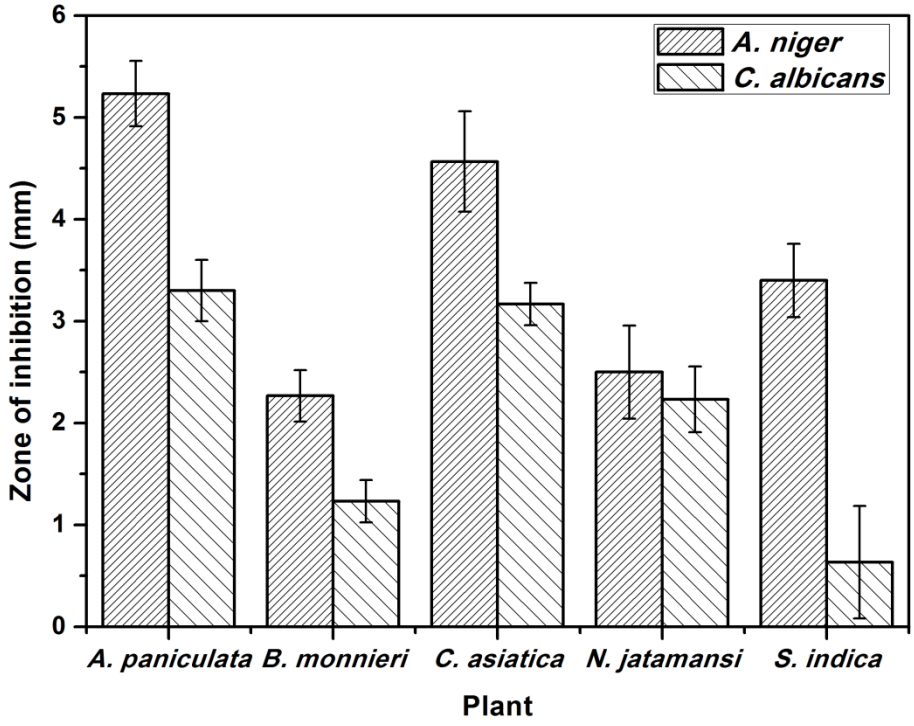

Fig 3. Antifungal activity of aqueous plant extracts of selected plants against *A.niger* and *C.albicans*

Antifungal activity

The zones of inhibition of aqueous extract in millimeters of fungal strains are shown (Fig 3) which revealed that these test fungal are not very sensitive towards these plants. It explained that highest antifungal activity was 5mm for *A. paniculata* whereas lowest activity was found of 2mm for *B. monnieri* and *N. jatamansi* both against *A.niger*. *C. asiatica* (4mm) and *S. indica* (3mm) has significant values. The antifungal activity against *C.albicans* was

revealed that *A. paniculata* and *C. asiatica* have maximum activity of 3mm, but *B. monnieri* and *N. jatamansi* showed less activity. *C.albicans* is resistant towards aqueous extract of *S. indica*. So an average all these plants showed better result against *A.niger* than *C.albicans*.

CONCLUSION

This comparative study exhibits the information about the ethano properties of these plants which can be uses as medicine. Further studies should be undertaken to elucidate the exact mechanism of action by which extracts exert their antimicrobial effect to identify the active ingredients which can be used in drug development program for safe health care services.

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