IOSR Journal Of Pharmacy And Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 17, Issue 5 Ser. I (Sep. – Oct. 2022), PP 74-80
www.Iosrjournals.Org

Studies on a Potential Ingredient in Herbal Respiratory Infection Treatments: Methanolic Extract of *Angelica Glauca* Root and Stem

Kavita Kumari
Research Scholar, Department of Biotechnology Glocal University Mirzapur Pole , Saharanpur (Uttar Pradesh) India.

Dr.Krishan Pal
Research Supervisor, Department of Biotechnology Glocal University Mirzapur Pole , Saharanpur (Uttar Pradesh) India.

ABSTRACT
It has been discovered that plants manufacture a wide range of substances to defend themselves from a wide range of diseases. This study evaluated the ability of the medicinal plant Angelica glauca (Choru) root and stem extracts to fight off respiratory tract pathogens like Staphylococcus aureus MTCC 1144, Streptococcus pneumoniae MTCC 655, Streptococcus pyogenes MTCC 442, Pseudomonas aeruginosa MTCC 2474, and Klebsiella pneumoniae MTCC 4030. The plant material was gathered in Tungnath, 3,800 meters above sea level, in the Garhwal Himalaya. Plant roots and stems were cleaned, dried in the shade, ground into a fine powder, and then extracted in polarity-varying organic solvents (petroleum ether, chloroform, methanol, and water).
Using the two-fold serial dilution method of MIC determination and agar well diffusion, the antibacterial activity of produced extracts were assessed. Utilizing the DPPH assay, antioxidant potential was calculated, and many phytochemicals underwent qualitative phytochemical investigation. The results of the experiments showed that methanol extract had the highest levels of antibacterial and antioxidant activity, as well as the presence of the greatest number of phytochemical groups. The ZOIs formed by the methanol extract ranged in diameter from 11.0 0.54 mm to 30.3 1.58 mm, and the MIC ranges against all of the studied bacteria were 3.12 mg/ml to 25 mg/ml. The extracts of A. glauca contained alkaloids, flavonoids, glycosides, tannins, steroids, and saponins. In order to increase the effectiveness of herbal medications against respiratory tract disorders, this study supports the use of A. glauca as a natural antioxidant, either in pure form or as a supplement with other herbal formulations already on the market. The usage of A. glauca in the current COVID-19 pandemic crisis may be advantageous because its methanolic extract shown positive antibacterial activity against bacterial infections of the human respiratory system.

Key words: *Angelica glauca, Antibacterial, Two-fold serial dilution, Natural antioxidant, Phytomedicine, Respiratory tract pathogens, DPPH.*

I. INTRODUCTION

Since the dawn of human civilization, plant resources have been essential to the growth and maintenance of human life and will continue to be so.

In addition to the three basic necessities of human life—food, clothing, and shelter—the other most significant component of a person's life—their health—also depends heavily on plant resources. Plants are miraculously healing and continue to be a key component of nutraceuticals, Ayurvedic, Unani, homeopathic, herbal, and allopathic treatments. One-fourth of all medications are either derived directly from plants or are plant products. Microbial disease is not an exception to the use of plant-derived therapies in treating a variety of nutrient deficiency-related, physiological, and pathogen-based ailments.1,2 Plant-derived extracts and bioactive phytochemicals have reportedly played a significant influence in the creation of novel drugs in recent decades.3 All five components, or "Panchaang" (root, stem, leaves, and flowers), are beneficial medicinally. Alkaloids, tannins, flavonoids, steroids, phenolics, and other secondary metabolites found in plants are essential sources of diverse secondary metabolites with specific bioactive qualities, such as antibacterial activity, which serve as the foundation for the use of plants in pharmaceuticals.4-6 Essential oils are known to include phenolics, which have the potential to be insecticidal substances.7,8

Reactive oxygen species (ROS), such as the superoxide anion, the hydroxyl radical, and hydrogen peroxide, are crucial in the onset of many diseases, including Parkinson's disease, rheumatoid arthritis, asthma,

and bronchitis. Due to the presence of natural antioxidant chemicals that protect cells from the detrimental effects of ROS and reactive nitrogen species (RNS), plants offer a good chance for the treatment of diseases caused by ROS. Clinical evidence indicates that antibiotics are the primary tool against microbial (bacterial and fungal) infections, but antibiotic misuse has led to the issue of multi-drug resistance among different pathogenic microbes, and plants are predicted to provide new therapeutic molecules to combat drug-resistant microbes.

Infections in the respiratory tract are a leading cause of illness and mortality.10 The greatest public health issues around the world include respiratory disorders such allergies, asthma, and chronic obstructive pulmonary disease (COPD).

The most recent COVID-19 pandemic is a virus-driven respiratory tract infection.11 The most frequent causal agents of these infections are the microorganisms Streptococcus pneumoniae, Klebsiella pneumoniae, Staphylococcus aureus, Haemophilus influenzae, Pseudomonas aeruginosa, Acinetobacter baumannii, and Stenotrophomonas maltophilia.The opportunistic microorganisms utilized in this study—Streptococcus pyogenes, Streptococcus pneumoniae, Klebsiella pneumoniae, Staphylococcus aureus, and Pseudomonas aeruginosa—cause mild to severe infections. Streptococcus pneumoniae (MTCC-655), Klebsiella pneumoniae (MTCC-4030), Staphylococcus aureus (MTCC 1144)16,17, and Pseudomonas aeruginosa (MTCC 2474) are MTCC strains of these diseases.Numerous research teams have employed 14, 18 as pathogens for urine and respiratory tracts in anti-bacterial testing. The family Apiaceae's genus Angelica is well known for its applications in both conventional and contemporary medical practices. There are between 110 and 115 different species of angelica known to exist, 87 of which are found in Asia.19 There have been reports of Angelica glauca edgew, Angelica archangelica L., and Angelica nubigena Cl. in the Indian Himalaya.20 of these were Angelica glauca Edgew, also known as Choru in the local dialect. Choru; Sanskrti name- Gandrayan; English name- smooth Angelica), is widely distributed in the Himalayan regions of Uttarakhand, Jammu and Kashmir and Himachal Pradesh along amsl of 2000 to 3,800m.21.

II. MATERIALS AND METHODS

Plant Material

The healthy plants of *A. glauca* were collected from Uttarakhand. Plant identification was performed by authentication of collected specimens at Glocal University Herbarium (GUH), Root and stem were properly washed under running water, shade dried and grinded together into fine powder for further use.

Preparation of Extract

Four different solvents *i.e.*, Petroleum Ether (PET), Chloroform (CHF), methanol (MeOH) and water, were used for preparation of *A. glauca* extracts. Extracts were prepared by soaking 100g of powdered plant material in 300 ml of each solvent. Soaking plant material along with the solvent, was filled in Soxhlet apparatus and extracted by successive method for 72 h.34 The recovered plant extracts were passed through Whatman No. 1 filter to remove insoluble materials and the filtrate was concentrated using rotatory vacuum evaporator to obtain thick gummy extract. For antibacterial assays, the extracts were dissolved in 'dimethyl sulfoxide' (DMSO) to achieve 200mg/ml concentration.

Test Micro-organisms

Staphylococcus aureus (MTCC 1144), Streptococcus pneumoniae (MTCC 655), Streptococcus pyogenes (MTCC 442), Pseudomonas aeruginosa (MTCC 2474), and Klebsiella pneumoniae (MTCC 4030) were the bacterial pathogens used in the current investigation.These bacterial strains were bought from the Chandigarh-based Institute of Microbial Technology (CSIR-IMTECH).Chandra et al.35's normal bacteriological techniques were used to cultivate and maintain the bacterial strains after acquisition.

Inoculum Preparation

Standard cultures were maintained on agar-slant at 4°C. Active experimental cultures were prepared by adding a loopful of bacterial cells from standard cultures to Mueller-Hinton broth (MHB) tubes, followed by incubation at 37°C for 24 hrs.

Antibacterial testing

Antibacterial activity of 'root and stem' extract of different solvents was determined by agar well-diffusion method.34 100 µl of 12-16 hrs grown cultures of bacteria were mixed with 20 ml of molten Mueller Hinton Agar (MHA) (Medium no. 173, HiMedia Pvt. Ltd., Mumbai, India) and poured into pre-sterilized petri plates. Wells were punched in solidified MHA media using sterile cork borer (6.0 mm diameter) and filled with of 45µl of extracts solutions (dissolved in DMSO having final concentration of 200 mg/ml). Erythromycin, a broad-spectrum antibiotic and pure DMSO were used as positive and negative controls, respectively. Plates were incubated at 37°C for 24 hrs, and the diameter of the clear zone (Zone of Inhibition, ZOI), appearing

around each well was measured. Experiments were performed in triplicates and mean values with ± SD were recorded. The antibacterial activity was interpreted from the ZOIs, measured in nearest millimeter (mm).

Determination of Minimum Inhibitory Concentrations (MICs)

Two-fold serial dilution method was used for determining the minimum inhibitory concentrations (MICs) against selected bacterial species,35,36 with slight modifications. Six serial dilutions of each extract (50, 25, 12.5, 6.25, 3.12 and 1.56 mg/ml) were used for MIC determination. Tube containing equal volume of normal saline in place of bacterial inoculum was used as negative control. All tubes were incubated for 24 hrs at 37°C. The lowest concentration of the extract in the tube showing no visible growth (turbidity) was considered as MIC of that extract.

Evaluation of Antioxidant Potential using DPPH method

One ml methanol solution of extract was mixed with 3 ml solution of 2×10-4 mol/L ethanolic 2,2-diphenyl- 1-picrylhydrazyl (DPPH) solution and final volume was maintained to 10 ml using methanol. The mixture was vigorously shaken, and absorbance was instantly assessed at 517nm. The absorbance decrease was estimated at 15 and 30 min before the absorbance reached a steady state (after almost 30 min). Reduction in colour of DPPH was indication of positive indication of antioxidant activity in plant extracts and degree of colour reduction was directly proportional to antioxidant activity. The sample without plant extract but pure solvent and DPPH was used as blank.45

III. RESULTS AND DISCUSSION

Antibacterial activities of A. glauca 'root and stem' extracts

The essential oil obtained from different species of *Angelica* had been reported to show potent antibacterial and antifungal activities. Essential oil from *A. archangelica* roots was found effective in controlling the growth of bacteria (*Clostridium difficile, Clostridium perfringens, Enterococcus faecalis, Eubacterium limosum* and *Peptostreptococcus anaerobius*) and fungi (*Fusarium genus, Botrytis cinerea, Alternaria solani,* and *Candida albicans*).29,46 Growth of *S. aureus, Staphylococcus chromogenes*, and *Streptococcus uberis* was controlled by essential oil obtained from roots of *A. sinensis and A. dahurica.*30 Besides antibacterial activity, the essential oil obtained from roots of *A. archangelica, A. pubescentis Maxim* and *A. koreana Maxim.* showed potent antifungal activities.47,48

The essential oil obtained from different species of *Angelica* had been reported to show potent antibacterial and antifungal activities. Essential oil from *A. archangelica* roots was found effective in controlling the growth of bacteria (*Clostridium difficile, Clostridium perfringens, Enterococcus faecalis, Eubacterium limosum* and *Peptostreptococcus anaerobius*) and fungi (*Fusarium genus, Botrytis cinerea, Alternaria solani,* and *Candida albicans*).29,46 Growth of *S. aureus, Staphylococcus chromogenes*, and *Streptococcus uberis* was controlled by essential oil obtained from roots of *A. sinensis and A. dahurica.*30 Besides antibacterial activity, the essential oil obtained from roots of *A. archangelica, A. pubescentis Maxim* and *A. koreana Maxim.* showed potent antifungal activities.47,48 *Klebsiella pneumoniae.* Han and Guo assayed antibacterial activity of *Angelica sinensis* roots against *E. coli, S. aureus, Monilia albicans, Shigella* and *Salmonella typhimurium* but no significant activity was reported by them.50 Almost similar to their finding the least antibacterial activity of aqueous extract was reported in present work. Antibacterial activity of different extracts of *A. glauca* was compared with standard antibiotics (Erythromycin). In case of *S. aureus* crude MeOH extract of *A. glauca* produced 30.3±1.58 mm ZOI, equivalent to the commercial use broad spectrum antibiotic erythromycin 30.3±0.87 mm (Table 1). But in other cases, the positive control (erythromycin) was found to be slightly more effective than extracts of *A. glauca*. Erythromycin is an antimicrobial macrolide with broad spectrum design. It has better and wide spectrum of action against respiratory tract infections particularly for atypical species like mycoplasma and legionellosis. The MICs result showed the range of MIC values from 3.12 to 25 mg/ml (Figure 2). The lowest MIC of MeOH extract of *A. glauca* was observed against *S. pneumoniae* and *S. aureus* (3.12 mg/ml). MIC value of the same extract against *S. pyogenes*, *P. aeruginosa* and *K. pneumonia* were 6.25 mg/ ml, 12.5mg/ml and 25 mg/ml, respectively. The MIC values of other extracts were not determined because the ZOIs shown by them were smaller compared to MeOH extracts against all the tested pathogen under consideration.

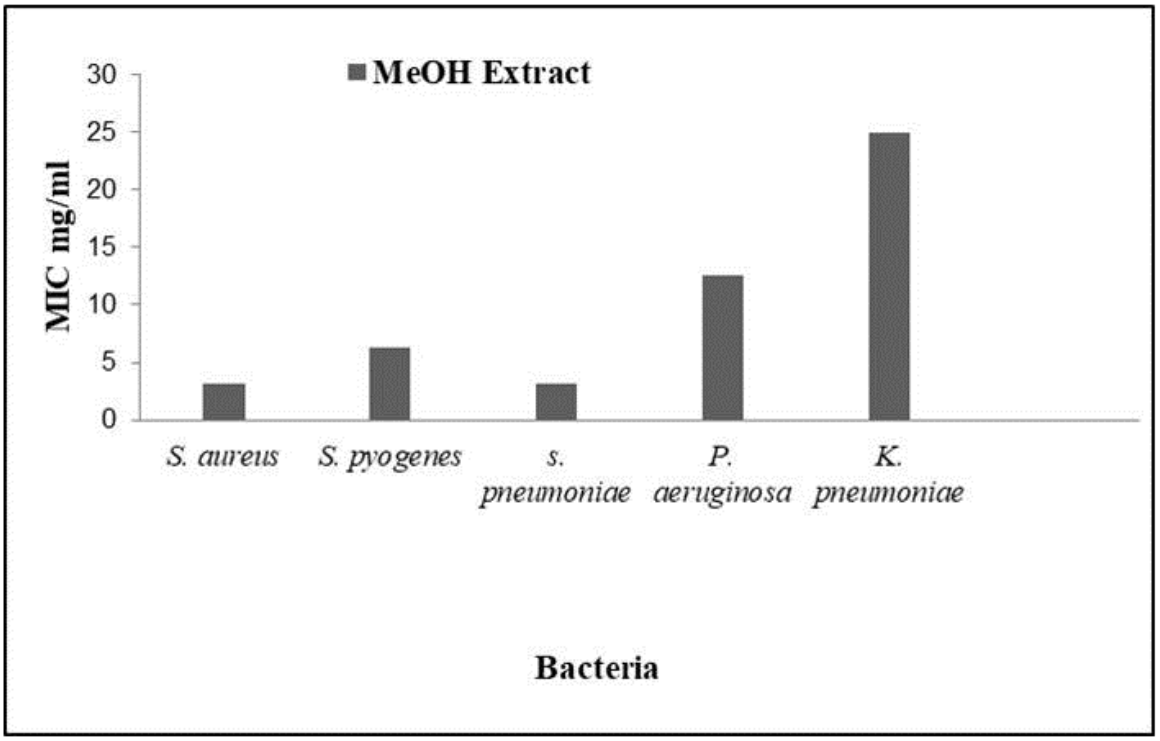

Figure 1: Minimum inhibitory concentrations (MICs) mg/ml of methanol extract of A. glauca.

The results of this study corroborated very well with studies by other research groups on *A. glauca* or other species of *Angelica*. The MeOH extract of *A. glauca* (Stem and Root) exhibited 30.3±1.58 mm ZOI against standard strains of *S. aureus* and its MIC was found 3.12 mg/ml. The sensitivity of *S. aureus*, *S. pyogenes*, *P. aeruginosa*, *S. pneumoniae* and *K. pneumoniae* are quite remarkable. The involvement of these micro-organisms in respiratory diseases is fairly notable and well known. *S. pyogenes* colonize the throat or skin and causes pharyngitis, impetigo, rheumatic fever, and acute glomerulonephritis.51 *S. pneumoniae* causes mild respiratory tract mucosal infections such as otitis media and sinusitis. Sometime it may cause more severe diseases such as pneumonia, septicemia, and meningitis.52 *Klebsiella pneumoniae* is an opportunistic pathogen, which mostly affects immune-compromised patients and causes nosocomial urinary tract infections, pneumonia, septicemias, and soft tissue infections. It also causes life-threatening community-acquired infections, such as pyogenic liver abscess, meningitis, fasciitis, endophthalmitis and severe pneumonia.53 *S. aureus* colonizes skin, but sometime causes different pyogenic and systemic infections. Most of *S. aureus* infections are not serious, but sometimes can be serious such as bloodstream infections, pneumonia, or bone and joint infections.54 *P. aeruginosa* is a nosocomial pathogen that affects immuno-compromised patients. It causes urinary tract infections, respiratory tract and other soft tissue infections.55

Table 1: The diameters of inhibition zones with various extracts of *Angelica glauca.*

Micro-organism	**Inhibition zone diameter (mm)**				**Positive control**	**Negative Control**
	PET	CHF	MeOH	H_2O	Erythromycin	DMSO
Staphylococcus aureus	23.6±0.38	24.3±0.25	30.3±1.58	19.3±1.73	30.3±0.87	0
Streptococcus pyogenes	19.0±0.57	16.6±0.76	22.3±0.42	11.0±0.54	24.6±0.76	0
Streptococcus pneumoniae	20.3±0.28	15.6±0.50	21.3±1.28	14.6±0.23	23.0±1.32	0
Pseudomonas aeruginosa	17.3±0.24	19.6±0.56	22.3±0.38	15.6±0.78	24.3±0.51	0
Klebsiella pneumoniae	16.6±0.97	15.6±0.25	19.0±0.54	11.6±0.47	21.6±0.76	0

Antioxidant activity of A. glauca root and stem extracts

The results of antioxidant activity assay showed the presence of natural antioxidants in 'root and stem' extract of *A. glauca*. At 100µg/ml concentration the MeOH, aqueous, CHF and PET showed 92.5%, 40%, 80% and 30% reduction of DPPH, while at 400µg/ml aqueous extract reduced 95.81% of DPPH (Figure 3 to Figure

6). The potential to scavenge DPPH radical was measured for determining IC50 value which indicate the concentration required to inhibit 50% of DPPH free radicals. Lower values of IC50 indicate higher potency to scavenging DPPH free radicals of plants extract. IC50 value of the MeOH extract (69.42 µg/ml) was much lower in comparison to CHF extract (100.71 µg/ ml), PET extract (261.35 µg/ml) and aqueous extract (231.65 µg/ml) of *A. glauca* (Figure 7). In comparison to standard antioxidants like BHA, ascorbic acid and rutin, antioxidant ability of methanolic extract was significantly lower than rutin and ascorbic acid, while it was higher than BHA. IC50 value of MeOH extract (69.42 µg/ ml) was half of the BHA (157.63 µg/ml), while it was significantly higher than the IC50s of rutin (45.19 µg/ml) and ascorbic acid (21.43 µg/ml) (Figure 7).

The results showed that highest anti-oxidant potential was present in MeOH extract followed by CHF, aqueous and PET. Phytochemical analysis of the different extracts showed that flavonoids, terpenoids and tannins, which are strong anti-oxidants were present in MeOH extract only. The remaining three extracts were either devoid of (aqueous) or were having only one of these phytochemicals (PET and CHF). Joshi *et al.*56 reported

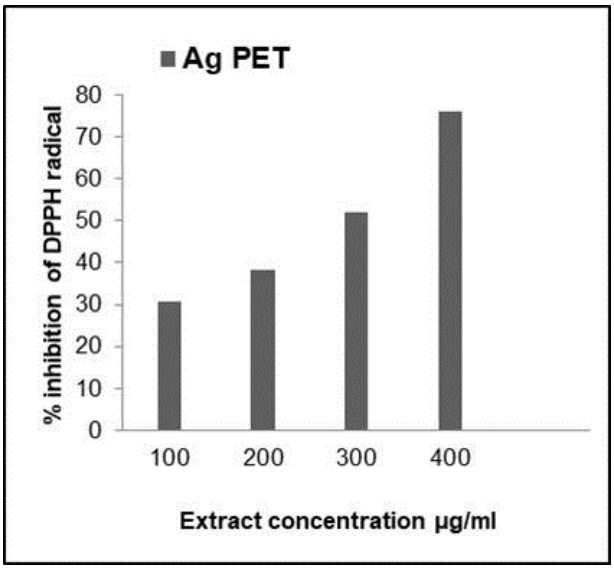

Fig .2 . % Inhibition of DPPH free radicals by *A. glauca* petroleum ether extract (Ag PET).

antioxidant activity of water extract of *A. glauca*, the scavenging activity of the water extract ranged from 14.58% to 71.53% when amount of extract increased from 5 to 25 mg, which was much lower compared to the antioxidant activities of aqueous extract of A. glauca reported by us. In present study 100 to 400 µg of extract showed 50.94% to 69.36% inhibition of DPPH, this could be due to different in extract preparation methods, in which we don't dried the extract at 50°C under vaccum, which may cause degradation of phytochemicals. Irshad et al.26 reported that the essential oil of A. glauca exhibited good DPPH radical scavenging activity showing (93.4% of inhibition and 45.05% inhibition of peroxidation). In present study the PET extract also showed similar range of antioxidant activity.

IV. CONCLUSION

In this study, methanol extract of root and stem of A. glauca have demonstrated strong antibacterial activity against the selected pathogenic bacteria of respiratory tract which may be due to the presence of major phytochemicals present in it. Furthermore, the results suggest a strong likelihood of developing safe effective and cheap antibacterial agent from various parts of A. glauca. The methanolic fraction of A. glauca can also be used as a new source of natural strong antioxidants. The future aspects of this study include isolating and identifying pure active compounds which are responsible for antibacterial action. The conclusion specifies that scientific studies performed on medicinal plants that have conventional efficacy may warrant fruitful results. Root and stem of A. glauca could be powerful source of novel antibiotics and major antioxidant compounds.

ABBREVIATIONS

%: Percentage; µg: Microgram; ml: Millilitre; µl: Microliter; mm: Millimetre; mg: Milligram; g: Gram; °C: Degree Celsius; hrs: Hours; PET: Petroleum Ether; CHF: Chloroform; MIC: Mininum Inhibitory Concentration; MeOH: Methanol; DMSO: Dimethyl Sulphoxide.

REFERENCES

[1]. Bhavnani SM, Ballow CH. New agents for gram-positive bacteria. Current Opinion of Microbiology. 2000;3(5):528-34.
[2]. Chariandy CM, Seaforth CE, Phelps RH, Pollard GV, Khambay BPS. Screening of medicinal plants from Trinidad and Tobago for antimicrobial andinsecticidal properties. Journal of Ethnopharmacology. 1999;64(3):265-70.
[3]. Bankole MA, Shittu LAJ, Ahmed TA, Bankole MN, Shittu RK, Terkula K, et al. Synergistic antimicrobial activities of phytoestrogens in crude extracts of two sesame species against some common pathogenic microorganisms. African Journal of Traditional, Complementary and Alternative Medicines. 2007;4(4):427-33.
[4]. Martindale W. Martindale: The extra pharmacopoeia, 31st edition edn. London: Royal Pharmaceutical Society; 1996.
[5]. Lis-Balchin M, Deans SG. Bioactivity of selected plant essential oils against
[6]. Listeria monocytogenes. Journal of Applied Microbiology. 1997;82(6):759-62.
[7]. Rios JL, Recio MC. Medicinal plants and antimicrobial activity. Journal of Ethnopharmacology. 2005;100(1-2):80-4.
[8]. Kambu K, Phanzu ND, Coune C, Wauters JN, Angenot L. Contribution à l'étude des propriétés insecticides et chimiques d'Eucalyptus saligna du Zaïre. Plantes Médicinales et Phytothérapie. 1982;16(1):34-8
[9]. Said-Al Ahl HA, Hikal WM, Tkachenko KG. Essential oils with potential as insecticidal agents: A review. Int J Environ Plan Manag. 2017;34:23-33.
[10]. Harbottle H, Thakur S, Zhao S, White DG. Genetics of Antimicrobial Resistance. Animal Biotechnology. 2006;17(2):111-24.
[11]. Cardoso TC, Lopes IM, Carneiro AH. A case-control study on risk factors for early-onset respiratory tract infection in patients admitted in ICU. BMC Pulmonary Medicine. 2007;7(1):12.
[12]. Subbarao K, Mahanty S. Respiratory virus infections: Understanding COVID-19. Immunity. 2020;52(6):905-9.
[13]. Ibrahim EH, Ward S, Sherman G, Kollef MH. A comparative analysis of patients with early-onset vs late-onset nosocomial pneumonia in the ICU setting. Chest. 2000;117(5):1434-42.
[14]. Alagumuthu M, Arumugam S. Molecular docking, discovery, synthesis, and pharmacological properties of new 6-substituted-2-(3-phenoxyphenyl)- 4-phenyl quinoline derivatives; an approach to developing potent DNA gyrase inhibitors/antibacterial agents. Bioorganic and Medicinal Chemistry. 2017;25(4):1448-55.
[15]. Gautam SS, Kumar S. The antibacterial and phytochemical aspects of Viola odorata Linn. Extracts against respiratory tract pathogens. Proceedings of the National Academy of Sciences, India Section B: Biological Sciences. 2012;82(4):567-72.
[16]. Bai S, Bharti P, Seasotiya L, Malik A, Dalal S. In vitro screening and evaluation of some Indian medicinal plants for their potential to inhibit Jack bean and bacterial ureases causing urinary infections. Pharmaceutical Biology. 2014;53(3):326-33.
[17]. Abboud Y, Saffaj T, Chagraoui A, ElBouari A, Brouzi K, Tanane O, et al. Biosynthesis, characterization and antimicrobial activity of copper oxide nanoparticles (CONPs) produced using brown alga extract (Bifurcaria bifurcata). Applied Nanoscience. 2014;4(5):571-6.
[18]. Raut SV, Pingle YA. Screening and characterization of antimicrobial substances produced by Bacillus species. Journal of Pure and Applied Microbiology. 2010;4(1):321-31.
[19]. Jayapriya J, Ramamurthy V. Use of non-native phenazines to improve the performance of Pseudomonas aeruginosa MTCC 2474 catalysed fuel cells. Bioresource Technology. 2012;124:23-8.
[20]. Pimenov MG, Leonov MV. The Asian Umbelliferae biodiversity database (ASIUM) with particular reference to South-West Asian taxa. Turkish Journal of Botany. 2004;28(1-2):139-45.
[21]. Samant SS, Dhar U, Palni LMS. Medicinal Plants of Indian Himalaya: Diversity, Distribution Potential Values. Nainatal: Gyanodaya Prakashan; 1998.
[22]. Butola JS, Badola HK. Effect of pre-sowing treatment on seed germination and seedling vigour in Angelica glauca, a threatened medicinal herb. Current Science. 2004;796-9.
[23]. Agnihotri VK, Thappa RK, Meena B, Kapahi BK, Saxena RK, Qazi GN, et al. Essential oil composition of aerial parts of Angelica glauca growing wild in North-West Himalaya (India). Phytochemistry. 2004;65(16):2411-3.
[24]. Collett H. Flora Simlensis: A handbook of the flowering plants of Shimla and the neighbourhood. Calcutta and Shimla: Thacker Spink and Company; 1921.
[25]. Irshad M, Aziz S, Habib UR, Hussain H. GC-MS Analysis and Antifungal Activity of Essential oils of Angelica glauca, Plectranthus rugosus, and Valeriana wallichii. Journal of Essential Oil Bearing Plants. 2012;15(1):15-21.
[26]. Irshad M, Shahid M, Aziz S, Ghous T. Antioxidant, antimicrobial and phytotoxic activities of essential oil of Angelica glauca. Asian Journal of Chemistry. 2011;23(5):1947.
[27]. Purohit VK, Andola HC, Haider SZ, Tiwari D, Bahuguna YM, Gairola KC, et al. Essential oil constituents of Angelica glauca Edgew. Roots: An endangered species from Uttarakhand Himalaya (India). National Academy Science Letters. 2015;38(5):445-7.
[28]. Sowndhararajan K, Deepa P, Kim M, Park SJ, Kim S. A review of the composition of the essential oils and biological activities of Angelica species. Scientia Pharmaceutica. 2017;85(3):33.
[29]. Fraternale D, Flamini G, Ricci D. Essential oil composition and antimicrobial activity of Angelica archangelica L.(Apiaceae) roots. Journal of Medicinal Food. 2014;17(9):1043-7.
[30]. Mullen KAE, Lee AR, Lyman RL, Mason SE, Washburn SP, Anderson KL. An in vitro assessment of the antibacterial activity of plant-derived oils. Journal of Dairy Science. 2014;97(9):5587-91.
[31]. Prakash B, Singh P, Goni R, Raina AKP, Dubey NK. Efficacy of Angelica archangelica essential oil, phenyl ethyl alcohol and Î±-terpineol against isolated molds from walnut and their antiaflatoxigenic and antioxidant activity. Journal of Food Science and Technology. 2014;52(4):2220-8.
[32]. Sharma S, Rasal VP, Patil PA, Joshi RK. Effect of Angelica glauca essential oil on allergic airway changes induced by histamine and ovalbumin in experimental animals. Indian Journal of Pharmacology. 2017;49(1):55.
[33]. Negi A, Maurya VK. Antibacterial potential of Antimicrobial peptides containing whole proteins of two Adiantum species from Himalaya against selected human bacterial pathogens. International Journal of Research in Pharmaceutical Sciences. 2020;11:2603-13.
[34]. Ahmad I, Mehmood Z, Mohammad F. Screening of some Indian medicinal plants for their antimicrobial properties. Journal of Ethnopharmacology. 1998;62(2):183-93.
[35]. Chandra H, Srivastava J, Agarwal RK. Fundamental Techniques in Microbiology Publisher John Publisher Pvt. Ltd, New Delhi; 2016.
[36]. Schwalbe R, Steele-Moore L, Goodwin AC. Antimicrobial susceptibility testing protocols CRC Press; 2007.
[37]. Clarke EGC. Clarke's Isolation and Identification of Drugs in Pharmaceuticals, Body Fluids and Post-mortem Material, 2nd Edition edn. London: The Pharmaceutical Press; 1986.
[38]. Gul R, Jan SU, Faridullah S, Sherani S, Jahan N. Preliminary phytochemical screening, quantitative analysis of alkaloids, and antioxidant activity of crudeplant extracts from Ephedra intermedia indigenous to Balochistan. The Scientific World Journal. 2017.

[39]. Hossain MA, Al-Raqmi KAS, Al-Mijizy ZH, Weli AM, Al-Riyami Q. Study of total phenol, flavonoids contents and phytochemical screening of various leaves crude extracts of locally grown Thymus vulgaris. Asian Pacific Journal of Tropical Biomedicine. 2013;3(9):705-10.
[40]. Evans WC, Trease GE. Trease and Evans' pharmacognosy, 13 edn. Philadelphia, London: Baillière Tindall; 1989.
[41]. Maria R, Shirley M, Xavier C, Jaime S, David V, Rosa S, et al. Preliminary phytochemical screening, total phenolic content and antibacterial activity of thirteen native species from Guayas province Ecuador. Journal of King Saud University-Science. 2018;30(4):500-5.
[42]. Hikino H, Kiso Y, Wagner H, Fiebig M. Antihepatotoxic actions of flavonolignans from Silybum marianum fruits. Planta Medica. 1984;50(03):248-50.