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Antibacterial and phytochemical action of medicinal plant *Peristrophe bicalculata* against human pathogens

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Abstract
The *in vitro* study of antimicrobial properties of *peristrophe bicalculata* was conducted using five different leaves extracts against five different human pathogens. *Peristrophe bicalculata* belong to the largest medicinal plant family acanthaceae and possess great value as a phytomedicine source. Positive results from antibacterial activity can prove these plant derived phytochemicals to be effective agents to be developed in phytomedicne in future which can overcome antibiotic resistance which is one the biggest threat in medical sciences. *Peristrophe bicalculata* showed maximum antibacterial activity in ethyl acetate solvent against selected human pathogens such as *Escherichia coli, Streptococcus aureus* and *Bacillus subtilis. Different solvent systems were employed for extract preparation*. For antibacterial activity agar well diffusion method was used and zone of inhibition measured. Thus, effective phytomedicine can be developed using *Peristrophe bicalculata* leaves extracts which can serve inordinate therapeutic value.

Keywords: antibacterial activity, antibiotic resistance, phytochemicals, phytomedicine, human pathogens

Introduction

The risk of opportunistic infections in individuals treated with immunosuppressive medications or severe chemotherapy has been steadily rising during the last few decades [1]. Antibiotic resistance is a critical issue in the treatment of illnesses that are caused by bacteria. In recent years, several novel techniques to combat such diseases have been proposed. Combination of different antibiotics, the discovery of new members of current antibiotic classes, and the introduction of novel drugs are the examples of novel techniques [2, 3]. The rise in antimicrobial resistance to drugs, as well as their toxicity and costs of treatment, necessitate the search for novel approaches. As a result, antibiotics are becoming increasingly vital in health care, and screening traditional plants for the development of new drugs is becoming increasingly relevant. Phytobiology considers medicinal plants to be a source of biologically active substances that have been traced since the beginning of evolution. Plants have performed a wide range of functions on the biosphere from the dawn of time, long before scientific understanding. However, plants gradually became a source of therapeutic agents only after the improvements in technology and scientific knowledge. They were identified to have fewer side effects compared to synthetic antibiotics [4]. The worldwide market for plant-derived pharmaceuticals is projected to be of more than $20 billion, and is still continuing to grow [5]. In India's ancient treatment systems such as Ayurveda, Unani, Homeopathy, and Siddha, about 95 percent of prescriptions were plant-based. As a result, researchers are focusing on the characterization of numerous plant ingredients in relation to a variety of ailments, based on Ayurvedic claims about the plants. The discovery of biologically active compounds in plants that can be used as a potential drug, has always been a difficult problem for scientists. Herbal medicine and, tribal and traditional remedies have been appreciated increasingly for the prevention and treatment of a variety of major human illnesses [6]. *Acanthaceae*, also stated as the Acanthus family, is a dicotyledon flowering plant in the Lamiales order, having 250 genera and 2500 number of species, among which most of them are tropical shrubs or herbs. They also consist of vines, that are found in temperate regions, primarily in Central America, Indonesia, Africa and Malaysia [7]. They may be found on the plains as well as at higher elevations in the central, south, north, east, and west Indian mountains. Members of the Acanthaceae can be found in arid, semi-arid, rocky, buried, and marshy areas across Rajasthan [8]. Leaves are aligned opposite and stipules are absent from the leaves. lowers are bisexual in nature, zygomorphic to sub-actinomorphic, and are frequently distributed in racemes or panicles, and terminal or axillary spikes. The calyx has 4-5 lobes.

Fig 1: *P. Bicalculata* plant with flower

The corolla is sympetalous, with five limbs. It has two or four epipetalous stamens, and hence is didynamous. Superior, 2-loculed, and axile placentation ovary is present. Fruit is generally loculicidal and in the form of a capsule. Normally, seeds are compressed [7]. These plants contain phytochemicals that can modulate the immune system a can

also act as a potential antioxidant. The phytochemicals are said to be anti-inflammatory, anti-viral and antifungal [9]. The genus *Peristrophe* is of the family *Acanthaceae*. It is a vertical herb with slender stem. It has ovate-lanceolate shaped leaves. The plant bears purple-colored flowers. Corolla is pink or purple in color, measures 1-1.5 cm in length, and has 2-lipped hairy capsules. The plant grows to a height of 60-180 cm and may be found in forest undergrowth, hedges, and waste bands nearly everywhere in India, as well as Afghanistan and Africa. In the Garhwal Himalaya, *P. bicalyculata* may be found at an altitude of 600-1,400m. In India, there are just eight species of the genus *Peristrophe*. Flowering occurs from July to September, with fruits occurring from September to November. *P. bicalyculata* is used as an antibacterial herb that can prevent the growth of Mycobacterium tuberculosis. It is also used as an antidote for snake poisoning, and as a treatment for bone fractures, sprains, fever, cold, cough, asthma, and eye and ear infections. The leaves of *P. bicalyculata* were used in the treatment of skin ailments, which includes healing of wounds. The paste is useful on wounds, and the flowers are utilized as a source of forage by the bees. In vitro, the essential oils from *P. bicalyculata* have tuberculostatic action on diverse strains of *Mycobacterium tuberculosis* [10].

Materials and Methodology

1. Preparation of plant extract

Desired plant leaves were washed under the tap water for proper removal of dust and impurities bounded on the surface. Then grind 200g fresh leaves into small particles and immersed them into 600ml of four different solvents having polarity low to high i.e., petroleum ether (PET), chloroform (CHF), methanol (MeOH) and aqueous (H2O), loaded in Soxhlet apparatus and extracted for 72 h through hot successive method. Then filter the plant extract through Whatman no. 1 filter paper and obtain the crude extracts by removing the solvent by vacuum evaporator at temperature 30 °C. The remaining plant residues were stored at 4 °C until further use. Extracts were dissolved in dimethyl sulphoxide to make a final concentration 200 mg/ml for antibacterial assay [11].

2. Phytochemical analysis

1. **Test for alkaloids:** Specific amount of sample was taken and was dissolved in a mixture of 10% acetic acid and ethanol (1:10) and allow it to stand for 4 hours at 28oC. Then this mixture was filtered by filter paper. Take out the filtrate and treat it with the drop wise addition of NH4OH until the alkaloid was precipitated. Then wash it with the 10% ammonia and dry it at 80oc.
2. **Test for flavonoids:** 5g of sample was taken in a flask and boiled it in 50 ml of 2M HCL solution for 30 minutes under reflux. After boiling, allow it to cool for some time and filtered it with filter paper. Then treat this filtrate with equal volume of ethyl acetate.
3. **2.3 Test for tannin:** Take 5g sample in a test tube and dissolve it in a 50 ml of distilled water and allow it to stand for 30 minutes at 28oc. Then take two 50 ml volumetric flask in which 2ml plant extract was added in one flask and 2ml standard solution with same amount of distilled water was added in another flask. Then add 2.5 ml of saturated Na_2CO_3 and reagent in both the flask and make up the volume 50 ml with distilled water. Then put both flasks in incubator at 28oc for 90 minutes. Take absorbance of both the flask by using spectrophotometer (spectrophotometer set at 260 nm and reagent blank was used for the calibration). This test shows the presence of tennis in plant extract.
4. **Test for steroids:** Take specific amount of sample and dissolve it in 100ml distilled water. Then homogenized this sample with using laboratory blender. Then filter this sample and wash the filtrate with normal ammonium hydroxide solution (PH 9). Then take out this filtrate in a fresh flask and mixed it with 2ml chloroform. Add 3ml ice cold acetic anhydride and 2 drops of concentrated H_2SO_4 in a mixture to cool it down. Check the absorbance of a mixture at 420 nm by using spectrophotometer. [12]
5. **Test for Saponins:** Crude extract were added to 5ml distilled water in a test tube and vigorously shaken to mix well. Few drops of olive oil were added. Stable foam formed denotes presence of saponins.
6. **Test for terpenoids (Salkowski test):** In a test-tube, 5ml of crude extract and 2ml of chloroform was taken. 3ml of concentrated sulphuric acid H2SO4 was added slowly via side wall of test tube to form a layer. Reddish brown coloured was formed at the interphase which explained the presence for terpenoids.
7. **Test for Cardiac Glycosides (Keller-Kiliani test):** In 5ml of crude extract; 2ml of glacial acetic acid containing one drop of ferric chloride solution was added. This was under-layered by 1ml concentrated H2SO4. A brown ring at the interface indicated the presence of deoxy sugar characteristics of cardenolides. A violet ring may appear below the bring ring while a greenish ring may form in the acetic acid layer throughout thin layer.

8. **Test for carbohydrates**

1. **Molisch test:** few drops of 1% alpha-naphthol were added to 1ml test solution. Then, 2-3ml concentrated sulphuric acid was added via side wall of test tube. This test was confirmed positive via presence of reddish violet or purple ring forming at the junction of two liquids.
2. **Barfoerd's test:** 2ml of reagent was added to 2ml test sample; mixed well and subjected to boiling water bath for 1 min. The red precipitate formed indicated presence of monosaccharides.
3. **Seliwanoff's test:** 1ml test sample was taken. To it, 3ml of Seliwanoff's reagent was added and subjected to heated water bath for 1 min. This test was confirmed positive by formation of Rose red colour for carbohydrate.
4. **Fehling's test:** Dissolve 2mg dry extract in 1ml distilled water and add 1ml of Fehling's(A+B) solution, shaken and heated on a water bath for 10 minutes. Presence of carbohydrates was confirmed by Brick red colour precipitate.

3. **Bacterial cultures and growth conditions:** There are main five common pathogenic bacterial stains which can cause serious disease in humans are *staphylococcus aureus, klebsilla pneumoniae*, *Salmonella typhimurium, Shigella desentarie* and *Escherichia coli*. All these cultures are used to check the antimicrobial activity of

peristrohe bicalculata plant extract against bacterial growth. These strains were generally maintained under 4oc on nutrient agar slant. Take loop-full of cells from stock cultures and suspend these cultures in Muller-Hinton broth (MHB) to activate the culture. Then incubate this culture at 37oc for 24-48 h before testing. This cultured bacteria suspension was further used for performing 'Antibacterial' activity assay [11].

Antibacterial activity assay: The antibacterial activity of *P. bicalculata* was analyzed by agar well-diffusion method [13]. To prepare the agar plates, O.1ml of 12-16 h pre incubated bacterial cultures were suspended in molten muller Hinton agar medium. Then sterilize the Petri plates and pour this medium in it and allow the agar medium to solidify. After solidification of agar, make puncture well (6mm in diameter) in it by using cork-borer. Then fill these wells with plant extracts (45 µl) having final concentration of 200 mg/ml. Then put these plates in BOD incubator in an upward direction at 37oc for 24 hours and allow them for diffusion. Dimethyl sulphoxide was used as negative control whereas antibiotic erythromycin was used as positive control. Therefore, the effectiveness of extracts against test bacteria can be compared with the antibiotic erythromycin. The zone of inhibition can be measured by measuring the diameter of clear zone in millimeter form edge of the well. Each sample has three replicates to confirm their accuracy and the data is expressed in mean±SD values [11].

Results

1. Preparation of plant leaves extract

Fig 2: Leaves extracts a. Methanol, b. ethanol, c. n-hexane, d. chloroform, e.ethyl acetate respectively

2. Result for phytochemical analysis

Table 1: Result for phytochemical analysis

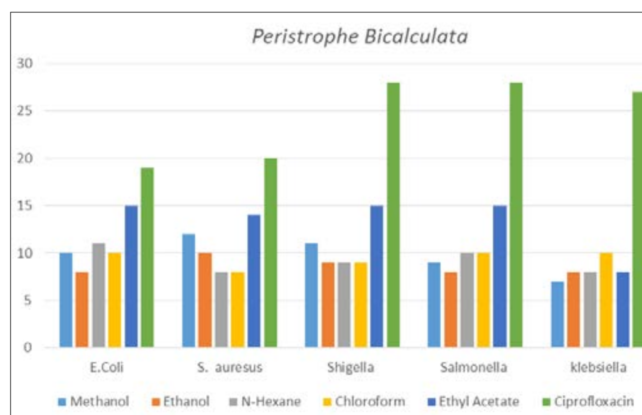
Phytochemicals	*Peristrophe bicalyculata*
Tannins	+
Saponins	+
Alkaloids	+
Terpenoids	+
Flavanoids	+
Steroids	+
Glycosides	+
Carbohydrates	+

3. Antimicrobial Activity by well diffusion *method-Peristrophe bicalyculata–*

- Peristrophe bicalyculata (Zone of Inhibition in mm)

Table 2: Antimicrobial activity of all 5 extracts

Extract	*E.Coli*	*S. auresus*	*Shigella*	*Salmonella*	*klebsiella*
Methanol	10±0.05	12±0.16	11±0.01	9±0.56	7±0.05
Ethanol	8±0.10	10±0.25	9±0.41	8±0.22	8±0.04
N-Hexane	11±0.15	8±0.36	9±0.06	10±0.52	8±0.01
Chloroform	10±0.26	8±0.45	9±0.07	10±0.14	10±0.00
Ethyl Acetate	15±0.05	14±0.14	15±0.05	15±0.52	8±0.05
Ciprofloxacin	19±0.06	20±0.04	28±0.01	28±0.25	27±0.05



Graph 1: Comparative analysis of extracts for antimicrobial activity

Discussion

The presence of saponins, alkaloids, tannin, steroid and flavonoid in the plant as shown in Table1 may be collectively or individually responsible for the observed antimicrobial activities. This result also corresponds with the results of phytochemicals of plants and fruit common in the region [14].

Table 2 shows the result of the antimicrobial potency of all the five extracts of *Peritrophe bicalyculata* against some selected microorganisms. The diameters of the zone of inhibition of this extracts were compared with Ciprofloxacin. The effect of the ethyl acetate extract of the *Peritrophe bicalyculata* was strongly effective against *salmonella typhimurium* whereas chloroform extract was most effective against *Shigella dysenterie*. There is a need for further study to ascertain if the yield in this species will be increased by using stronger fractionating solvent such as ethyl acetone or methyl acetone. These solvents have been reported to be more vigorous than other solvents used in crude extraction of plants [15].

Conclusion

In conclusion, the results from this study have shown the effectiveness of the plant crude extract on the tested microorganisms, which is indication of the medicinal value of the plant extract as antimicrobial agents. The extracts with proper formulations can be developed in phytomedicine to treat antibiotic resistance against these human pathogens.

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