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Research Paper

Chemical Composition and Antimicrobial Properties of Essential Oil of Root Extract of *Angelica glauca* from Uttarakhand Region (India)

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A B S T R A C T

The purpose of this study was to evaluate the essential oil composition and antibacterial activity of root extract of Angelica *glauca* against four bacterial strains. Essential oil was obtained by hydro-distillation from the roots and analyzed using Gas Chromatography (GC) and Gas Chromatography- Mass Spectroscopy (GC-MS). The antibacterial activities of root extracts were tested using disc diffusion method against four bacterial strains. Twenty-six compounds were identified representing 93.20% of total oil, of which non-terpenoids (42.76%) and monoterpene hydrocarbons (42.10%) were found in large amount, i.e., terpinene-4-ol (20.13%) and trans-ligustilide (29.93%), phthalide- butylidene (11.67%). The antibacterial results revealed that the root extracts exhibited notable antimicrobial activity against *Staphylococcus aureus, Salmonella typhii, Bacillus* aureus and *Klebsiella pneumonia* except *Escherichia coli*. The extract of root obtained through methanol, ethyl acetate and aqueous exhibited good to moderate antimicrobial activity against tested bacterial strains. *n*-Butanol showed no antimicrobial activity. The above analysis suggests that B. *aureus* showed the best zone of inhibition for methanol extract.

***Key words*:** Angelica, Bacillus, Essential oil, Hydro-distillation, Terpenoids, Antimicrobial, Gas chromatography

Plants are a versatile source of bioactive metabolites, including polysaccharides, phenolics, alkaloids, essential oils (EOs), steroids, lignins, resins, tannins, etc. [1]. Essential oils (EOs) are complex mixtures of volatile compounds, used as additives to protect food materials against microbial aggression and as a flavour to enhance taste. EOs obtained from plants have various applications, especially in the health, agriculture, food, and cosmetic industries. So far, more than 3000 EOs have been isolated from about 2000 plant species, out of which 300 have been commercially used for various purposes [2]. Medicinal plants have a rich diversity of chemical and biological active chemical constituents that have a vital role in human welfare working as a remedy against diseases. These biologically active compounds found in plants also account for their antibacterial properties [3], [4], [5]. [6], [7]. Plant-produced active compounds have gained a lot of interest due to their reliable sources and renowned antimicrobial agents, which are more effective than their synthetic substitutes [8]. Medicinal plants with antimicrobial properties are being exhaustively used in different parts of the world. According to an estimate by the World Health Organization, 80% of the total world's population uses extracts of a plant for their biological active constituents as folk medicine in their tradition therapies [9].

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Angelica glauca commonly name as *Choru* or *Gandhrayan* belongs to the Apiaceae family. It is endemic to India and distributed between 2550 to 3800 m in Uttarakhand, Himachal Pradesh and Jammu and Kashmir [10]. Itt has high medicinal and aromatic properties that makes it suitable to be used as a stimulant, appetizer, carminative, cardioactive, diaphoretic, etc. It is also used in dyspepsia, bilious complaints, stomach difficulty, renal disorders, digestive disorders, lungs disorders, rheumatism, menorrhea, etc. [11], [12], [13]. Its roots are rich source of essential oils, which are useful in modern medicine including aromatherapy [11]. The species is reported to have huge pharmaceutical and therapeutic importance and anti-inflammatory property due to the occurrence of active constituents like butylidene phthalide and ligustilide [14], [15]. Steroids, alkaloids and terpenoids are the major components present in essential oil and extract of the plant. However, terpenes, monoterpenes, sesquiterpenes with alpha-phellandrene, beta cymene, alpha pinene, germacrene and caryophyllene are the major constituents [16]. In the present study, the evaluation of chemical composition and antimicrobial properties of essential oil of root extract of Angelica *glauca*. The seeds were extracted and procured from Gangotri, Uttarakhand (India) for use in the present study.

MATERIALS AND METHODS

Plant material and isolation of essential oil

The *Angelica glauca* roots samples were collected from the Gangotri region of Uttarakhand in the month of October. Hydro-distillation of dried roots of *A. glauca* was further preceded for experiment by using a Clevenger-type apparatus for 4-5 h [17]. The oil was dried by anhydrous sodium

sulphate and kept at 4°C till the GC and GC-MS was carried out.

Antimicrobial activity

Preparation of extracts and bacterial cultures used

The roots were oven dried again at 35°C for few hours for complete drying. Dried roots were ground and weighed before loading into Soxhlet assembly. The process takes up to 4 to 5 hours for complete extraction per solvent. Distillation was done under reduced pressure for removal of solvent and the liquid was dried by evaporation further. Four solvents are used on the basis of their polarity, and the temperature of the mantle was regulated according to the boiling point of the respective solvent. The boiling ranges of the solvents used during the extraction given in (Table 1).

Table 1 List of solvents

Solvents	Boiling range
Ethyl Acetate	77°C
Methanol	64°C
n-butanol	117°C
Distilled water	100°C

Muller Hilton agar media (Hi-media, India) was used to identify the antibacterial activity according to the standard method [18]. These strains were procured from the Microbial Type Culture Collection (MTCC, Chandigarh, India). The test organisms in this study were *Staphylococcus aureus* (MTCC3160), *Escherichia coli* (MTCC40), *Salmonella typhi* (MTCC3224), *Bacillus aureus* (MTCC4079) and *Klebsiella pneumoniae* (MTCC7028). These cultures were maintained on nutrient agar slants at first being incubated at 37°C for about 16-24 hours and then stored at 4°C as stock cultures for further antimicrobial assay. The stock cultures were sub-cultured at regular intervals.

Experimental procedure

Disc-diffusion method was used for the estimation of antimicrobial activity [19]. The extracts were dissolved in DMSO (1% v/v) to yield the final concentration of 100 mg/ml. The prepared extracts were soaked in sterile filter paper discs (Himedia, India). The bacteria strains were cultured in nutrient broth at 37°C, 200 rpm for 24 hours. One hundred microliters of prepared culture were plated on the surface of Muller-Hinton agar (Hi-Media). Previously prepared extract impregnated discs placed on the rooted plates. The plates were kept at ambient temperature for 30 min to enable diffusion of extracts and then incubated at 37°C for bacterial strains, respectively. Discs impregnated with only solvents used as negative controls and antibiotic discs of streptomycin (10 µg) (Hi-Media) for bacteria and Amphotericin B were used as positive controls. Antimicrobial activity was calculated by measuring the diameter of the zone of inhibition. Each experiment was repeated at three times and the mean of the diameter of inhibition zones around the discs were observed and recorded.

Minimum inhibitory concentration

For the determination of MIC of the sensitive bacterial strains, two-fold liquid dilution tests were made using Muller Hilton broth (Hi-media, India). Whether bacterial growth occurred or not was determined by observing the turbidity of cultures. The tube with the lowest dilution in which no growth occurred was evaluated as minimum inhibitory concentration.

GC and GC-MS analysis

Instrumentation and analytical conditions

The GC estimation of essential oil was performed by applying an Agilent Technology 6890 N gas chromatography data handling system equipped with a split-splitless injector and fitted with an FID using N2 as the carrier gas. The HP-5 capillary column (30 m × 0.32 mm, 0.25 μm film thickness) and temperature program was used. Initial temperature of 60°C (hold: 2 min) programmed at a rate of 3°C /min to a final temperature of 220°C (hold: 5 min). The injector and FID temperature were maintained at 210°C and 250° C, respectively. The Perkin Elmer Clarus 500 (Shelton, CT 06484, USA) gas chromatograph equipped with a split-splitless injector (split ratio 50: 1) data handling system was used to analysis GC-MS of essential oil. Rtx®-5 capillary column (60 m × 0.32 mm, 0.25 μm film thicknesses) was used and at a flow rate 1.0 m*l*/min helium was the carrier gas. The GC was interfaced with (Perkin Elmer Clarus 500) mass detector operating in the EI+ mode. The mass spectra were generally recorded over 40-500 amu that revealed the total ion current (TIC) chromatograms. The Temperature program was used as the same as described above for GC analysis. The temperatures of the injector, transfer line, and ion source were maintained at 210°C, 210°C and 200°C, respectively [20].

Qualitative and quantitative analysis

The individual components were identified by matching their recorded mass spectra with the library (NIST/ Pfleger /Wiley) provided by the instrument software, and by comparing their calculated retention indices with literature values [21]. Relative area percentages of the individual components were obtained from GC-FID analysis

RESULTS AND DISCUSSION

Chemical composition of essential oil

GC and GC-MS analyses of hydro-distilled compounds, roots of *A. glauca* revealed 26 different constituents, characterized 93.205% of total oil (Table 2). The study showed that monoterpene hydrocarbons (42.106%) and non-terpenoids (42.761%) were the major constituents of the oil. Terpinene-4-ol (20.13) and Trans-Ligustillide (29.935) was found in highest percentage among monoterpene hydrocarbons and non-terpenoids. The sesquiterpene hydrocarbons (5.242%) and oxygenated monoterpene (2.628) were also found in the oil. The remaining chemical constituents detected were in substantially lower quantities. It was reported that A. *glauca* collected from Himalayan region of Uttarakhand has high content of (Z) Ligustillide and (Z) butylidene phthalide [22].

Antimicrobial activity

Methanol, ethyl acetate, *n*-butanol and aqueous extracts were checked for their antimicrobial activity against bacterial pathogens. As shown in (Fig 1-3), the methanol extract showed antibacterial activity against *Bacillus aureus, Klebsiella pneumoniae and Staphylococcus aureus*, with inhibition zone diameters of 23 mm, 21 mm and 18 mm, respectively, in contrast, the zone of inhibition observed was much smaller in the case of ethyl acetate and aqueous extract. *Salmonella typhii* show a reduced zone of inhibition of 15 mm in methanol extract seen in (Fig 4). *n*-Butanol showed no antimicrobial activity against any bacterial pathogens as shown in (Table 3).

Table 2 Chemical constituents present in Angelica *glauca.*

S. No.	Components	RI	% in oil	Method of Identification
1	α – Pinene	939	1.270	RI, MS
2	α – Phellandrene	1003	0.016	RI, MS
3	P – Cymene	1025	0.308	RI, MS
4	O – Cymene	1026	2.499	RI, MS
5	Limonene	1029	6.165	RI, MS
6	β Phellandrene	1030	3.516	RI, MS
7	Thujene	1031	2.500	RI, MS
8	Cis-ocimene	1037	0.584	RI, MS
9	Transocimene	1050	0.440	RI, MS
10	Gamma– Terpinene	1060	3.342	RI, MS
11	Linalool	1097	0.567	RI, MS
12	Trans, Sabinene hydrate	1098	1.327	RI, MS
13	Terpinene-4-ol	1177	20.13	RI, MS
14	Carvone	1239	1.584	RI, MS
15.	Carene	1243	0.171	RI, MS
16.	terphenyl acetate	1349	0.306	RI, MS
17.	Germacrene-D	1485	0.307	RI, MS
18.	Delta cadinene	1523	0.534	RI, MS
19.	Trans- nerolidol	1550	1.500	RI, MS
20.	Spathulenol	1578	0.920	RI, MS
21.	Caryophyllene oxide	1583	1.393	RI, MS
22.	Eudesmol.	1649	0.588	RI, MS
23.	α – Cardinol	1652	0.468	RI, MS
24.	Phthalide- butylidene	1671	11.617	RI, MS
25.	Cis-ligustilide	1736	1.209	RI, MS
26.	Trans-Ligustillide	1797	29.935	RI, MS
	Monoterpene hydrocarbons	42.106		
	Oxygenated monoterpenes	2.628		
	Sesquiterpene hydrocarbons	5.242		
	Oxygenated sesquiterpene	0.468		
	Non-terpenoids	42.761		
	Total identified	93.205		

Result of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration of methanolic extract of *Angelica glauca* against *Bacillus aureus* was found to be 6.25 mg/m*l*. It was found 12.5 mg/m*l* against both *Klebsiella pneumoniae* and *Staphylococcus aureus*. The result obtained in this study suggests a potential application of *Angelica glauca* for treatment of several disorders. The most active constituents and extract could be useful in understanding the relationship between traditional cures and current medicines.

Table 3 *In-vitro* antimicrobial activity of different extract of *Angelica glauca*

Test organism	Methanol extract (100 mg/m*l*)	Ethyl acetate extract (100 mg/m*l*)	Aqueous extract (100 mg/m*l*)	*n*-butanol extract (100 mg/m*l*)	Standard drug streptpmycin[a] / amphotercin B[b] (10 μg/m*l*)
Bacillus aureus (MTCC 4079)	23 ± 0.57735	13 ± 1.15	-	-	32[a]
K*lebsiella pneumoniae* (MTCC 7028)	21 ± 1.57	11 ± 0.57	-	-	30[a]
Staphylococcus aureus (MTCC 3160)	18 ± 1.52	14 ± 1.15	4 ± 0.57	-	31[a]
Escherichia coli (MTCC 40)	-	-	-	-	32[a]
Salmonella typhi (MTCC 3224)	15 ± 1.527	11 ± 1.57	-	-	36[b]

Values are average of three replicates ± standard error
Mean inhibition zone diameter (mm)

Antimicrobial Activity studies on disk diffusion and the minimal inhibitory concentration of *A. glauca* essential oil demonstrated inhibition of the growth of bacteria and fungi [22]. The essential oils can be used as food additives for food preservation. Earlier some investigations have reported of Angelica *glauca* from Himalayan region including (Kashmir, Himanchal Pradesh and Kuamoun) of India which shows the qualitative and quantitative differences in the essential oil components [23], [24], [25], [26], [27]. The Angelica glauca root oil has been found to be rich in phthalides butylidene and Ligustillide [28], [29]. It was reported in earlier studies that Z-ligustilide has a protective effect on the brain from damage induced through transient forebrain cerebral ischemia [30]. Similarly, these two compounds were also isolated in abundance from essential oil obtained from the roots of Angelica glauca in present study. Further, herb essential oil

was found to be rich in monoterpenoids which were common to root oil [31]. It was also found in past studies that environmental factors, altitude, seasons, habitats and harvesting and drying methods influenced the primary and secondary metabolites such as alkaloids, terpenoids and essential oil compounds [32], [33], [34].

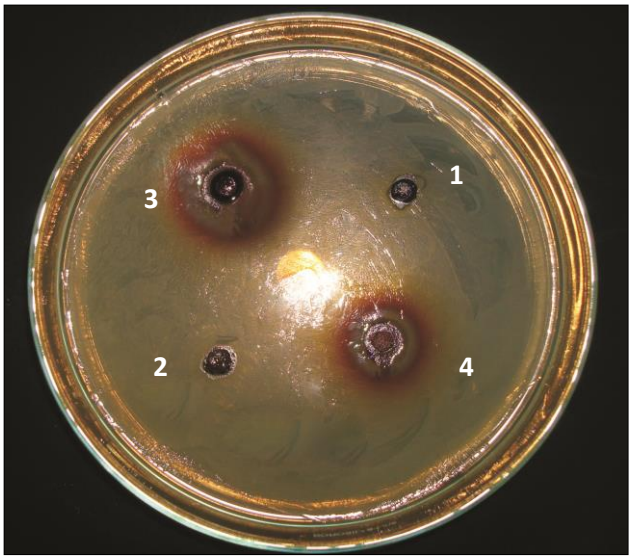

Fig 1 IZD shown by different solvent extract of *Angelica glauca* against *Bacillus aureus*
Where, 1 = Methanol, 2 = Ethyl Acetate, 3 = Aqueous, 4 = Control

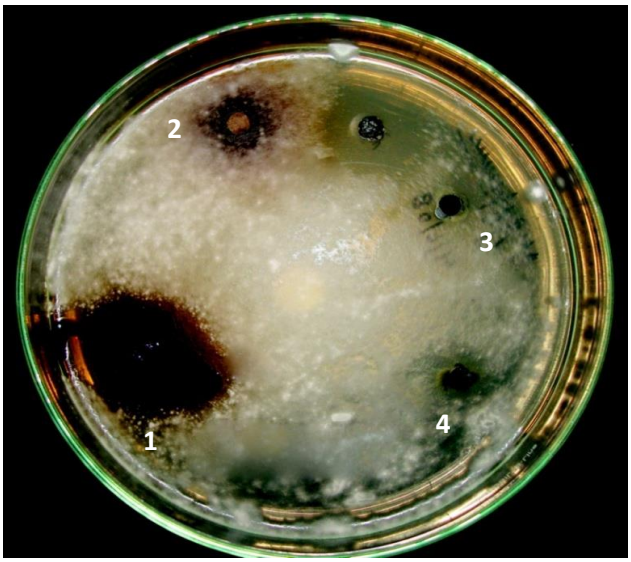

Fig 2 IZD shown by different solvent extract of *Angelica glauca* against *Klebsiella pneumoniae*
Where, 1 = Methanol, 2 = Ethyl acetate, 3 = Aqueous, 4 = Control

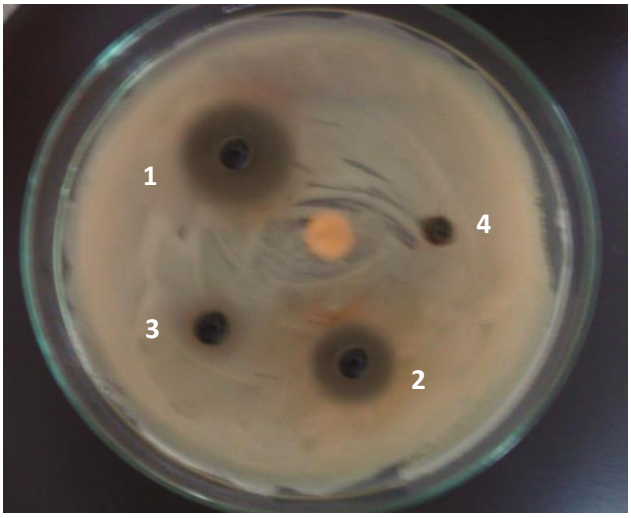

Fig 3 IZD shown by different solvent extract of *Angelica glauca* against *Staphylococcus aureus*
Where, 1 = Methanol, 2 = Ethyl Acetate, 3 = Aqueous, 4 = Control

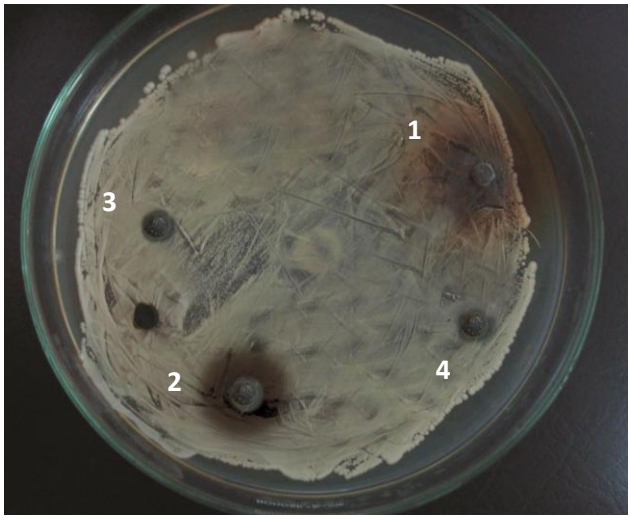

Fig 4 IZD shown by different solvent extract of *Angelica glauca* against *Salmonella typhi*
Where, 1 = Methanol, 2 = Ethyl Acetate, 3 = Aqueous, 4 = Control

CONCLUSIONS

The present study concluded that nonterpenoids and monoterpenoids were the dominant parts of the essential oil. The extract possesses antibacterial activity against all tested microbial strains, whereas *B. aureus* showed the best zone of inhibition for methanol extract. The antibacterial activity of extract may be due to the presence of active constituents in their roots. The most active constituents and extract could be useful in understanding the relationship between traditional cures and current pharmaceutical approach with high aroma properties.

Conflict of statement

The authors declare that there is no conflict of interest.

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Authors' contributions

TR designed the experiment and analyzed the data. GC-MS operated by TR. SG supervised the entire project and guided the experimental work. All authors wrote and finalized the manuscript.

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