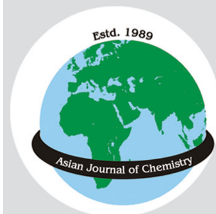

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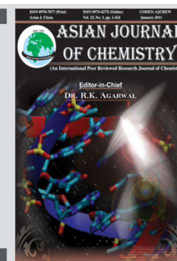

Antioxidant, Antimicrobial and Phytotoxic Activities of Essential Oil of *Angelica glauca*

MUHAMMAD IRSHAD[1], HABIB-UR-REHMAN[1], MUHAMMAD SHAHID[2,*], SHAHID AZIZ[1] and TAHSIN GHOUS[1]

[1]Department of Chemistry, University of Azad Jammu and Kashmir, Muzaffarabad-13100, Pakistan
[2]Department of Chemistry and Biochemistry, University of Agriculture, Faisalabad-38040, Pakistan

*Corresponding author: E-mail: mshahiduaf@yahoo.com

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Fresh plant material of *Angelica glauca* growing wild in the state of Jammu and Kashmir, collected and subjected to Clevenger-type hydro distillation apparatus for essential oil extraction. The essential oil was analyzed by GC/MS. *Angelica glauca* yielded about 0.17 % refreshing pale coloured oil on moisture free basis with characteristics floral woody flavour. Thirty compounds were identified, which yields about 97.8 % of the essential oils. The major constituents of *A. glauca* herb essential oil were characterized as terpene hydrocarbon like α-phellandrene (18.0 %), β-pinene (14.0 %), β-caryophyllene (8.6 %), γ-terpinene (6.7 %), β-bisaboline (5.2 %), germicene-D (4.5 %) with oxygenated terpenes like *trans*-carvol (16.4 %), β-caryophyllene oxide (8.0 %). Essential oil also exhibited good DPPH radical scavenging activity showing 93.4 % of inhibition and inhibition of peroxidation 45.05 %. The essential oil of *Angelica glauca* showed appreciable antimicrobial activity against battery of selected strains of bacteria and fungi, assessed by disc diffusion and measurement of minimum inhibitory by microdilution method. The plant was selected because of their reported medicinal importance in the indigenous system of medicines from ancient times and is abundantly available in various parts of Pakistan and particularly in State of Jammu and Kashmir.

Key Words: *Angelica glauca*, Essential oils composition and *in vitro* antimicrobial and antioxidant activity.

INTRODUCTION

Angelica a genus belong to family Apiaceae of tall, perennial herbs, ornamental, native to the North-temperate region and Newzeland, are cultivated by vegetative and also from seeds. *Angelica archangelica*, *Angelica glauca* and *Angelica cyclocarpa* are the three species present in India. The dominate species *Angelica glauca* occurs[1] wild in North-West Himalyas from Kashmir to Garhwal at an altitude of approximately 1500-3700 m. All parts of this plant were reported to be useful as stimulant, appetizer dysprosia, cordial, cardio active, diaphoretic, carminative, expectorant and also in stomach troubles, for treating constipation. The native of Kashmir valley used the roots part of this plant as a spice and condiment. Efforts are being made by some tribal peoples use to cultivate it in farms. The present work on the plant is on the chemical composition of the essential oil extracted from the plant at flowering stage[2] . Lee *et al.*[3] systematically isolated five different linear furano (pyrano) coumarin from *Angelica gigas* roots are bergapten, decursinol angelate, decursin, nodakenetin and nodakenin. The antibacterial activities of those compounds against pathogenic bacteria were investigated. Compounds decursinol angelate and decursin exhibited significant antibacterial activity against *Bacillus subtilis*. Saeed and Sabir[4] reported the irritant and cytotoxic potentiality of six coumarins, isolated from the roots of *Angelica glauca*. Even the volatile and molecular characterization of two Portuguese endemic species *Angelica lignescens* and *Melanoselinum decipiens* are reported by Marta *et al.*[5].

After the intensive review of literature we plan this piece of research work. This work is in continuation of the screening programme and chemical investigation of unexploited aromatic flora of state of Jammu and Kashmir a part of Himalaya for new sources of aromatic oils or aroma chemicals. The present work being appears to be the first report in literature on the chemical composition of the essential oil extracted from the whole plant of *Angelica glauca*.

EXPERIMENTAL

Fresh plant material was collected from different locations of the state of Jammu and Kashmir. The plant was identified by the plant taxonomist.

GC analysis: GC analysis were performed on a Shimadzu gas chromatograph, GC-9A with a FID and using two different capillary columns SE-30 (25 m × 0.25 µm film thickness) and supelcowax 10 (25 m × 0.32 mm, 0.25 µm film thickness). The oven temperature programmed from 60-220 ºC at 3 ºC/

min for SE-30 column 60-250 ºC at 5 ºC/min and supelcowax 10, carrier gas N_2.

GC/MS analysis: The oil was analyzed by GC-MS using a Hewlett-Packard GC-MSD 5890 Series 2 mass spectrometer (70 eV) on a SE-30 colmun (25 m × 0.25 mm) was used with helium as a carrier gas (1.8 mL/min). GC oven temperature was kept at 50 ºC for 2 min and programmed to 250 ºC at a rate of 5 ºC/min, then kept constant at 250 ºC for 2 min and then programmed to 250 ºC at a rate of 1 ºC/min split ratio was adjusted at 50:1. The injector and detector temperatures were at 260 ºC. MS were taken at 70 eV. Mass range was from 35-425 m/z.

The constituents were identified by comparison of their retention indices with literature values, library search was also carried out using Wiley GC/MS Library and TBAM Library of essential oil constituents. Retention indices (RI) of the sample components were determined on the basis of homologous *n*-alkanes hydrocarbons under the same condition. Relative percentage amount were calculated from TIC (Total ION Current) by the computer[6].

Hydrodistillation method: Air-dried plant material (500 g) were ground and subjected to hydrodistillation for 3 h, using a Clevenger-type apparatus as recommended by British Pharmacopeia[7]. Briefly, the plant was immersed in water and heated to boiling, after which the essential oil was evaporated together with water vapours and finally collected in a condenser. The distillate was isolated and dried over anhydrous sodium sulfate[8].

Antimicrobial activity

Microbial strains: The synthetic compounds were individually tested against a set of microorganisms, including two gram-positive bacteria: *Staphylococcus aureus*, API Staph TAC 6736152 and *Bacillus subtilis* JS 2004, two gram-negative bacteria: *Escherichia coli* ATCC 25922 and *Pasteurella multocida* (local isolate) and four pathogenic fungi, *Candida albicans*, *Microsporum canis*, *Aspergillus flavus* and *Fusarium solani*. The pure bacterial and fungal strains were obtained from Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad, Pakistan. Purity and identity were verified by the Department of Microbiology, University of Agriculture, Faisalabad, Pakistan. Bacterial strains were cultured overnight at 37 ºC in Nutrient agar (NA, Oxoid) while fungal strains were cultured overnight at 28 ºC using Potato dextrose agar (PDA, Oxoid).

Disc diffusion method: The antimicrobial activity of the essential oil was determined by disc diffusion method. Briefly, 100 µL of suspension of tested microorganisms, containing 10^8 colony-forming units (CFU)/mL of bacteria cells and 10^5 spores/mL of fungi spread on NA and PDA medium, respectively. The filter discs (6 mm in diameter) were individually impregnated with 15 µL of essential oil, placed on the agar plates which had previously been inoculated with the tested microorganisms. Discs without samples were used as a negative control. Amoxycillin (30 µg/dish) (Oxoid) and flumequine (30 µg/disk) (Oxoid, UK) were used as positive reference for bacteria and fungi, respectively to compare sensitivity of strain/isolate in analyzed microbial species. Plates, after 2 h at 4 ºC, were incubated at 37 ºC for 24 h for bacteria and at 28 ºC for 48 h for fungal strains. Antimicrobial activity was evaluated by measuring the diameter of the growth inhibition zones (zone reader) in millimeters for the organisms and comparing to the controls[9].

Determination of minimum inhibitory concentration: For the determination of minimum inhibitory concentration (MIC), which represents the concentration that completely inhibit the growth of microorganisms, a micro-dilution broth susceptibility assay was used, as recommended by National Committee for Clinical Laboratory Standards[10]. All tests were performed in nutrient broth (NB, Oxoid) for bacterial and sabouraud dextrose broth (SDB, Oxoid) for fungal strains supplemented with Tween 80 detergent to a final concentration of 0.5 % (v/v). Bacterial strains were cultured overnight at 37 ºC in nutrient broth and the fungi were cultured overnight at 28 ºC in sabouraud dextrose broth. Dilutions series were prepared from 0.03-72.0 mg/mL of the compounds in a 96-well microtiter palte, 160 µL of nutrient broth and sabouraud dextrose broth for bacteria and fungi, respectively were added onto microplates and 20 µL of tested solution. Then, 20 µL of 5×10^5 cfu/mL of standard microorganism suspension were inoculated on to microplates. Plates were incubated at 37 ºC for 24 h for bacteria and at 28 ºC for 48 h for fungi. The same test was performed simultaneously for the growth control (NB + Tween 80) and sterility control (NB + Tween 80 + test compound). Amoxycillin was used as a reference compound for antibacterial and flumequine for antifungal activities. The growth was indicated by the presence of a white "pellet" on the well bottom.

Antioxidant activity

DPPH radical scavenging assay: The antioxidant activity of the *Angelica glauca* oil was assessed by measuring their scavenging abilities to 2,2'-diphenyl-1-picrylhydrazyl stable radicals. The DPPH assay was performed as described by Mimica-Dukic *et al.*[11]. The samples from 0.5-15.5 µg/mL were mixed with 1 mL of 90 µM DPPH solution followed by addition of 95 % MeOH up to final volume of 4 mL. The absorbance of the resulting solutions and the blank were recorded after 1 h at room temperature. Synthetic antioxidant, BHT was used as a positive control. The disappearance of DPPH was read spectrophotometrically at 515 nm (U-2001, model 121-0032 Hitachi, Tokyo, Japan). Inhibition of free radical by DPPH in per cent (%) was calculated in following way:

$$I (\%) = 100 \times (A_{blank} - A_{sample}/A_{blank})$$

where A_{blank} = absorbance of the control reaction mixture excluding the test compounds and A_{sample} = absorbance of the test samples. IC_{50} values, which represented the concentration of essential oil that caused 50 % neutralization of DPPH radicals, were calculated from the plot of inhibition percentage against concentration.

Per cent inhibition in linoleic acid system: The antioxidant activity of *Angelica glauca* oil was determined by using inhibition of linoleic acid oxidation, following the method described by Singh *et al.*[12] with modification. The test samples (50 µg) were mixed with 1 mL of ethanol (v/v), linoleic acid (2.5 %, v/v), 99.5 % ethanol (4 mL) and 4 mL of 0.05 M sodium phosphate buffer (pH 7). The solution was incubated at 40 ºC for 175 h. The extent of oxidation was measured by

peroxide value using the colorimetric method described by Yen *et al.*[13]. To 0.2 mL sample solution, 10 mL of ethanol (75 %), 0.2 mL of an aqueous solution of ammonium thiocyanate (30 %) and 0.2 mL of ferrous chloride solution (20 mM in 3.5 % HCl) were added sequentially. After 3 min of stirring, the absorbance was measured at 500 nm, using spectrophotometer (U-2001, Hitachi Instruments, Inc., Tokyo, Japan). A control was performed with linoleic acid but without samples. Butylated hydroxytoluene (BHT) was used as positive control. Per cent inhibition of linoleic acid oxidation expressed as per cent was calculated as follows:

Inhibition of linoleic acid oxidation =

$$100-\left[\frac{\text{Abs. increase of sample at 175 h}}{\text{Abs. increase of control at 175 h}}\times 100\right]$$

Phytotoxic activity: The phytotoxic activity was studied through applying modified protocol of McLaughlin[14] *Lemna minor* was used as test plant.

Statistical analysis: All the experiments were conducted in triplicate unless stated otherwise and statistical analysis of the data were performed by analysis of variance (ANOVA) using Statistix 8.1 (Stat Soft Inc, Tulsa, OK, USA) software. A probability value of $p \leq 0.05$ was considered to denote a statistical significance difference. Data are presented as mean values ± standard deviation calculated from triplicate determinations.

RESULTS AND DISCUSSION

Angelica glauca is found sparingly distributed in North West Himalayas part of India and State of Jammu and Kashmir. The essential oil obtained from the whole plant at flowering stage is being characterized for its chemical constituents. The plant material were collected and subjected to hydrodistillation yielded about 0.17 % refreshing pale coloured oil on moisture free basis. The gas chromatogram of the oil revealed the presence of 45 components, of which 30 were identified, representing 97.8 % of the oil. The essential oil extracted from the whole herb was found to be a mixture of complex mono- and sesquiterpenes. The essential oil composition reported from aerial parts was found to be widely different from the root essential oil collected from the same area[15]. The essential oil of *A. glauca* root has been found to be rich in ligustide and butylidene phthalides[16] while these compounds were absent in *A. glauca* herb essential oil. The major compounds of *A. glauca* herb essential oil were characterized as terpene hydrocarbon like α-phellandrene (18.0 %), β-pinene (14.0 %), β-caryophyllene (8.6 %), thujene (6.7%), γ-terpinene (6.7 %), β-bisaboline (5.2 %). Germicene D (4.5 %) with oxygenated terpenes like *trans*-carvol (16.4 %), β-caryophylleneoxide (8.0 %). The quality of essential oils extracted from different locations at high altitudes of the state of Jammu and Kashmir showed that is no significant variation in chemical composition[15]. The constituents spiked in the essential oil of present sample are shown in the Table-1.

Antioxidant activity: The antioxidant activity of the oil of *A. glauca*, as assessed by DPPH radical scavenging assay and expressed in terms of 50 % inhibition (IC_{50}) is given in Table-2. Free radical scavenging capacity of the essential oil was noted to be increased in a concentration dependent manner. In the DPPH assay, the ability of the examined oil to act as donor of hydrogen atoms or electrons in transformation of DPPH$^{\hat{}}$ into its reduced form DPPH-H was investigated. The oil sample was able to reduce the stable, purple coloured radical DPPH into yellow coloured DPPH-H. The *A. glauca* oil exhibited considerable free radical scavenging activity with IC_{50} value 32.32 μg/mL. When DPPH scavenging activity of oil was compared with synthetic antioxidant BHT, the oil provided weaker activity (Table-2). No earlier studies were reported in the literature regard to the DPPH radical scavenging capacity of *A. glauca* oil. Similarly, DPPH inhibition percentage of oil was recorded lower (93.9 %) as compared to synthetic BHT (98.4 %).

TABLE-1
PERCENTAGE COMPOSITION OF THE ESSENTIAL OIL of *Angelica glauca* BY GC-MS

Compounds	*Angelica glauca*	RI	Method of identification
α-Phallendrene	18.0	995	RI, CO, MS
trans-Carvol	16.4	1196	RI, CO, MS
β-Pinene	14.0	974	RI,CO, MS
β-Caryophyllene	8.6	1405	RI, CO, MS
β-Caryophyllene oxide	8.0	1556	RI, CO, MS
Thujene	6.7	1052	RI, CO, MS
γ-Terpinene	6.7	1049	RI, CO, MS
β-Bisabolene	5.7	1496	RI, CO, MS
Germecene-D	4.5	1471	RI, CO, MS
β-Eudesmol	2.0	1629	RI, CO, MS
Sabinene	1.2	934	RI, MS
β-Elemene	0.7	1390	RI, MS
3-carene	0.7	1006	RI, MS
Verticiol	0.7	-	MS
Camphor	0.6	1126	RI, MS
α-Pinene	0.5	936	RI, MS
Caryophyllene	0.4	1400	RI, MS
cis-Ocimene	0.3	1264	RI, MS
Citronellol	0.3	1138	RI, MS
cis-Piperitol	0.3	–	MS
Ledol	0.2	1542	RI, MS
β-Phellendrene	0.2	1023	RI, MS
Lemonene	0.2	1020	RI, MS
Ledene	0.2	–	MS
Menthol	0.2	1174	RI, MS
L-Carvone	0.2	1242	RI, MS
Linalool	0.2	1085	RI, MS
Borneol	0.1	1155	RI, MS
p-Cymene	0.1	1015	RI, MS
Myrecene	0.1	983	RI, MS

RI: Retention indices, MS: Mass fragmentation, CO: Co-injection with authentic samples.

TABLE-2
ANTIOXIDANT ACTIVITIES OF *Angelica glauca* ESSENTIAL OIL BY DPPH RADICAL SCAVENGING AND LINOLEIC ACID INHIBITION ASSAY

Assay method	Essential oil	BHT
DPPH inhibition (%)	93.9[a]	98.4[b]
DPPH, IC_{50} (μg/mL)	32.32 ± 0.67[a]	19.23 ± 0.87[b]
Inhibition in linoleic acid system (%)	44.03 ± 0.96[a]	93.04 ± 1.95[b]

Values are mean ± standard deviation of three separate experiments. Different letters in superscript indicate significant differences within solvents.

A. glauca oil exhibited 44.03 % inhibition of peroxidation, that was significantly ($p < 0.05$) lower than BHT (93.04). Table-2 shows the level of percentage inhibition of linoleic acid oxidation as exhibited by the essential oil of *A. glauca*. Linoleic acid is a polyunsaturated fatty acid, upon oxidation peroxides are formed which oxidize Fe^{2+}-Fe^{3+}, the later forms complex with SCN^-, concentration of which is determined spectrophotometrically by measuring absorbance at 500 nm. Higher the absorbance, higher will be the concentration with control BHT (93.04 %).

Antimicrobial activity: The antimicrobial activity of the oil of *A. glauca* against a set of microbes like gram positive and gram negative bacteria and selected fungal strains is shown in Table-3. *A. glauca* oil exhibited variable degree of antimicrobial activity against the all microorganism tested. Results obtained from disc diffusion method, followed by measurement of minimum inhibitory concentration (MIC), showing the following sensitivity order *Escherichia coli* > *Staphylococcus aureus* > *Pasturella multocida* > *Bacillus subtilis*. The results indicated that *E. coli* and *S. aureus* were the most sensitive bacteria among selected bacterial strain tested, the zone of inhibition (mm) were 24.6 and 22.8 and the lowest MIC values (141.3 and 159.3 µg/mL), respectively. The lowest activity was observed for *Bacillus subtilis* with inhibition zones (20.3 mm) and highest MIC value (182.6 µg/mL). Overall, *A. glauca* oil, possessed antibacterial activity comparable with the standard drug, amoxycillin. Present results also showed that the oil exhibited significantly ($p < 0.05$) different activity against the entire selected bacterial microorganism with different zone of inhibition. Lee *et al.*[3] also reported the antibacterial activity and MIC of coumarins isolated from the root of *Angelica gigas*.

TABLE-3
ANTIMICROBIAL ACTIVITY OF THE ESSENTIAL OIL OF *Angelica glauca* AGAINST SELECTED BACTERIAL AND FUNGAL STRAINS

Selected organism	Essential oil		Antibiotic[A]	
	DD[B]	MIC	DD	MIC[C]
Bacterial strain				
S. aureus	22.8±1.2	159.3±3.9	28.8±0.8	103.3±3.9
B. subtilis	20.6±0.6	182.6±2.9	29.6±0.7	87.6±2.9
P. multocida	21.8±0.8	178.6±1.4	31.8±0.2	78.6±1.4
E. coli	24.6±1.6	141.3±2.3	28.6±1.9	99.3±2.3
Fungal strains				
C. albicans,	18.3±1.2	239.3±2.6	27.3±1.2	114.3±3.9
A. flavus	12.3±1.2	282.6±1.7	25.3±1.3	121.6±2.9
F. solani	18.5±4.6	239.6±1.4	29.5±2.6	89.6±1.4
M. canis	21.3±0.2	178.1±1.2	27.35±0.3	117.3±2.3

Values are of three independent experiments. A: Amoxiclin (30 µg/disk) for bacterial and flumequine (30 µg/disk) for fungal strains. B: DD, diameter of inhibition zone (mm) including disc diameter of 6 mm. C: MIC, minimum inhibitory concentration (µg/mL).

The results of antifungal activity of *A. glauca* oil against fungal strains are presented in Table-3. The oil from *A. glauca* exhibited antifungal activities comparable with the standard drug (flumequinene). The sensitivity order of selected fungal strains is *Microsporum canis* > *Fusarium solani* > *Candida albicans* > *Aspergillus flavus*. Among these fungal strain *Microsporum canis* was the most sensitive organism showing the highest zone of inhibition (21.3 mm) with lowest MIC value of 178.1 µg/mL while *Aspergillus flavus* was noted to be the most resistant fungus tested with the lowest inhibition zones (12.3 mm) and highest MIC (282.6 µg/mL). Based on these results, it is possible to conclude that the essential oil of *A. glauca* has stronger and broader spectrum of antimicrobial activity. But the overall antimicrobial profile of *A. glauca* oil evidented that this oil is more potent against bacterial as compared to fungal strains. Dukic[17] reported the antifungal activity of different solvent extract this plant against *Candida albicans* and *Candida tropicalis*.

Phytotoxic activity: The plants also exhibit *in vitro* phytotoxic activity. The essential of oil of *Angelica glauca* shows good phytotoxic activity against *Lemana minor*, activity shown in Table-4. Saeed and Sabir[4] reported six coumarins from *Angelica glauca* that possess potential irritant and cytotoxic activities.

TABLE-4
IN VITRO PHYTOTOXIC BIOASSAY OF *Angelica galuca* ESSENTIAL OIL

Name of plant	Conc. of compound (µg/mL)	No. of fronds		Growth regulation (%)	Conc. of std. drug (µg/mL)
		Sample	Control		
Lemna minor	1000	10	20	61	0.015
	100	16		12	
	10	18		04	

Keys: Std. drug paraquat, Incubation condition = 28 ± 1 ºC.

In conclusion, it is believed that this study provides useful information regarding the composition, antioxidant and antimicrobial attributes of *A. glauca* essential oil from Pakistan. From the results of the present investigation it can be concluded that oil can exhibit relatively better antimicrobial activity than antioxidant activity and cytotoxic activity. The utilization of indigenously grown *A. glauca* as potential source of natural antioxidants, as well an antimicrobial, food preservating and flavouring agents ought to be encouraged. However, further investigations involving more detailed *in vitro* and *in vivo* studies to establish how components interact to offer the antioxidant and antimicrobial activities are recommended. Studies should also be performed in order to evaluate the practical effectiveness of *A. glauca* oil using specific food substrates under particular environmental and storage conditions.

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ProCOngress (Thailand) Co., Ltd., 4/383 Moo6, Soi Nakniwas 37, Nakniwas Rd.,
Ladprao, Bangkok Thailand 10230.
Tel:+662-956-1580, Fax:+662-932-4454,
E-mail:alcharat@procongress.net, http://www.14acc.org/index.html