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PHTYTO-CHEMICAL COMPOSITION, ANTIMICROBIAL AND PHYTO-TOXIC ACTIVITY OF *ANGELICA GLAUCA* (APIACEAE)

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Abstract

Medicinal and aromatic plants produce different bio-compounds which are used in pharmaceuticals, cosmetics and drug industries. *In vitro* phytotoxic, antibacterial activities as well as phytochemical screening of various fractions from the root of *Angelica glauca* were investigated**.** The data revealed that all the extracted fractions showed variable antibacterial activities. *Klebsiella pneumonia* was the most resistant bacterial strain and *Xanthomonas compestris* was the most sensitive. Ethyl acetate, chloroform, methanol and petroleum ether fractions showed inhibitory activities against all the tested strains. Chloroform fraction showed highest activity followed by ethyl acetate while butanol and aqueous extracted samples were found least responsive against the tested strains. Ethyl acetate and chloroform extracts measured maximum antifungal activity. Butanol and n-hexane extracted samples showed maximum phytotoxic activity while water fractions showed lowest phytotoxic activity. Phyto-chemical analysis revealed the presence of proteins and carbohydrates in all the extracts, however, the presence of tannins was not confirmed in any fraction. Alkaloids were reported in different fractions excluding n-hexane, water and ethyl acetate. Similarly, saponines were present only in butanol and water extracted samples. Lipids were found in all the extracted samples except methanol, water and petroleum ether extracted samples while sterols were found in all extracts except butanol and water.

Keywords: Antibacterial, Antifungal, Phytotoxic activity, Phytochemical, *Angelica glauca,* Disc diffusion assay

Introduction

Traditional medicines are mainly derived from the herbs and plants since ages and are widely used across the globe for the cure of a number of infectious diseases. Medicines based on plants and herbs are generally considered safe, less toxic and economical. It is the source of treatment for various health problems. Hakeem and tribal local people generally uses traditional medicines for the cure of significant number of health problems (Al-Essa *et al.,* 1998). It is normally believed that traditional herbs and plants are curative due to the presence of bioactive compounds (Iwu, 1993). Since ancient time humans are dependent on various products from the biological system (Parekh & Chanda, 2007). Due to the pharmacological properties of bioactive compounds, medicinal plants are commonly used in the manufacturing and production of safe and powerful drugs. In food industries too derived natural compounds are routinely used for the control of microbial contaminations (Delaquis & Mazza, 1995; Parveen and Bakht, 2015). Medicinal plants and their essential oil contain appreciable amount of naturally derived antimicrobial compounds. They retard the growth of food borne and spoilage bacterial pathogens and have tremendous potential as antimicrobial agents. The antimicrobial potential of medicinal plants and traditional herbs have been reported in various studies (Mari *et al.,* 2003; Obagwu & Korsten, 2003; Ayaz *et al.,* 2017; Wajid *et al.,* 2017).

The genus *Angelica* belongs to the family *Apiaceae. Angelica archangelica* and *Angelica glauca* are reported from Hazara, Gilgit and Kashmir (Nasir, 1972). *Angelica glauca* is the dominant specie which grows wild in North-West Himalyas at an altitude of approximately 2-4000 m (Agarwal, 1986). Almost all parts of the plants were found to be having potential as appetizer dysprosia, cordial, cardio active, diaphoretic and carminative, expectorant. The same plant can also be used in stomach problems and treating constipation. Roots of *Angelica glauca* are also used as a spice and condiment in Kashmir valley. The current investigation was carried out to screen the chemical composition, antibacterial and phyto-toxic activity of *Angelica glauca* root.

Materials and Methods

The proposed study was carried out at 'The Institute of Biotechnology and Genetic Engineering, The University of Agriculture, Peshawar, Pakistan. Plants roots were obtained from the mountainous areas of District Battagram, Tehsil Allai KPK, Pakistan and identified by plant taxonomists at the Department of Botany University of Peshawar, Pakistan. *Angelica glauca* roots were chopped, dried in the shade and grinded by tissue homogenizer.

Extraction and fractionation: The chopped and shade dried roots of *Angelica glauca* (1000 g) were placed in the drum containing methanol (5 L) for a period of two weeks at room temperature. After filtration the crude methanolic extract was dried using rotary evaporator that yielded a brownish gummy residue (110 g). The initial crude methanolic extract (100 g) was subsequently fractionated with different solvents.

Bacterial strains used: The antibacterial activity of the solvents extracted from the roots tissues of *Angelica glauca* was carried out against the selected bacterial strains through disc diffusion protocol. Fresh cultures were obtained from the stock cultures by streaking the sterilized and aseptic loop on the nutrients agar medium in the laminar flow hood while performing inoculation. The streaked fresh cultures were maintained in incubators for twenty (24) hrs at 37ºC for 24 hrs. Flasks containing

nutrients broths were subsequently inoculated with doubled streaked culture and placed in shaking incubators (GLSC-SBR-04-28) at 200 rpm for eighteen (18) hrs at a temperature of 37ºC.

Disc diffusion assay: Disc diffusion assay was carried for the determination of antibacterial activity (Bauer *et al*., 1966) and antifungal activity (Ramdas *et al*., 1998). Standardized inoculums of microbial cultures were streaked on the nutrients agar medium plates. On the solidified and sterilized nutrients agar media petri plates, autoclaved 3 mm discs of Whatman filter paper were placed. Subsequently plant extracts were applied on the disc in concentrations of 0.5, 1 and 2 mg per disc in 6, 12 and 18 µl volume respectively. Azithromycin was used as positive control in case of gram positive bacteria, ciprofloxacin for gram negative (50 µg per six micro liter) and DMSO as negative control at the same concentration. The inoculated plates were incubated at 37ºC overnight. Zone of inhibition was measured in millimeter in each plate against different microbes (Table 1).

Phytochemical screening: Phyto-chemical tests were carried for the confirmation of alkaloids, tannins, phenolic compounds, flavonoids, saponins, sterols and carbohydrates (Shome & Joshi, 1984).

Phytotoxic activity*:* Extracts were dissolved in ethyl acetate to prepare stock solutions and placed in falcon tubes overnight. After one day, 20 ml of enriched medium (basic pH) was added to evaporated falcon tubes. Ten healthy plants of *Lemna minor* each with three fronds were added to the flasks and kept in growth cabinet/chamber for one week (at 30ºC; light intensity of 9000 lux and 60% humidity). One week later the percent growth was calculated (Rehman *et al.,* 2012). Herbicide Parquet was used as positive control.

$$\text{Growth inhibition \%} = \frac{\text{Fronds in sample}}{\text{Fronds in –ve control}} \times 100$$

Statistical analysis: For statistical analysis, MSTATC computer software was used (Russel & Eisensmith, 1983). Significant differences among means were separated by Least Significant Difference (LSD) test (Steel *et al*., 1997).

Results

Results indicated that methanol, ethyl acetate, chloroform and pet ethers extracted samples showed good activities against *K. Pneumonia* (Fig. 1). However, it was resistant to the remaining solvents extracted fraction at all concentrations in comparison to positive control. Ethyl acetate extracted samples were the most effective one and recorded highest response at all concentrations (57.86 % at 18ul, 51.89 % at 12ul and 49.42 % at 6ul disc^{-1}) as compared to the other extracts. Fig. 2 presents the antibacterial activity of various extracted samples from *Angelica glauca* roots against *X. compestris.* Chloroform extracted fraction was the most effective one and recorded maximum antibacterial activity (69.87 % at 18 µl, 60.74 % at 12 µl and 58.06 % at 6 µl disc^{-1}) as compared to the other extracted samples. Similarly, all other solvents extracted samples also repressed the growth of bacteria at each concentration used while water fraction did not measure zone of inhibition at any concentration.

The growth of *E. coli* was reduced by crude methonolic extract, n-hexane, petroleum ether, chloroform and ethyl acetate fractions at 6, 12 and 18µl disc^{-1}. Crude methanolic extract and chloroform fractions were more effective to control the activity of *E. coli*. Water and butanol fraction were in effective to reduce the activity at any concentration recording 0% zone of inhibition (Fig. 3). Fig. 4 revealed that the activity of *B. Subtilis* was inhibited by crude methonolic extract, n-hexane, petroleum ether, chloroform and ethyl acetate fractions at all concentrations. Ethyl acetate fraction was more effective (57.86 %, 48.23 % and 41.21% at 18, 12 and 6 µl disc^{-1} respectively) in controlling the activity of *B. Subtilis* as compared to other extracts. *B. Subtilis* proliferation was also inhibited by methanolic, n-hexane, petroleum ether and chloroform fractions at all concentrations while water and butanol fraction was not effective to control the activity *B. Subtilis* at any concentration.

Proliferation of *S. aureus* was efficiently retarded by methonolic extract, n-hexane, petroleum ether, chloroform and ethyl acetate fractions at all concentration (Fig. 5). The most efficient extract in controlling the activity of *S. aureus* was metahnolic extract (55.35 % at 18 µl disc^{-1}) and ethyl acetate fraction (54.38 % at 18 µl disc^{-1}). Crude methanolic, n-hexane, petroleum ether, chloroforms and ethyl acetate extracts effectively inhibited the activity of *S. aureus* at all concentrations. Water and butanol fraction did not repress the growth of *S. aureus* at any concentration (0% ZI).

Table 1. Microbial strains tested during the present experiment.

Microbial species	Gram strain type	Details of the microbial strains used
Klebsiella pneumoniae	Negative	Clinical isolate obtained from Microbiology lab., QAU, Islamabad, Pakistan
Pseudomonas aeruginosa	Negative	ATCC # 9721
Staphylococcus aureus	Positive	ATCC # 6538
Bacillus subtilis	Positive	Clinical isolate obtained The Department of Microbiology, Quaid-I-Azam University Islamabad Pakistan
Escherichia coli	Negative	ATCC # 25922
Xanthomonas campestris	Negative	ATCC # 33913
Candida albicans		ATCC # 10231. Plant Pathology Department, The University of Agriculture Peshawar KPK Pakistan

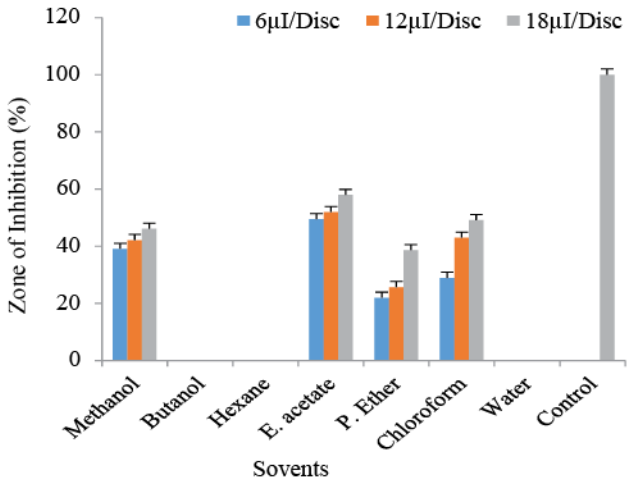

Fig. 1. Antibacterial activity of crude methanol, butanol, n-hexane, ethyl acetate, petroleum ether, chloroform and water extracted samples from the roots of *Angelica glauca* against *Klebsiella pneumoniae*by disc diffusion assay (Bar shows LSD at p<0.05).

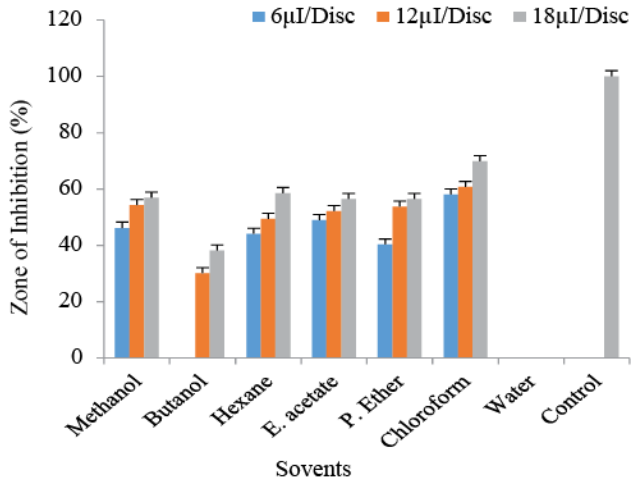

Fig. 2. Antibacterial activity of crude methanol, butanol, n-hexane, ethyl acetate, petroleum ether, chloroform and water extracted samples from the roots of *Angelica glauca*a gainst *Xanthomonas campestris* by disc diffusion assay (Bar shows LSD at p<0.05).

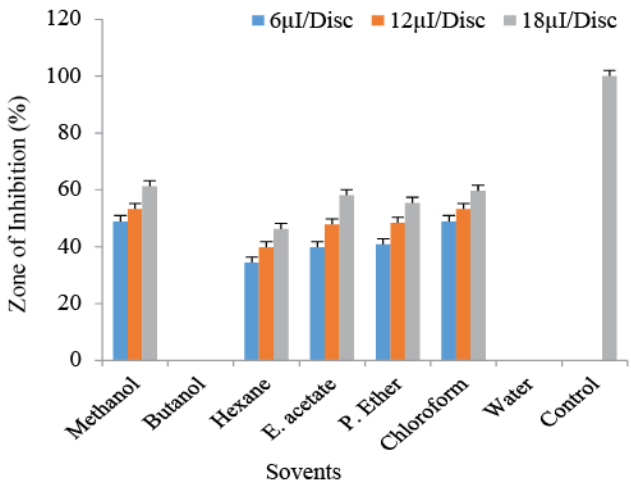

Fig. 3. Antibacterial activity of crude methanol, butanol, n-hexane, ethyl acetate, petroleum ether, chloroform and water extracted samples from the roots of *Angelica glauca* against *Escherichia coli* by disc diffusion assay (Bar shows LSD at p<0.05).

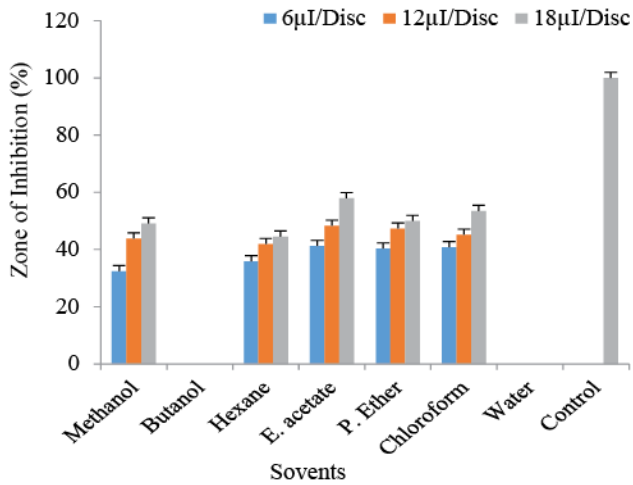

Fig. 4. Antibacterial activity of crude methanol, butanol, n-hexane, ethyl acetate, petroleum ether, chloroform and water extracted samples from the roots of *Angelica glauca* against *Bacillus subtilis* by disc diffusion assay (Bar shows LSD at p<0.05).

In comparison to the positive control, *P. aeruginosa* was highly susceptible to crude methanol, hexane, ethyl acetate, chloroform, butanol and pet ether fractions at all concentrations and was resistant to water extracted samples at the same concentration (Fig. 6). *P. aeruginosa* was susceptible to butanol extracted samples at higher concentrations (49.29 % at 18 µland 34% 12 µl disc^{-1}) and resistant at lower concentration showing 0% zone of inhibition. In the tested extracts, highest activity was measured for ethyl acetate (79.83 % at 18 µl disc^{-1}) while water fraction was not effective to repressed the activity of *P. aeruginosa* at any concentration. Fig. 7 indicated that different extracts from the roots of *Angelica glauca* reduced the growth of *C. albicans*. Maximum growth inhibition of 64.43% was measured by ethyl acetate and chloroform extracts at 12 and 18 µl disc^{-1} respectively. Butanol, hexane and water fractions did not reduce the activity of the same microbe at the tested concentrations.

Phytotoxic activity: All extracted samples exhibited good phytotoxic potential against *Lemna minor* (Fig. 8). Among these samples, butanol and n-hexane extracted sample showed maximum phytotoxic activity (30% at 18 µl disc^{-1}) as compared to control, while crude methanolic extract and water fractions at 18 µl disc^{-1} showed lowest phytotoxic activity (26.00%). The growth of *Lemna minor* was effectively controlled by chloroform, n-hexane, petroleum ether, butanol and ethyl acetate extracted samples as compared to the positive control while methanol and aqueous extracted samples demonstrated lowest activity.

Phytochemical sceening: Table 2 shows various secondary metabolites obtained from six different solvents extracted samples of the *Angelica glauca* root. The phytochemical screening confirmed the presence of variety of the secondary metabolites including alkaloids, carbohydrates, flavonoids, proteins, saponins, sterols and lipids and the presence of tannins was not confirmed in any of the samples. Phytochemical screening further

exhibited that alkaloid were available in majority of the solvents extracted samples except n-hexane, water and ethyl acetate. The existence of saponines was recorded in butanol and water extracted samples. The presence of lipids was confirmed in all samples except aqueous and petroleum ether extracted samples while the presence of sterols was not observed in pet ether and ethyl acetate extracted samples.

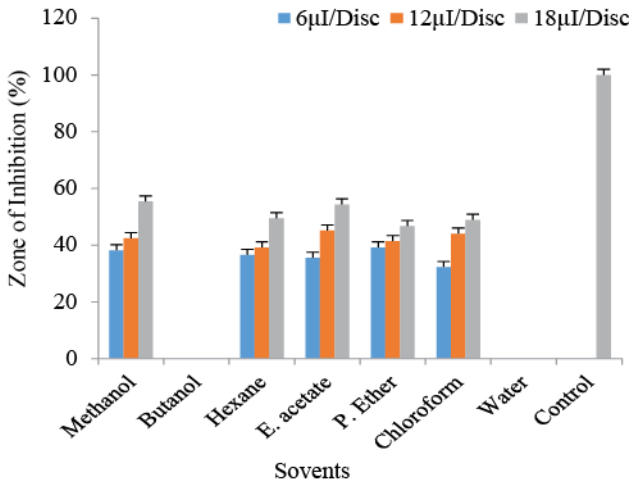

Fig. 5. Antibacterial activity of crude methanol, butanol, n-hexane, ethyl acetate, petroleum ether, chloroform and water extracted samples from the roots of *Angelica glauca* against *Staphylococcus aureus* by disc diffusion assay (Bar shows LSD at $p<0.05$).

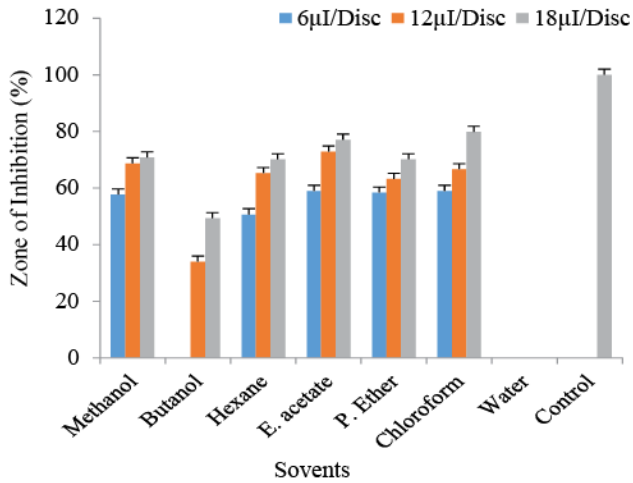

Fig. 6. Antibacterial activity of crude methanol, butanol, n-hexane, ethyl acetate, petroleum ether, chloroform and water extracted samples from the roots of *Angelica glauca* against *Pseudomonas aeruginosa* by disc diffusion assay (Bar shows LSD at $p<0.05$).

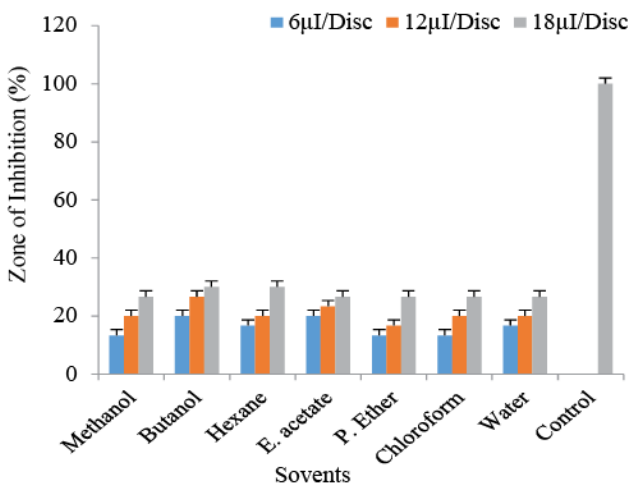

Fig. 7. Antibacterial activity of crude methanol, butanol, n-hexane, ethyl acetate, petroleum ether, chloroform and water extracted samples from the roots of *Angelica glauca* against *Candida albicans* by disc diffusion assay (Bar shows LSD at $p<0.05$).

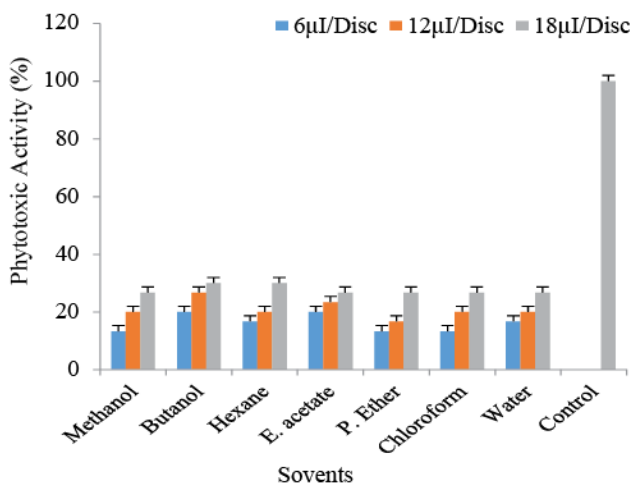

Fig. 8. Phytotoxic activity (%) of different extracted samples from the roots tissue of *Angelica glauca* against *Lemna minor* (Bar shows LSD at $p<0.05$).

Table 2. Phytochemical screening of differents solvent extracted fractions of *Arisaemajacquemontii*.

Plant Extract	Alkaloid	Saponin	Tannin	Sterols	Flavonoid	Protein	Carbohy-drates	Lipids
Methanol	+	–	–	+	+	+	+	+
n-Hexane	–	–	–	+	+	+	+	+
Ethyl acetate	–	–	–	–	–	+	+	–
n-Butanol	+	+	–	+	–	+	+	–
Pet. Ether	+	–	–	–	+	+	+	+
Chloroform	+	–	–	+	+	+	+	+
Water	+	–	–	+	+	+	+	–

+: Shows presence but in less amount

–: Shows complete absence of the compound

Discussion

A range of secondary metabolites and aromatic compounds including flavonoids, alkaloids and saponine play a vital role in defense against different microbial infections (Lutterodt *et al.,* 1999; Marjorie, 1999). Metabolites and compounds found in plants also reduce the growth of different microbes (Jager *et al.,* 1996). The inherited potential of various types of plants extracts depends mainly on plant specie, procedure of extraction and the reagents used for the extraction (Al-Zoreky, 2009). Low or no activity is mainly due to the degradation or complete insolubility of various bio-compounds during the isolation and extraction (Premanath *et al.,* 2011). Solvents extracted methodologies are used mainly to study the different pharmacological activities including antimicrobial activities against different microbes.

The antibacterial activity of the roots tissues of the *Angelica glauca* was carried out against selected bacterial strains. Different solvents extracted samples had variable degree of antibacterial activity which depended on dose, strain and the solvent used. Seven different extracts of the roots tissues from *A. glauca* were studied against the Gram negative *K. pneumoniae.* Crude methanolic, ethyl acetate, chloroform and ether extracts showed good potentail against *K. Pneumonia* at all concentrations in comparison to the positive control. Ethyl acetate extracted sample was the most effective one and recorded highest response at all the tested concentrations. These results are similar to Mushtaq *et al.,* (2011) who suggested that ethyl acetate and methanolic extracts were highly responsive in the inhibition of *K. pneumoniae* at each concentration used. The activity of *K. pneumoniae* was highly affected by methanolic and ethyl acetate fractions and recorded maximum ZI in comparison to the positive control. The results also showed that *K. pneumoniae* was resistant to butanol and water extracted samples.

In comparison to the positive control, *P. aeruginosa* was highly susceptible to crude methanolic, hexane, ethyl acetate, chloroform, butanol and ether extracted fractions and resistant to water extracted samples at all concentrations. The activity of *P. aeruginosa* was highly reduced by butanol extracted sample at higher concentration, however, was resistant at lower concentration showing zero percent zone of inhibition. In the tested extracts, maximum zone of inhibition was recorded in the case of ethyl acetate, while water fraction was not effective to control the activity of *P. aeruginosa* at any concentrations $disc^{-1}$ (Benkeblia, 2004; Santas *et al.,* 2010). *S. aureus* proliferation was efficiently retarded by crude methonolic extract, n-hexane, petroleum ether, chloroform and ethyl acetate fractions at all the concentration. The most efficient sample in controlling the activity *S. aureus* was crude metahnolic extract. Water and butanol fraction was not ineffective to control the activity of *S. aureus* at any concentration. These results are similar to Hughes & Lawson (1991) and Chathradhyunthi *et al.,* (2009). Petroleum ether, ethyl acetate and chloroform extracts effectively reduced the growth of *S. aureus.* The growth of *S. aureus* was not affected by butanol, ethanol and water extracts at any concentration. *B. Subtilis* growth was inhibited by crude methonolic extract, n-hexane, petroleum ether, chloroform and ethyl acetate fractions at all concentrations. Ethyl acetate fraction was more effective in controlling the growth of *B. subtilis*. Water and butanol fraction was not effective to control the activity of *B. Subtilis* at any concentration (Santas *et al.,* 2010; Rauf *et al.,* 2012).

The growth of *E. coli* was effectively repressed by crude methonolic extract, n-hexane, petroleum ether, chloroform and ethyl acetate fractions. Crude methanolic and chloroform extracts were effective to reduce the activity of *E. coli*. Water and butanol fractions did not reduce the growth of *E.coli* at any concentration. These results are similar to Hughes & Lawson (1991) and Chathradhyunthi *et al.,* (2009). Wang *et al.,* (2010) obtained contradictory results while working on *P. sepium.* Chloroform extracted fraction was the most effective one to control the activity of *X. compestris* at all the tested concentrations. Water fraction did not reduce the growth of *X. compestris* in comparison to the positive control. Fazal *et al.,* (2012) Hughes & Lawson, (1991) and Chathradhyunthi *et al.,* (2009) demonstrated that *X. compestris* was susceptible to ethyl acetate extracted samples. Antifungal activity was also measured in different extracts from the roots of *Angelica glauca*. Chloroform and ethyl acetate recorded highest activity. Butanol, hexane and water fractions were not effective to control the activity of *C. albicans* at any concentrations.

Butanol and n-hexane fractions showed maximum phytotoxic activity as compared to controls, while crude methanolic extracts and water fractions showed lowest phytotoxic activity. The growth of *Lemna minor* was effectively controlled by chloroform, n-hexane, petroleum ether, butanol and ethyl acetate extracted samples as compared to the positive controls, while crude methanolic and aqueous extracted samples showed least phytotoxic activity against *Lemna minor*. Rauf *et al.,* (2012) and Grisi *et al.,* (2013) reported good phytotoxic activity for crude methanolic extract, n-hexane and acetate fractions.

The phytochemical screening demonstrated the presence of variety of the secondary metabolites including alkaloids, carbohydrates, flavonoids, proteins, saponins, sterols and lipids. All the extracted samples contain proteins and carbohydrates while the presence of tannins was not confirmed in any of the extracted samples. Alkaloids were available in majority of the solvents extracted samples except n-hexane, water and ethyl acetate. The existence of the saponines was recorded in butanol and water extracts. The presences of lipids were revealed all extracts except aqueous and petroleum ether while sterols was not found in ether and ethyl acetate extracted samples.

Plants contain variety of bioactive compounds which are used since ancient times for the treatment of various diseases (Ayodele, 2003). These metabolites possess variety of properties and activities such as antioxidants, anti-diarrheal activities (Agbor *et al.,* 2004) and are also helpful in controlling disorder concerning oxidative stress (Vinson *et al.,* 1995). Immune responses of the human body against the pathogens are initiated by tannins (Tiger, 1980). Flavonoids have free radical scavenging capacities and are known for its anticancer potentials (Okwu & Josaiah, 2006). Antimicrobial activity of the plant extracts is mainly due to the presence of alkaloids (Ramkumar *et al.,* 2007). Various parts of the plants are rich sources of these biologically important compounds (Ara & Nur, 2009).

Conclusion

Different solvent extracted samples revealed varying degree of antibacterial, antifungal and phytotoxic activity

depending on the dose and solvent. *A. glauca* contain appreciable amount of different bioactive compounds and can be utilized in curing health related problems.

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