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 valuable 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 compared to recent synthetic drugs. Thus the central factor needed is to take the maximum benefit from the traditional system of medicine for providing 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 2.45x1.3 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 600-1,400 m asl in Garhwal Himalaya. The genus Peristrophe only eight species 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 asml 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 (H2O), 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.
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 for 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).

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

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 (1.5×10⁶) 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 diphenyl 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 radical scavenging activity of the plant extract was calculated as the percentage inhibition according to the given formula $[7]$. 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 $[7]$. 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 | H₂O | 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, H₂O-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) (1.43±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).
Table 2: Phytochemical screening of various leaves extracts of *P. bicalyculata*

| Phytoconstituents | Solvent            | Petroleum ether (PET) | Chloroform (CHF) | Methanol (MeOH) | Aqueous (H₂O) |
|-------------------|--------------------|-----------------------|-------------------|-----------------|---------------|
| 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 IC₅₀ of the extract showed the strong antioxidant activity presence in the extracts. IC₅₀ 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). IC₅₀ 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).
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 aerogenes (E. aerogenes), 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 with antibiotic chloramphenicol. The ethanolic extracts of P. bicalyculata showed the highest zone of inhibition against E. coli (18.0±0.90 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 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±0.21 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 Positive control chloramphenicol showed 15.0±0.88 mm against E. aerogenes, 10.0±0.80 mm against S. aureus, 11.0±0.66 mm against B. cereus, 13.0±0.04 mm against E. coli and 12.0±0.12 mm against S. typhi. The acetone 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 typhimurium isolated from contaminating fish and Salmonella typhimurium isolated from contaminated salad. Stereotyped strains used were: Bacillus cereus ATCC 9144, Escherichia coli ATCC 25922, Escherichia coli CPI 105182, Proteus mirabilis ATCC 35659, Shigella dysenteria CPI 5451 and Staphylococcus aureus ATCC 6538B. The antibacterial activity was performed by using agar diffusion method using different solvent 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) (24.53 mm) followed by E. coli (CPI: 105182) (15.33 mm), S. aureus (ATCC: 6538B) (14.67 mm) and B. cereus (ATCC: 9144) (1.67 mm) [9].

Another worker studied the antimicrobial activity by agar diffusion method 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 epithelial carcinoma (KB) cells (anticanerous). 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 the methanol 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. Chloroform extract of P. bicalyculata have slightly less antibacterial activity than 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, Microlitre-µl, Millimetre-mm, Milligram-mg, Gram-g, Temperature- °C, Hours-h, PET-Petroleum Ether Extract, CHF-Chloroform Extract, MeOH-Methanol Extract, H2O-Aqueous Extract, DMSO-Dimethyl Sulphoxide.

CONFLICT OF INTERESTS
Author declares there is no conflict of interest

REFERENCES
1. Srivastava J, Lambert G, Vietmeyer V. Medicinal plants: an expanding role in development. World Bank Technical Paper 2006;320−2. Doi:10.1596/0-8213-3613-4
2. Ghani A. In: Traditional Medicine. Jahangirnagar University Savar Dhaka; 1999. p. 15−9.
3. Gaur RD. Flora of the District Garhwal North-West Himalaya (with Ethno Botanical Uses). Transmedia Publication Srinagar; 1999. p. 234−5.
4. Ahmad 1, Mehmood Z, Mohammad F. Screening of some Indian medicinal plants for their antimicrobial properties. J Ethnopharmacol 1998;62:183−3.
5. Aboaba OO, Smith SI, Olude FO. Antibacterial effect of edible plant extract on Escherichia coli. Pak J Nutr 2006;5:325−7.
6. Evans WC. Trease and evans pharmacognosy. Harcourt Brace Company India 1996;15:341−1.
7. Sheng ZW, Gao JH, Bil Y, Zhang WM, Dou HT, Jin ZQ. Antioxidant properties of the banana flower of two cultivars in China using 2,2-diphenyl-1-picrylhydrazyl (DPPH) reducing power 2,2‘-azino-bis-(3-ethylbenzthiazoline-6-sulphonate (ABTS) and inhibition of lipid peroxidation assays. Afr J Biotechnol 2011;10:4470−7.
8. Janakiraman N, Johnson M, Sahaya SS. G C-M S analysis of bioactive constituents of Peristrope bicalyculata (Retz) nees (Acanthaceae). Asian Pac J Trop Biomed; 2012. p. 546−9.
9. Nabere O, Hilou A, Gueme S, Konate K, Zerbo P, Meda Nag-Tierro R, et al. Antibacterial and phytochemical studies of three Acanthaceae species used in Burkina Faso traditional medicine. J Appl Pharm Sci 2013;3:49−5.
10. Giwa OE, Seyifunmi OE, Adewumi BL, Adebote VT, Aladejimokun AO. Screening of antimicrobial ethanolic extract of Peristrope bicalyculata. Ethnobot Leaflets 2010;14:766−3.
11. Abdulazeez MA, Ibrahim AD, Ayo JO, Carvalho LGC. Anticancer activities of extracts of Peristrope bicalyculata (RETZ) NEES. Rom Biotechnol Lett 2013;18:7995−8006.
12. Selvamohan TV, Ramadas S, Kishore SS. Antimicrobial activity of selected medicinal plants against some selected human pathogenic bacteria. Adv Appl Sci Res 2012;3:3374−1.
13. Johaley IR, Joshi P. In vitro antioxidant activity of various extracts of the whole plant of Peristrope bicalyculata forssk. Int J Pharm Drug Anal 2014;2:705−9.
14. Krishnamoorthy K, Krishnaswamy T, Subramaniam P, Sellamuthu M. Quantification of phytochemicals and in vitro antioxidant potential of various solvent extracts of certain species of Acanthaceae. Int J Green Pharm 2014;8:58−4.