International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 10, Issue 3, 2018

Original Article

ANTIOXIDANT, PHYTOCHEMICAL AND ANTIBACTERIAL ACTION OF HIMALAYAN MEDICINAL HERBS *PERISTROPHE BICALYCULATA* LEAVES EXTRACT AGAINST RESPIRATORY TRACT PATHOGENS

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Received: 25 Nov 2017 Revised and Accepted: 13 Feb 2018

ABSTRACT

Objective: Evaluate the antioxidant, phytochemicals and antibacterial screening of leaves extracts of *Peristrophe bicalyculata*.

Methods: The antibacterial activity against the respiratory tract pathogens i.e., *Staphylococcus aureus* (MTCC 1144), *Streptococcus pneumoniae* (MTCC 655), *Streptococcus pyogenes* (MTCC 442), *Pseudomonas aeruginosa* (MTCC 2474) and *Klebsiella pneumoniae* (MTCC 4030) was examined by the method agar well diffusion and the minimum inhibitory concentration (MICs) was determined by the method of twofold serial dilution. Broad spectrum antibiotic erythromycin was used as positive control and dimethyl sulphoxide (DMSO) used as negative control. The qualitative method was adapted for phytoconstituents screening and antioxidant activity of plant extract was examined by DPPH free radical scavenging method.

Results: The results showed that the chloroform (CHF) extract has a higher degree of antibacterial potency then the other extract. The zone of inhibition showed by chloroform extract against tested bacteria ranged between 9.3±0.59 mm to 26.6±0.66 mm, respectively. MICs values were recorded between 6.25 mg/ml to 25 mg/ml against all the test organisms. Phytoconstituents analysis of *P. bicalyculata* extract exposed the presence of alkaloids, flavonoids, glycosides, steroids, saponins and tannins. The methanolic extract of *P. bicalyculata* % inhibition of DPPH radical is up to 86.33%. The *P. bicalyculata* (Methanolic extract) gives best antioxidant activity than another extract.

Conclusion: This investigation ropes a good answer to the use of *P. bicalyculata* as a natural antioxidant and in herbal medicine as a support for the development of new drugs and phytomedicine in the foundation for its use in remedial of respiratory infectious diseases.

Keywords: Antibacterial activity, Agar well diffusion method, Twofold serial dilution method, Antioxidant, Phytomedicine, Respiratory tract pathogens

DOI: http://dx.doi.org/10.22159/ijpps.2018v10i3.23961

INTRODUCTION

Medicinal plants represent a prime source of antimicrobial agents. Plants are used medicinally in different countries and are a basis of many effective and controlling drugs. A broad range of medicinal plant parts is used for extract as untreated drugs and they possess varied medicinal properties [1]. Medicinal plants are supposed to be a central source of new chemical substances with possible therapeutic effects. The secondary metabolites of plants were found to be a source of various phytoconstituents that could be openly used as intermediates for the production of new drugs. The conventional medicine should be able to play an even greater role in the current prime healthcare system of the developing countries. The natural medicines are understood to be more acceptable to the human body when compare to recent synthetic drugs. Thus the central factor needed is to take the maximum benefit from the traditional system of medicine for provided that sufficient healthcare service to pastoral people [2].

The genus *Peristrophe* belongs to the family Acanthaceae, is a vertical herbs, stem slender, divided 30-70 cm, the shape of leaves is ovate-lanceolate, size is 2-8.5x1-3 cm, rounded at base acute or acuminate; petioles. Flower colour is purple in greatly branched, lax panicles; bract 2 opposite, unequal linear bractoles linear-lanceolate, ciliate. Colour of Corolla pink or purple, length is 1-1.5 cm, with 2-lipped hairy capsules. The height of plant is 60-180 cm found in forest undergrowth, hedges and waste band almost throughout India, it's also found in Afghanistan and Africa. *P. bicalyculata* is found on the altitude of 600-1,400m amsl in Garhwal Himalaya. The genus *Peristrophe* only eight spices found in India. Flowering time is start from July to September and fruiting start in September to November [3]. The herb *P. bicalyculata* is used as anti-bacterial property (tuberculostatic), the antidote for snake poison, use in bone fracture, sprain, fever, cold, cough, asthma and for ear and eye infection. The leaves of *P. bicalyculata* were used for curing of many skin related problems like the healing of the wound; paste of *P. bicalyculata* valuable on the wound, flowers use as a source of *bee-forage*. The essential oil shows tuberculostatic activity *in vitro*. It inhibits the growth of different strains of *Mycobacterium tuberculosis*. The main aim of present study was to investigate the antioxidant activity, phytochemicals screening and antibacterial potential of leaves extracts of Himalayan medicinal herbs *Peristrophe bicalyculata* against some common respiratory tract pathogens.

MATERIALS AND METHODS

Plant material

The leaves of *P. bicalyculata* was collected from, Srinagar, Srikot and Pauri at 600-1000m amsl district Pauri Garhwal in Uttarakhand (Fig.-1), from the month of October to December 2016. The plant sample was authenticated at Garhwal University Herbarium (GUH), H. N. B. Garhwal University Srinagar (Garhwal) where a herbarium voucher specimen was deposited (Accession No. GUH 20750). Collected plant leaves were appropriately washed with tap water, dried under shade at room temperature and crushed to small pieces by using an electric grinder.

Preparation of crude extract

Plant extracts were prepared by immersing 200g of grinded plant material in 600 ml of four different solvents according to polarity low to high i.e. petroleum ether (PET), chloroform (CHF), methanol (MeOH) and aqueous (H_2O), loaded in Soxhlet apparatus and extracted for 72 h through hot successive method. Plant extracts were filtered through Whatman No. 1 filter paper and crude extracts obtained by remove the solvent by vacuum evaporator at temperature 30 °C. The residues of plant material were stored at 4 °C until further use. Extracts were dissolved in dimethyl sulphoxide to make a final concentration of 200 mg/ml for antibacterial assay.

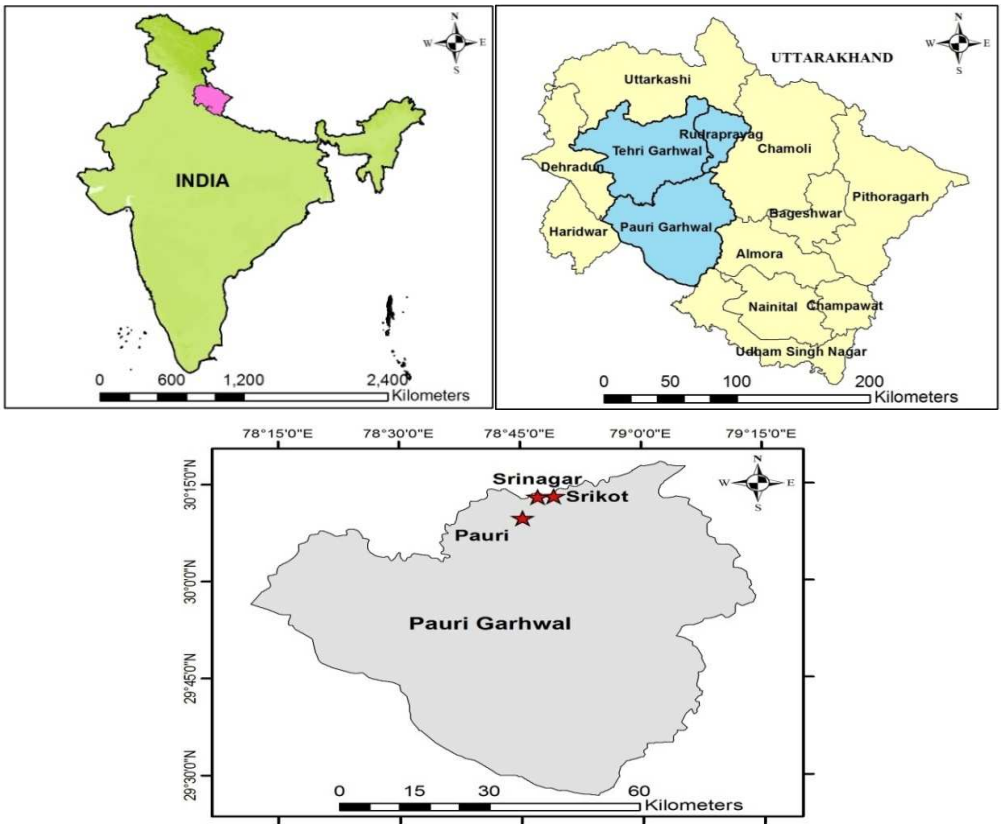

Fig. 1: Collection site of *P. bicalyculata*

Test microorganism

The five common pathogenic bacterial strains causing respiratory infections used in this study i.e. *Staphylococcus aureus* MTCC 1144, *Streptococcus pneumoniae* MTCC 655, *Streptococcus pyogenes* MTCC 442, *Pseudomonas aeruginosa* MTCC 2474, *Klebsiella pneumoniae* MTCC 4030. These standard bacterial strains were purchased from Institute of Microbial Technology (IMTECH), Chandigarh.

Preparation of inoculums

Stock cultures were maintained at 4 °C on nutrient agar slant. Active cultures for the experiment were prepared by transferring a loopful of cells from stock cultures to test tubes containing Mueller-Hinton broth (MHB) for bacteria that were incubated without agitation for 24–48 h at 37 °C.

Antibacterial testing

The antibacterial activity of different extracts of *P. bicalyculata* was examined by agar well-diffusion method [4]. 0.1 ml of 12-16 h pre-incubated cultures of bacterial species were mixed in molten Mueller Hinton Agar medium no. 173 (Hi-media Pvt. Ltd., Mumbai, India) and poured in pre-sterilized petri plates. A cork borer (Size of cork borer is 6 mm in diameter) used to punch wells in solidified medium and filled with extracts of 45 µl of 200 mg/ml final concentration of plant extracts. Di-Methyl Sulphoxide was used as negative control. The effectiveness of extracts against test bacteria was compared with the antibiotic erythromycin (positive control). The plates were incubated at 37 °C up to 24 h in BOD incubator and the diameter of the zone of inhibition was measured in millimeter. Each sample was arranged in triplicate and the mean±SD values were observed. The antibacterial activity was interpreted from the size of the diameter of the zone of inhibition measured to the nearest millimeter (mm) as observed from the clear zones surrounding the wells.

Percentage of potency

The percentage of the potency of crude extracts was calculated by using following formula. Efficacy of extracts against bacteria was compared with a broad-spectrum antibiotic erythromycin (positive control).

$$\text{Percentage of potency} = 100 - \left(\frac{T}{C}\right) \times 100$$

Where, C = Control or standard, T = Test

Determination of minimum inhibitory concentration (MICs)

The minimum inhibitory concentrations (MICs) were determined by two-fold serial dilution method against the selected bacterial strain [5]. The methanol extract was diluted double fold (2:2) with nutrient broth in a series of six test tubes. The concentration of 50, 25, 12.5, 6.25, 3.12 and 1.56 mg/ml of crude Methanol extract were prepared separately and dissolved in 1 ml of DMSO. An aliquot of 1 ml of bacterial suspension (1.5×10^6) was inoculated into each culture tube. Control tubes were inoculated with a same quantity of sterile distilled water. All tubes were incubated at 37 °C for 24 h. The lowest concentration that did not permit any visible growth when compared with control was considered as the minimum inhibitory concentration. The MICs was considered as the lowest concentration that could not produce a single bacterial colony. The contents of all tubes that showed no visible growth in the form of turbidity were cultured on Mueller-Hinton agar, incubated at 37 °C for 24 h.

Phytochemical screening

The key phytocompounds, in the crude leaves extracts of *P. bicalyculata* were subjected to phytochemical screening to decide the presence of bioactive components by using standard qualitative methods [6].

Evaluation of antioxidant activity

By DPPH free radical scavenging activity method

DPPH (2, 2-diphenyl picrylhydrazyl) (RM2798-1G Hi-media Pvt. Ltd., Mumbai, India) is a commercially available stable free radical, which is purple in colour. The antioxidant molecules present in the herbal extracts, when incubated, react with DPPH and convert it into di-phenyl hydrazine, which is yellow in colour. The degree of

discolouration of purple to yellow was measured at 517 nm, which is a measure of scavenging potential of plant extracts.

A 2 ml aliquot of the solution was added to 2 ml of 2×10^{-4} mol/l ethanolic DPPH solution. The mixture was shaken vigorously and the absorbance was measured at 517 nm immediately. The decrease in absorbance was determined at 15 and 30 min until the absorbance reached a steady state (after nearly 30 minutes). The DPPH with corresponding solvents (without plant material) serves as the positive control. The respective solvent of plant extracts (without DPPH) serves as blank. All the tests were performed in triplicate and the DPPH radical scavenging activity of the plant extract was calculated as the percentage inhibition according to the given formula [7].

$$\% \text{ Inhibition of DPPH free radical} = \left[\frac{(A_{\text{blank}} - A_{\text{sample}})}{A_{\text{blank}}}\right] \times 100$$

RESULTS

Antibacterial activity

Chloroform extract of *P. bicalyculata* showed a high degree of antibacterial activity in comparison to other three extracts (table 1). The chloroform extract was highly active against *S. aureus* (MTCC 1144) (26.6±0.66 mm) and lowest inhibition against *K. pneumoniae* (MTCC 4030) (18.3±0.79 mm) in comparison to other solvent extracts. The minimum percentage of potency was found in chloroform extract (12.21%) against *S. Aureus*

Table 1: Zone of inhibition of *Peristrophe bicalyculata* extracts (leaves), antibiotic (Erythromycin) and negative control (Dimethyl Sulphoxide) against standard bacterial strains causing respiratory pathogens

Microorganism	Diameter of the inhibition zone (mm)				Percentage of potency				Positive control	Negative control
	PET	CHF	MeOH	H_2O	PET	CHF	MeOH	H_2O	Erythromycin	DMSO
Staphylococcus aureus	21.3±0.28	26.6±0.66	22.0±0.50	16.0±0.53	29.70	12.21	27.39	47.19	30.3±0.87	0
Streptococcus pyogenes	16.6±0.58	19.3±0.76	11.3±0.54	9.3±0.59	32.52	21.54	54.06	62.19	24.6±*0.76*	0
Streptococcus pneumoniae	14.3±0.50	19.6±0.50	14.3±0.36	10.0±1.56	37.82	14.78	37.82	56.52	23.0±*1.32*	0
Pseudomonas aeruginosa	16.6±0.28	20.0±0.45	14.0±0.50	11.6±0.59	32.92	17.69	42.38	52.26	24.3±*0.51*	0
Klebsiella pneumoniae	14.0±0.50	18.3±0.79	13.6±0.52	16.3±2.37	35.18	15.27	37.03	24.53	21.6±*0.76*	0

Values are mean±SD of three replicates; Cork borer diameter: 6 mm, PET-Petroleum Ether Extract, CHF-Chloroform Extract, MeOH-Methanol Extract, H_2O-Aqueous Extract, DMSO-Dimethyl Sulphoxide.

Methanolic extract of *P. bicalyculata* showed maximum activity against *S. aureus* (MTCC 1144) (22.0±0.50 mm) lowest against *S. pyogenes* (MTCC 442) (11.3±0.54 mm) respectively. Petroleum ether extract was found most active against *S. aureus* (MTCC 1144) (21.3±0.28 mm) and lowest inhibition against *S. pneumoniae* (MTCC 655) (14.3±0.50 mm).

The aqueous extract was found less active against all test pathogens. It was found most active against *K. pneumoniae* (MTCC 4030) (16.3±2.37 mm), H2O extract was found less active against *S. pyogenes* (MTCC 442) (11.3±0.54 mm) and *S. pneumoniae* (MTCC 655) (10.0±1.56 mm).

Minimum inhibitory concentrations (MICs)

The minimum inhibitory concentrations (MICs) were determined for most effective plant extracts showed maximum antibacterial activities. The MIC values for *P. bicalyculata* CHF extract were ranged between 6.25 to 25 mg/ml (fig. 2). The inhibition was noted at 6.25 mg/ml against *S. aureus, S. pneumoniae,* 12.5 mg/ml against the *S. pyogenes* and similar inhibition against *K. pneumoniae* and last *P. aeruginosa* give 25 mg/ml MIC value.

Phytochemical screening

Chloroform extract of *P. bicalyculata* showed the presence of alkaloids, glycosides, flavonoids, steroids/terpenes, tannins and saponins. Petroleum ether extract was found positive for alkaloids, saponins and terpenes, methanolic extract for alkaloids, flavonoids, glycoside and saponins and aqueous extract positive for alkaloids, glycosides, steroid, and sugar (table 2).

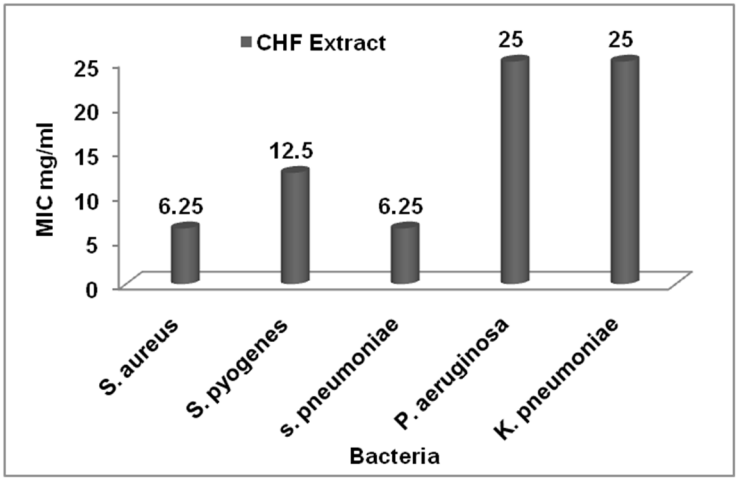

Fig. 2: Minimum Inhibitory Concentrations (MICs) of the chloroform extract of *P. bicalyculata*. The inhibition is noted that 6.25 mg/ml against *S. aureus,* 12.5 mg/ml against *S. pyogenes*, 6.25 mg/ml against *S. pneumoniae*, 25 mg/ml against *P. aeruginosa* and 25 mg/ml against *K. pneumoniae*

Table 2: Phytochemical screening of various leaves extracts of *P. bicalyculata*

Phytoconstituents	Solvent			
	Petroleum ether (PET)	Chloroform (CHF)	Methanol (MeOH)	Aqueous (H_2O)
Alkaloids	+	+	+	+
Flavonoids	–	+	+	–
Glycosides	–	+	+	+
Steroids/Terpenes	+	+	–	+
Sugars	–	–	–	+
Saponins	+	+	+	–
Tannins	–	+	–	–

+= Present, – = Absent

Antioxidant activity

The methanol extract of *P. bicalyculata* (fig. 5) exhibited maximum potency in scavenging DPPH radical in comparison to petroleum ether (fig. 3), chloroform (fig. 4) and aqueous extract (fig. 6). The methanolic extract of *P. bicalyculata* % inhibition of DPPH radical is up to 86.33%. The lower value of IC_{50} of the extract showed the strong antioxidant activity presence in the extracts. IC_{50} value of the methanolic extract (153.79 µg/ml) was much lower than that of chloroform (330.16 µg/ml), petroleum ether extract (243.79 µg/ml) and aqueous extract (272.26 µg/ml). IC_{50} value of methanolic extract of *P. bicalyculata* was comparable to BHA (157.79 µg/ml), however, it was much higher than other synthetic antioxidant like rutin (45.19 µg/ml) and ascorbic acid (21.43 µg/ml) (fig. 7).

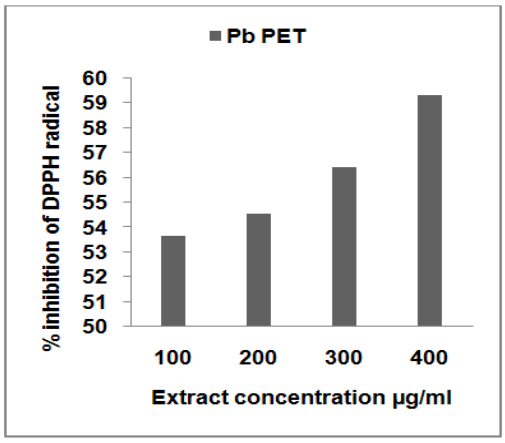

Fig. 3: % Inhibition of DPPH free radicals by *P. bicalyculata* petroleum ether extract

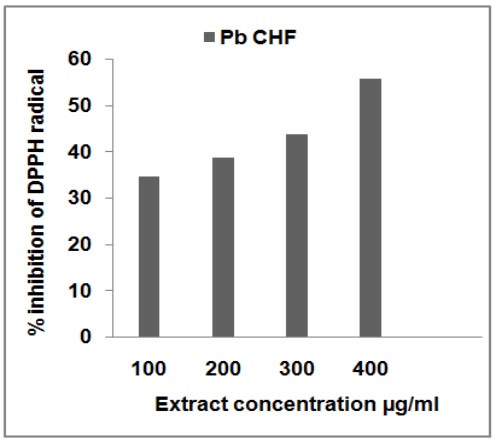

Fig. 4: % Inhibition of DPPH free radicals by *P. bicalyculata* chloroform extract

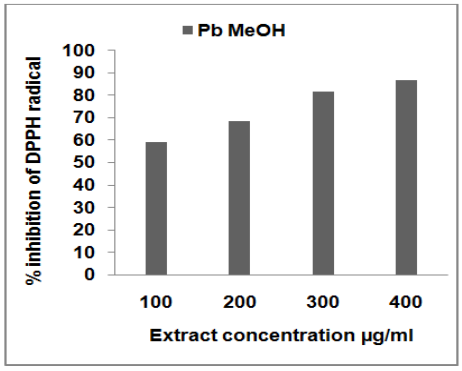

Fig. 5: % Inhibition of DPPH free radicals by *P. bicalyculata* methanol extract

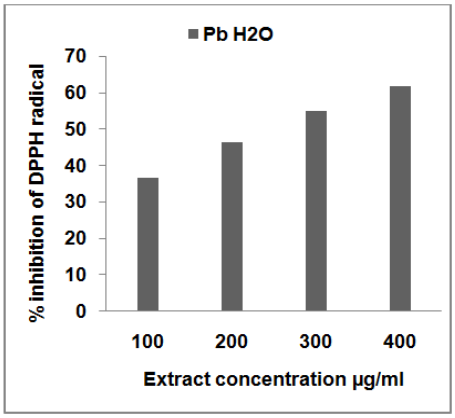

Fig. 6: % Inhibition of DPPH free radicals by *P. bicalyculata* aqueous extract

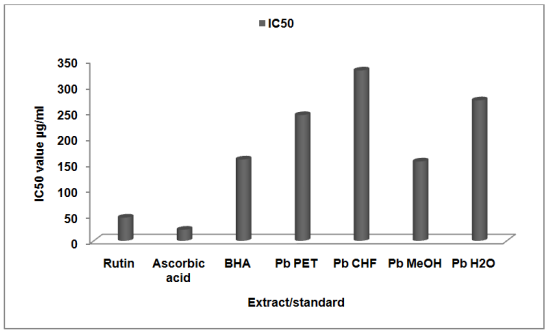

Fig. 7: Comparison of the DPPH free radicals scavenging ability by various extracts of *P. bicalyculata* with standard antioxidant. BHA= Butylated Hydroxyl Anisole, Pb PET= Petroleum ether extract of *P. bicalyculata*, Pb CHF= Chloroform extract of *P. bicalyculata*, Pb MeOH= Methanolic extract of *P. bicalyculata*, Pb H_2O= Aqueous extract of *P. bicalyculata*

DISCUSSION

The antibacterial properties of *P. bicalyculata* showed high activity against tested microorganisms. Some worker observed the antibacterial activity by disk diffusion method of ethanol, acetone and chloroform extracts of *P. bicalyculata* (Leaves) against *Bacillus cereus* (*B. cereus*), *Enterobacter aerogens* (*E. aerogens*), *Escherichia coli* (*E. coli*), *Salmonella typhi* (*S. typhi*) and *Staphylococcus aureus* (*S. aureus*). The ethanolic extract of *P. bicalyculata* exhibited a high degree of inhibition followed by chloroform and acetone. The zone of inhibition of various extracts of *P. bicalyculata* was compared antibiotic chloramphenicol. The ethanolic extracts of *P. bicalyculata* showed the highest zone of inhibition against *E. coli* (18.0±0.80 mm) followed by *B. cereus* (17.0±0.16 mm) and *S. typhi* (14.0±0.08 mm). The ethanolic extracts of *P. bicalyculata* did not show inhibition against *E. aerogenes* and *S. aureus.* The acetone extracts of *P. bicalyculata* illustrated maximum zone of inhibition against *S. aureus* (14.0±0.21 mm) followed by *B. cereus* (11.0±0.20 mm) and *S. typhi* (10.0±0.12 mm). The acetone extracts of *P. bicalyculata* were failed to demonstrate the inhibition of *E. aerogenes* and *E. coli.* The chloroform extracts of *P. bicalyculata* showed the highest zone of inhibition against *S. aureus* (14.0±0.08 mm) followed by *E. aerogenes* (13.0±0.04 mm) and *B. cereus* (12.0±0.21 mm) and failed to show the inhibition of *E. coli* and *S. typhi.* The Positive control chloramphenicol showed 15.0±0.08 mm against *E. aerogens*, 10.0±0.80 mm against *S. aureus*, 11.0±0.06 mm against *B. cereus*, 12.0±0.04 mm against *E. coli* and 12.0±0.12 mm against *S. typhi*, they could not calculate the minimum inhibition concentration (MICs) and ethanolic extract of *P. bicalyculata* failed to make zone of inhibition against *E. aerogenes* and *S. aureus.* But in the present study, the methanolic extract of *P. bicalyculata* gave 21.0±0.54 mm zone against the isolated strain of *S. aureus* and 22.0±0.50 mm zone against the standard strain of *S. aureus* (MTCC 1144) and MICs range in between 6.25 mg/ml to 25 mg/ml [8].

Study the antibacterial and phytochemical studies of three plants of family Acanthaceae used in Burkina Faso traditional medicine against eleven microorganisms comprising of clinical isolates and collection/stereotyped strains (gram positive and gram negative). The clinical isolates were obtained from biomedical laboratory i.e. *Escherichia coli, Vibrio cholera* isolated from contaminating water, *Vibrio cholera*, *Salmonella typhimirium* isolated from contaminating fish and *Salmonella typhimirium* isolated from contaminate salad. Stereotyped strains used were: *Bacillus cerus* ATCC 9144, *Escherichia coli* ATCC 25922, *Escherichia coli* CPI 105182, *Proteus mirabilis* ATCC 35659, *Shigella dysenteria* CPI 5451 and *Staphylococcus aureus* ATCC 6538. The antibacterial activity was performed by agar well diffusion method using different fraction viz., hexane, dichloromethane, ethyl acetate and butanol of *P. bicalyculata*. The hexane Fractions of *P. bicalyculata* is exhibited maximum inhibition zone against *S. typhimurium* (25 mm) followed by *V. cholerae* (14 mm), *E. coli* (12 mm) and *E. coli* (ATCC: 25922) (14.67 mm) while dichloromethane and butanol fraction showed maximum inhibition zone against *P. mirabilis* (ATCC: 35659) (28.33 mm) followed by *E. coli* (CPI: 105182) (15.33 mm), *S. aureus* (ATCC: 6538) (14.67 mm) and *B. cerus* (ATCC: 9144) (11.67 mm) [9].

Another worker studied the antimicrobial activity by agar diffusion steak methods of ethanolic extract of *Peristrophe bicalyculata* against clinically isolated microorganism (bacteria: *E. coli*, *Klebsiella* spp, *Staphylococcus aureus* and *Pseudomonas aeruginosa* and fungi: *Aspergillus niger*, *Aspergillus clavatus*, *Rhizopus stolonifer*) broad-spectrum antibiotic gentamycin was used as positive control. The diameter of the zone of inhibition of ethanolic extract of the *P. bicalyculata* against the test bacteria: *Staphylococcus aureus*, *Klebsiella* spp., *E. coli*, and *Pseudomonas aeruginosa* to be 21 mm, 19 mm, 20 mm, 21 mm respectively at a concentration of 100 mg/ml. The zone of inhibition against *Aspergillus niger*, *Aspergillus clavatus* and *Rhizopus stolonifer* was also 18 mm, 15 mm and 22 mm respectively. These results were comparable with gentamycin and other broad-spectrum antibiotics used as control [10]. Another article reported that the leaves fraction of *Peristrophe bicalyculata* with an IC50 of 15.60±0.52 µg/ml was potentially very toxic against human mouth epidermal carcinoma (KB) cells (anticancerous). The present study supports the traditional use of *P. bicalyculata* and indicated that chloroform extract contains some major phytochemicals which inhibit the growth of microorganisms thereby proving a very effective source of derived drugs. However, erythromycin was found a little bit more effective as compared to plant extracts [11].

The use of crude extracts of plants parts and phytochemicals, of known antimicrobial properties, can be of great significance in the therapeutic treatments. In recent years, a number of studies have been conducted in different countries to prove such efficiency. Many plants have been used because of their antimicrobial traits, which are due to the secondary metabolites synthesized by the plants. These products are known by their active substances like, phenolic compounds, alkaloids, flavonoids, etc. which are the part of the essential oils and extracts [12]. The present study investigated *in vitro* antibacterial activity of crude leaves extract of *P. bicalyculata* obtained using different solvent. The data characterizing the antibacterial activity of crude extract of *P. bicalyculata* leaves are effective against all tested microorganisms. Chloroform extract of *P. bicalyculata* exhibited good antibacterial efficiency in comparison to another extract. It was highly active against *S. aureus* (MTCC 1144) (26.6±0.66 mm) lowest inhibition by aqueous extract against *S. pyogenes* (MTCC 442) (9.3±0.59 mm) in comparison to other solvent extracts.

Some research article reported that the antioxidant activity of the whole plant of P. bicalyculata by DPPH method. They used the three solvent petroleum ether, methanol and ethyl acetate. The results showed highest antioxidant activity by methanol extract in 1000 µg/ml concentration (63.15%) followed by same concentration of petroleum ether (50.28%) and ethyl acetate (55.45%). The IC50 value of methanolic extract is (612 µg/ml), petroleum ether extract (1020 µg/ml) and ethyl acetate extract (830 µg/ml) in 1000 µg/ml concentration. Rutin used as standard give IC50 value (480 mg/ml) on methanol, petroleum ether and ethyl acetate.

Another researcher work on antioxidant activity of water, ethanol and acetone extract of P. bicalyculata by DPPH method on 50 µg/ml concentration. The IC50 value of water extract is (471 µg/ml), ethanol (501 µg/ml) and acetone extract (144.7 µg/ml). The maximum scavenging percentage was found in acetone extract than ethanol and water extract of P. bicalyculata [13]. Determine the antioxidant activity of water-ethanol and acetone extract of P. bicalyculata by DPPH method on 50 mg/ml concentration [14].

CONCLUSION

Therefore, it can be concluded that chloroform extract of *P. bicalyculata* has excellent antibacterial potential against tested respiratory tract pathogens than another extract. Crude chloroform extract of *P. bicalyculata* have slightly less antibacterial activity then broad spectrum antibiotic erythromycin. Leaves of *P. bicalyculata* can be used in the treatment of various respiratory diseases. The antibacterial properties of extracts may be endorsed to the presence of reported phytoconstituents, which is confirmed by the results of the phytochemical analysis. Chloroform extract of *P. bicalyculata* showed the presence of alkaloids, glycosides, flavonoids, steroids/terpenes, tannins and saponins. The methanolic leaves extract of *P. bicalyculata* showed the potent DPPH free radical scavenging ability then other extracts, which is better than the synthetic antioxidant BHA. The methanolic extract of *P. bicalyculata* showed effective antioxidant activity. The synergistic effect between the antibiotics and plant extracts against selected pathogens leads to the new choice of treatment. It is recommended that further research should be carried out to explore the bioactive component of these Himalayan medicinal herbs. The need for the establishment of standard dosage cannot be overemphasized. This is necessary to investigate the toxicity level of extract resulting from overdosage or from any of phytochemical component present in plant material.

ACKNOWLEDGEMENT

This work was financially supported by U G C New Delhi and Department of Botany and Microbiology H N B Garhwal University (A Central University) Srinagar (Garhwal). The author is also thankful to Curator of Garhwal University Herbarium (GUH), H. N. B. Garhwal University Srinagar (Garhwal) for medicinal plant identification.

AUTHOR CONTRIBUTION

The objective, experimental part of this work and writing, correction of the manuscript was done by the main author Dr. Prashant Arya.

ABBREVIATION

Percentage-%, Microgram-µg, Millilitre-ml, Microliter-ul, Millimetre-mm, Milligram-mg, Gram-g, Temperature- °C, Hours-h, PET-Petroleum Ether Extract, CHF-Chloroform Extract, MeOH-Methanol Extract, H_2O-Aqueous Extract, DMSO-Dimethyl Sulphoxide.

CONFLICT OF INTERESTS

Author declares there is no conflict of interest

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