Original Article

Methanolic Extract of *Angelica glauca* Edgew Root and Stem: A Possible Component of Herbal Medicines against Respiratory Infections

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ABSTRACT

Plants are identified to produce a variety of compounds to protect themselves against a diversity of pathogens. This study assessed the antibacterial, antioxidant potential and phytochemical screening of medicinal plant *Angelica glauca* (Choru) root and stem extracts against 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. The plant material was collected from Tungnath 3,800m amsl of Garhwal Himalaya. Root and stem of the plants were washed, shade dried, grinded into fine powder and extracted in the organic solvents of different polarity (petroleum ether, chloroform, methanol and water). Antibacterial activities of prepared extracts were assayed using agar well diffusion and two-fold serial dilution method of MIC determination. Antioxidant potential was determined using DPPH assay and phytochemical analysis was performed using qualitative methods for different phytochemicals. Experimental outcomes revealed maximum antibacterial and antioxidant activity of methanol extract; also the maximum groups of phytochemicals were present in the methanolic extract. The methanol extract produced ZOIs of 11.0±0.54mm to 30.3±1.58mm diameters and MICs ranges were recorded between 3.12 mg/ml to 25 mg/ml against all the tested bacteria. Alkaloids, flavonoids, glycosides, tannins, steroids and saponins were present in the extracts of *A. glauca*. This study favors a good response towards using *A. glauca* as natural antioxidant in herbal medicines, either in pure form or as supplement with other herbal formulations already available in the market, to enhance their potential against respiratory tract diseases. In the present COVID-19 pandemic situation, use of *A. glauca* can be beneficial, as it's methanolic extract showed promising antibacterial activity against bacterial pathogens of human respiratory tract.

Key words: *Angelica glauca*, Antibacterial, Two-fold serial dilution, Natural antioxidant, Phytomedicine, Respiratory tract pathogens, DPPH, COVID-19.

INTRODUCTION

Plants resources have played a pivotal role in development and sustaining human life since the inception of human civilization, and will remain indispensable part of it. Besides the three basic needs of human life i.e., food, fiber and shelter, another most important aspect of human's life i.e., health is also dependent on plant resources to great extent. Plants possess remarkable therapeutic properties and have remained important constituent of nutraceuticals, Ayurvedic, Unani, homeopathic, herbal and allopathic medicines. One-quarter of all drugs come either directly or as derivative from plants. Plant derived remedies are traditionally being used in fighting against various nutrient deficiency generated, physiological and pathogen based diseases, and microbial disease are not the exceptions of it.[1,2] In last few decades, plant derived extracts and bio-active phytochemicals have been reported to play a crucial role in development new drug.[3] All five parts *i.e.,* '*Panchaang*' (root, stem, leaves, flowers

Submission Date: 08-12-2020;
Revision Date: 03-03-2021;
Accepted Date: 03-05-2021

DOI: 10.5530/ijper.55.2s.127

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and seeds) either individually or in combinations are useful for therapeutic purposes. Plants are important source of various secondary metabolites like alkaloids, tannins, flavonoids, steroids, phenolics etc. which show distinct bioactive properties including antimicrobial activities that form the basis for applications of plants in pharmaceuticals.[4-6] Phenolics contained in essential oils are well recognized as potential insecticidal agents.[7,8] Reactive Oxygen Species (ROS) such as superoxide anion, hydroxyl radical and hydrogen peroxide, play the key role in the development of various illnesses such as arthritis, asthma, bronchitis, dementia, mongolism, carcinoma and Parkinson's disease in human. Plants provide a good hope in alleviating ROS generated disease because of containing natural antioxidant compounds which save cells from the harmful effects of ROS and reactive nitrogen species (RNS). Clinical data show that antibiotics are the main weapon against microbial (bacterial and fungal) infections but antibiotics misusage have given rise to the problem of presence and spread of multi-drug resistance among various pathogenic micro-organisms,[9] and plants are expected to provide new therapeutic molecules to counter drug resistant microbes.

Respiratory tract infections are a major cause of sickness and death.[10] Respiratory conditions such as allergies, asthma and chronic obstructive pulmonary disease (COPD) are the world's main public health concerns. Recent pandemic of COVID-19 is also a respiratory tract infection, but caused by virus.[11] The micro-organisms namely *Streptococcus pneumoniae, Klebsiella pneumoniae, Staphylococcus aureus, Haemophilus influenzae, Pseudomonas aeruginosa, Acinetobacter baumannii* and *Stenotrophomonas maltophilia* are the most common causative agents of such types of infections.[12] *Streptococcus pyogenes, Streptococcus pneumoniae, Klebsiella pneumoniae, Staphylococcus aureus* and *Pseudomonas aeruginosa,* used in this study, are opportunistic pathogens which causes mild to severe infections. MTCC strains of these pathogens; *Streptococcus pyogenes* (MTCC 442),[13,14] *Streptococcus pneumoniae* (MTCC-655),[14] *Klebsiella pneumoniae* (MTCC 4030),[15] *Staphylococcus aureus* (MTCC 1144)[16,17] and *Pseudomonas aeruginosa* (MTCC 2474)[14,18] have been used as respiratory tract, urinary tract pathogens by various research groups for anti-bacterial assays.

Genus Angelica of the family Apiaceae is globally recognized for its uses in traditional and modern medicine systems. 110–115 species of *Angelica* are world reported, and 87 species of them occur in Asia.[19] *Angelica glauca* edgew, *A. archangelica* L. and *A. nubigena* Cl. have been reported from the Indian Himalaya region.[20] Out of them *Angelica glauca* Edgew (locally called as Choru- vernacular name-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]

A. glauca is well known for its aromatic and therapeutic values. It has been grouped under 'Chandanadi varg' by Shodala nighantu which also indicates presence of volatile oils and flavonoids in it. In Charak Samhita *A. glauca* has been classified under '*Sanjasthapana varg'*, which means for bringing consciousness to fainted persons. Flowering period started from July to August and fruiting period is September to October.[22] Several *Angelica* species have been used in home-grown system of medicine as anti-inflammatory in stomachache, in skin diseases and as a diuretic. *A. glauca* leaves are used against fever, jaundice and the paste of leaves is useful in skin allergy.[23] Roots and stem of *A. glauca* are used for constipation, urinary disorders, rheumatism, bronchitis, cough, cold and asthma[21,22] and considered effective in cardiac disease. Local communities use its roots and stem as a spice especially for seasoning curry.[24] Pharmacological studies revealed the role of *A. glauca* in Unani and ayurvedic drugs against common cold, asthma, cough and other respiratory and stomach infection.[22]

Presence of essential oil in *A. glauca* has attracted many phytochemical and antibacterial studies on it. Z and E-ligustilide, Z and E-butylidene phthalide, caryophyllene oxide, thujene, terpinene, nerolidol, bisabolene, 6 types of coumurins and 23 other compounds have been isolated from essential oil of *Angelica* species.[23,25-28] Different biological activities like anti-oxidant, anti-seizure, cytotoxicity, Anti-aflatoxigenic, nicotine sensitizaiton, anti-inflammatory, mosquito repellent etc., have been reported in different species of *Angelica*.[28] Root extract of *A. archangelica L.* showed potent antimicrobial activities against four fungal and five bacterial pathogens.[29] *A. sinensis* and *A. dahurica* were found active against *Staphylococcus aureus, Staphylococcus chromogenes,* and *Streptococcus uberis*.[30] Essential oil of *A. archangelica* was also found effective in agricultural and food items preservations.[31] Antibacterial activities of *A. glauca* whole plant extract have been reported against bacteria (*Staphylococcus aureus, Bacillus subtilis, Escherichia coli,* and *Pasteurella multocida*) and fungi (*Candida albicans, Microsporum canis, Aspergillus flavus* and *Fusarium solani*),[25,26] which were comparable to Amoxicillin and Flumequinene, respectively. *Essential oil* of *A. galuca* was effective in treating histamine and ovalbumin induced allergic asthama in guinea pigs and mice.[32] Three antimicrobial peptides (AMPs) (a-phellandrene, trans-carveol, b-pinene caryophyllene) were isolated from dried aerial part of *A. glauca* which were found very

effective against *K. pneumoniae*, *P. aeruginosa*.[23] AMPs have their unique mechanism of antibacterial action and could be effective against drug resistant bacteria.[33]

Despite of reports on use of *A. glauca* in local herbal medicines, its use in herbal formulations for respiratory ailments, available in the market, is not observed. Even the recently proposed 'Arogya-Kashayam-20' an Ayurvedic medicine for asymptomatic patients of COVID-19 also don't have *A. glauca*. Several reports on medicinal properties of essential oil, obtained from *A. glauca* are available. The present work was based on antioxidant potential and antibacterial properties of *A. glauca* against respiratory tract pathogens, along with qualitative estimations of various phytochemicals present in it, to establish its further medicinal applications.

MATERIALS AND METHODS

Plant Material

The healthy plants of *A. glauca* were collected from Tungnath (District Rudraprayag, Uttarakhand), on the way of Chandrashila, at 3,800m amsl (Figure 1). Plant identification was performed by authentication of collected specimens at Garhwal University Herbarium (GUH), H. N. B. Garhwal University Srinagar (Garhwal) and the same voucher was deposited to the herbarium with 'Accession No. GUH-20748'. Root and stem were properly washed under running water, shade dried and grinded together into fine powder for further use.

Figure 1: Maps showing Collection site of *Angelica glauca*.

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

Following bacterial pathogens causing human respiratory tract infections were used in present study: *Staphylococcus aureus* (MTCC 1144), *Streptococcus pneumoniae* (MTCC 655), *Streptococcus pyogenes* (MTCC 442), *Pseudomonas aeruginosa* (MTCC 2474) and *Klebsiella pneumoniae* (MTCC 4030). These bacterial strains were purchased from 'Institute of Microbial Technology (CSIR-IMTECH), Chandigarh. After procurement the bacterial strains were cultured and maintained using standard bacteriological protocols described by Chandra, *et al.*[35]

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.

Phytochemical screening

The primary phytochemical analyses were performed for qualitative estimation of various phytochemicals present in root and stem extracts of *A. glauca*.

Test for alkaloids

Solvent free extract was dissolved in aqueous acidic solution (Hydrochloric acid or Acetic acid) and filtered. One or two drops of Mayer's reagent were added slowly by the wall of test-tube to 5 ml of filtrate. Formation of white creamy precipitate was a positive indication for presence of alkaloids in the extract.[37]

Test for flavonoids

Alkaline reagent test: Extract was dissolved in methanol and 1N NaOH solution was added to it. Appearance of intense yellow colour, which disappears upon adding an acid to it, confirms the presence of flavonoids in the sample.[38-41]

Shinoda test: Small amount of magnesium and concentrated hydrochloric acid were added to the plant extract. The appearance of pink color after few minutes indicates the presence of flavonoids in the extract.[40,41]

Test for glycosides

Plant extract was dissolved in water, heated and filtered. The appearance of brick red color, upon adding 0.2 ml of Fehling solutions to the 5.0 ml of filtrate, confirmed the presence of glycosides in it. If instead of water, diluted H_2SO_4 is added and process is repeated, then appearance of a thicker precipitate compared to previous one, further confirms the presence of glycosides in it.[42]

Test for Steroids

The extract was dissolved in chloroform. Appearance of green color upon adding few drops of conc. H_2SO_4 and acetic anhydride to it is the positive indicator for presence of steroids in the sample.[43]

Test for reducing Sugars

The extract was dissolved in water and 1:1 mixture of Fehling solutions A and B were added to it. Appearance of brick red colored precipitate upon heating indicated the presence of aldolase (reducing) sugars in the sample.

Test for Saponins

20 mg of extract was boiled and cooled with 10 ml of water in a test tube for two minutes. The mixture was shaken vigorously, and left for 3 min. Formation of 1 cm thick foam layer indicates for presence of saponins in the sample.[44]

Test for Tannins

Plant extract was dissolved in water and 10% Ferric chloride was added to it. Presence of blue green color indicates the presence of tannins in the sample.

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]

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]

In present study the 'root and stem' extract of *A. glauca* exhibited potential antibacterial activity against tested bacterial pathogens, the results of antibacterial activities of 'root and stem' extracts of *A. glauca,* in different organic solvents, are shown in Table 1. MeOH extract of *A. glauca* exhibited maximum antibacterial activity compared to aqueous, CHF and PET extracts. MeOH extract showed strongest activity against *S.aureus* (30.3±1.58 mm), followed by *S. pyogenes* (22.3±0.42 mm), *P. aeruginosa* (22.3±0.38 mm), *S. pneumoniae* (21.3±1.28 mm) and *K. pneumoniae* (19.0±0.54 mm). Aqueous extract showed minimum inhibition compared to other extracts. Aqueous extract showed maximum activity against *S.aureus* (19.3±1.73 mm) followed by *P. aeruginosa* (15.6±0.78 mm), *S. pneumoniae* (14.6±0.23 mm), *S. pyrogens* (11.0±0.54 mm) and *K. pneumoniae* (11.6±0.47 mm). Maximum and minimum inhibition by CHF extract was found against *S. aureus* (24.3±0.25 mm) and *K. pneumoniae* (15.6±0.25 mm). The PET extract showed maximum activity against *S. aureus* (23.6±0.38 mm), and least activity against *K. pneumonia*e (16.6±0.97 mm).

There are number of reports on antibacterial potential of medicinal plants extracts against the human bacterial pathogens. These activities indicate the presence of certain metabolites having wide spectrum of antibiotic activities. *A. glauca* has well known medicinal and aromatic values; the roots of *A. glauca* contains valeric acid, angelic acid and angelisine resin are valued in treatment of cough, cold, asthma and inflammation.[26,49] Irshal *et al.* (2011) reported that essential oil of *A. glauca* (whole plant) showed antimicrobial action against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pasteurell amultocida* and found that the essential oil was most effective against *E. coli* and *S. aureus.* Present work demonstrated that all the extracts were also most effective against *S. aureus.* The whole plant essential oil also showed weak antibacterial activity against *bifidobacteria* and *lactobacilli.* The aqueous 'root and stem' extract of *A. glauca* showed antibacterial activity in the order *Staphylococcus aureus*> *Pseudomonas aeruginosa*> *Streptococcus pneumoniae*> *Streptococcus pyogenes*≥ *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

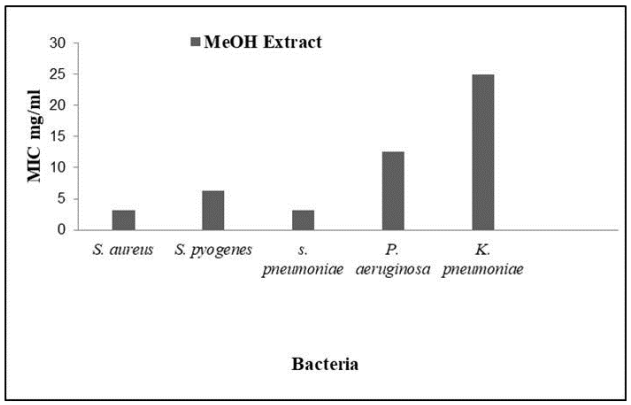

Figure 2: Minimum inhibitory concentrations (MICs) mg/ml of methanol extract of *A. glauca*.

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

Values of three replicates: Mean±SD; Cork borer diameter: 6 mm.

the ZOIs shown by them were smaller compared to MeOH extracts against all the tested pathogen under consideration.

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]

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 IC_{50} value which indicate the concentration required to inhibit 50% of DPPH free radicals. Lower values of IC_{50} indicate higher potency to scavenging DPPH free radicals of plants extract. IC_{50} 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,

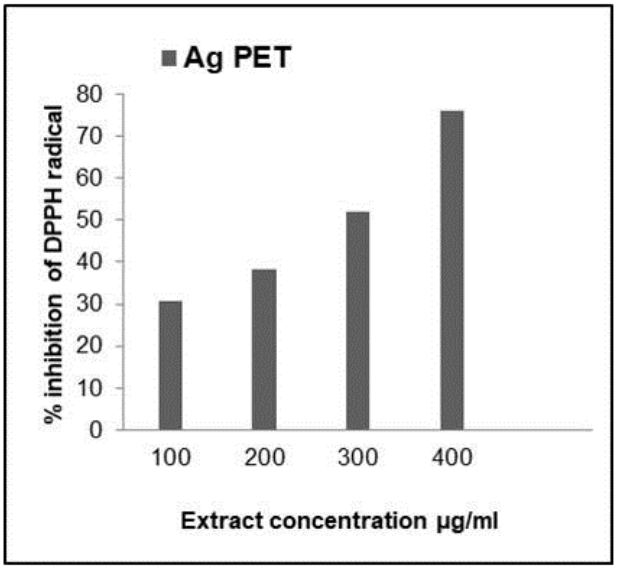

Figure 3: % Inhibition of DPPH free radicals by *A. glauca* petroleum ether extract (Ag PET).

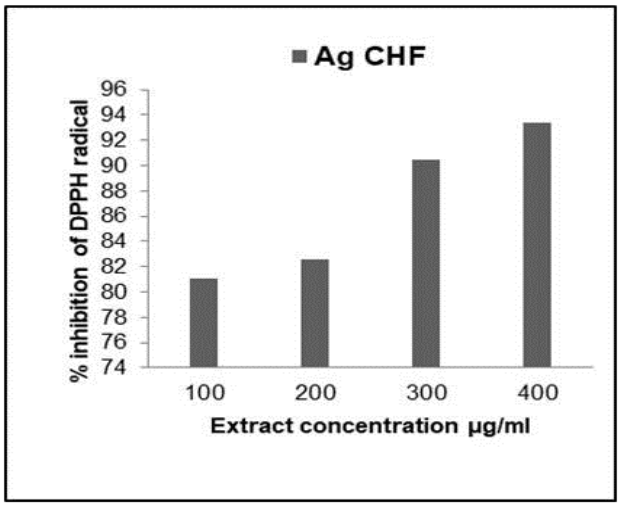

Figure 4: % Inhibition of DPPH free radicals by *A. glauca* chloroform extract (Ag CHF).

antioxidant ability of methanolic extract was significantly lower than rutin and ascorbic acid, while it was higher than BHA. IC_{50} value of MeOH extract (69.42 µg/ml) was half of the BHA (157.63 µg/ml), while it was significantly higher than the IC_{50}s 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

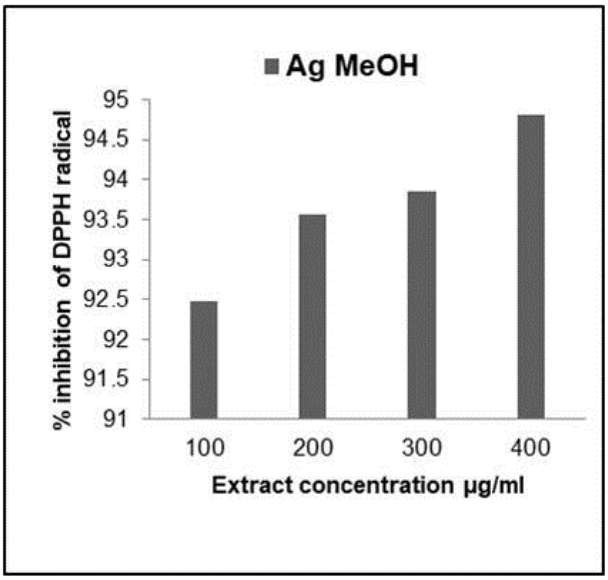

Figure 5: % Inhibition of DPPH free radicals by *A. glauca* methanol extract (Ag MeOH).

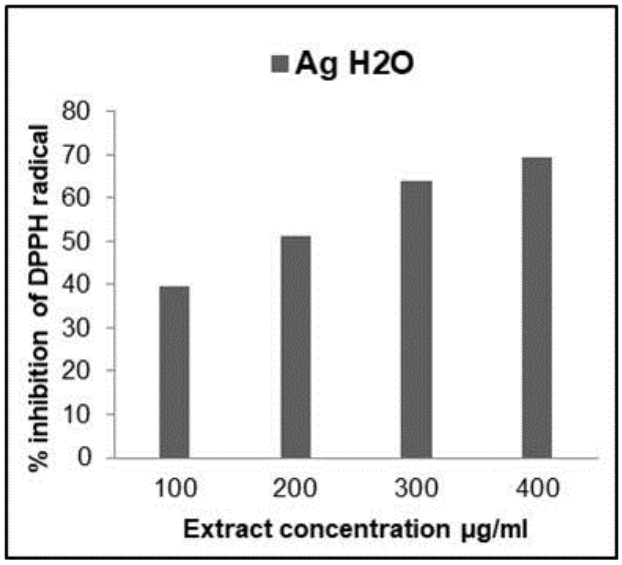

Figure 6: % Inhibition of DPPH free radicals by *A. glauca* aqueous extract (Ag H_2O).

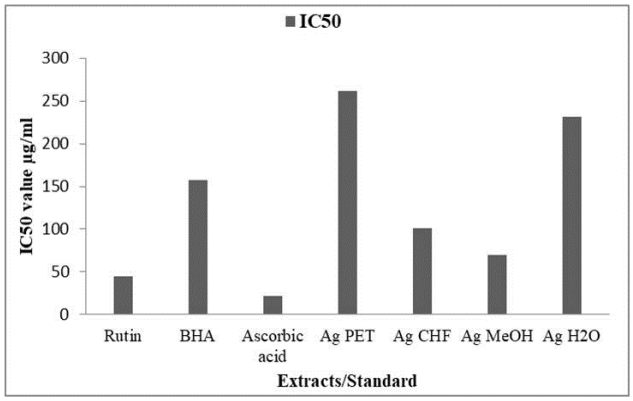

Figure 7: Comparison of the DPPH free radicals scavenging ability by various extracts of *A. glauca* with standard antioxidant. BHA= Butylated Hydroxyl Anisole, Ag PET= Petroleum ether extract of *A. glauca*, Ag CHF= Chloroform extract of *A. glauca*, Ag MeOH= Methanolic extract of *A. glauca* and Ag H_2O= Aqueous extract of *A. glauca*.

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.

Phytochemical analysis of *A. glauca* root and stem extracts

List of different phytochemicals present in the 'root and stem' extracts of *A. glauca* are shown in Table 2. Studies prior to this work analyzed the phytochemical

Table 2: Phytochemical analysis of root and stem extract of *A. glauca*.

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

+ = Present, – = Absent

BHA= Butylated Hydroxyl Anisole, Ag PET= Petroleum ether extract of *A. glauca*, Ag CHF= Chloroform extract of *A. glauca*, Ag MeOH= Methanolic extract of *A. glauca* and Ag H_2O= Aqueous extract of *A. glauca*.

composition of essential oil of root and aerial part of *A. glauca*, which would be equivalent to petroleum ether or hexane extract of the plant. Alkaloids, Flavonoids, Glycosides, Steroids, Terpenes, Sugars, Saponins and Tannins were present in aqueous and MeOH extract of *A. glauca*. Water and methanol are polar solvents and as expected polar compounds like sugar and saponins were present in it. CHF extract was found to contain only alkaloids and flavonoids, while alkaloids, flavonoids, glycosides and tannins were present in PET extract. Due to diverse compositions of alkaloids, flavonoids and tannins, these compounds were present in both polar (water and MeOH) and non-polar (PET) extracts. All these groups of compounds possess potent medicinal properties, and the antimicrobial potential of *A. glauca* is considered due to these chemicals.

Those primary and secondary metabolites present in *A. glauca* may be responsible for its antibacterial properties against tested micro-organisms, because the earlier pharmacology studies have publicized the role of *A. glauca* based herbal remedies in treatment of common respiratory problems like common cold, asthma, and cough. GC-MS analysis revealed the presence of 34 phytochemicals including Citronellol, Limonene, alpha and beta Pinene, alpha and beta Phellandrene, Thujene, Terpinene, Cis and trans-Ocimene, Germacrene D, b-Bisabolene, Alloaromadendrene, Piperitol, trans-Carveol and Carvone from the essential oil obtained from aerial parts of *A. glauca*.[23] Two compounds ligustilide and butylidene phthalides were specifically present in essential oil from root of *A. glauca*, while 16 monoterpenoids were common in both the oils.[57]

Flavonoids and terpenoids possess strong antioxidant activity and also show *in-vitro* antimicrobial activity. The possible mode of action of flavonoids is complex formation with soluble and extracellular proteins and inducing perturbations in cell wall of bacteria.[58] Saponins also affect the permeability of membrane owe to their detergent like actions. Due to presence of phenolic groups tannins also possess antibacterial activities. Tannins suppress bacterial multiplication by blocking essential metabolic enzymes required for normal growth. Presence of these phytochemicals in *A. glauca* can account for its antimicrobial and antioxidant activities.[59]

Although there are many reports on use the usage of *A. glauca* as local medicine and its antibacterial activities, but the available herbal formulations in the Indian market don't have this component. Even the famous Indian bioactive health supplement and immunity booster 'Chyawanprash' also lacks *A. glauca*.[60] The reason for this could be the very low availability of this herb to be used at commercial scale. The 'High Altitude Plant Physiology Research Center (HAPPRC) of H. N. B. Garhwal University, Srinagar Garhwal is engaged in *in-situ* cultivation at higher altitudes so that it could be made available in larger quantities.

Further investigations on phytochemical composition, followed by isolation of pure compound may provide some new antibacterial agent from *A. glauca*. Presence of AMPs is also reported from *A. glauca* and further investigation could provide new AMPs form *A. glauca*.

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.

ACKNOWLEDGEMENT

The PA is thankful to U.G.C., New Delhi and H. N. B. Garhwal University (A Central University) Srinagar (Garhwal), for financial support during his Ph.D. degree. All the authors are thankful to the University and Department of Botany and Microbiology, HNBGU for providing necessary workspace and laboratory facilities.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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.

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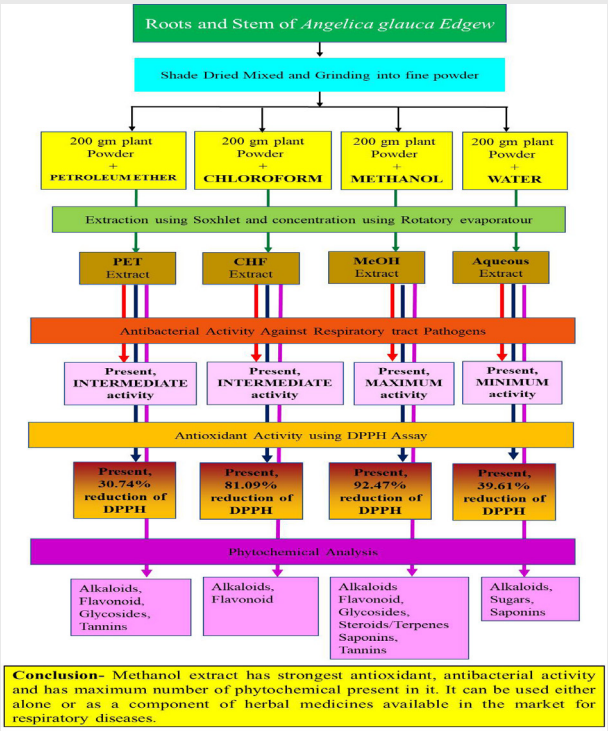
PICTORIAL ABSTRACT



SUMMARY

In the present study the 'root and stem' of *Angelica glauca* (Choru) were analyzed for their anti-bacterial, anti-oxidant potential and for the presence of bioactive phytochemicals in it. The PET, CHF, MeOH and H_2O extracts of 'root and stem' of *A. glauca* were prepared. The study confirmed good anti-bacterial activity of the plant extracts against the selected bacterial pathogens (*Staphylococcus aureus, Streptococcus pneumonia, Streptococcus pyogenes, Pseudomonas aeruginosa* and *Klebsiella pneumonia*) causing infections in human respiratory tract. MeOH extract showed the most potent anti-bacterial activity and maximum types of phytochemicals (Alkaloid, flavonoids, terpenes, tannins, saponins and glycosides) were present in it. Plant also showed good anti-oxidant activity using DPPH assay. Since the bacteria used in the study cause respiratory infections, and the plant showed good anti-oxidant activities, the use of *A. glauca*, either in pure form or in combination with other herbs, may be suggested in the marketed herbal formulations for respiratory diseases. This study was preliminary investigation on the phytochemical constituents of *A. glauca*, further studies on isolation, purification and other biological activities of this plants are awaited.

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Cite this article: Arya P, Mehta JP, Maurya VK. Methanolic Extract of *Angelica glauca* Edgew Root and Stem: A Possible Component of Herbal Medicines against Respiratory Infections. Indian J of Pharmaceutical Education and Research. 2021;55(2s):s552-s562.