



Differential Antimicrobial Potential of *Ajuga integrifolia* Buch. Ham. Ex D. Don Based on Extraction Solvents

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ABSTRACT

The current study aimed to evaluate the effect of extraction solvents on the anti-bacterial and anti-fungal efficacy of the stem and roots of *Ajuga integrifolia* Buch. Ham. ex D. Extracts were prepared in different solvents and tested against fungi and bacteria species including, *Agrobacterium tumefaciens*, *Xanthomonas oryzae*, *Citrobacter freundii*, *Alternaria alternata*, *A. solani* and *Aspergillus niger*. Antibacterial efficacy of the *Ajuga integrifolia* was carried out by disc diffusion susceptibility method and antifungal efficacy by well diffusion susceptibility method. Methanol stem extract revealed efficacy against *C. freundii* by producing a 63% zone of inhibition at 3000µg/disc while methanol roots extract produced 77% ZI against *A. tumefaciens*. Methanol root and stem extracts produced an equal zone of inhibition (97%) at 1000 µg/ml concentration against *A. alternata* and *A. solani*. The results of the study clearly stated that the polarity of the solvents used in the extraction procedure affects the bioactivities of the extracts.

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Introduction

Medicinal flora is a gift of nature for humanity and produces large numbers of bioactive compounds that possess remarkable biological activities, such as pharmaceuticals, dyes, pesticides, flavors, and scents (Kina et al., 2021; Akgül et al., 2022). Medicinal plants produced antimicrobial components to protect plants against viruses, bacteria, and fungi (Vohra and Kaur, 2011; Krupodorova et al., 2022). Antimicrobial components possess the phytotherapeutic potential to cure wide varieties of illnesses and ailments. Traditionally rural people used medicinal herbs in Ayurveda for curing their sicknesses (Rahman et al., 2015; Ahmadipour et al., 2016; Hussain et al., 2016; Mohammed et al., 2022; Unal et al., 2022). Fungi and bacteria have the abilities to show drug resistance that increase the rate of infections. Rapid increases in infections threaten human fitness, especially immune-compromised which needs rapid, modified, and accurate drugs (Fazal et al., 2012; Nasrullah et al., 2012). Medicinal plants are one

of the important sources of bioactive compounds which can be used as an alternative to anti-microbial chemotherapy and used for the production of new medication (Farnaz et al., 2011; Walter et al., 2011; Mothana et al., 2012; Sevindik et al., 2017; Pehlivan et al., 2021; Uysal et al., 2021).

Pakistan has a rich of medicinal plants, including *Ajuga integrifolia* Wall ex. Benth has therapeutic potential. *A. integrifolia* leaves, stem, barks, roots, and flowers possess pharmacological properties such as anti-bacterial, anti-fungal, anti-parasitic, anti-tumor, anti-inflammatory, antipyretic, anti-malarial, immune-regulatory and cytotoxic (Pal et al., 2011; Jan et al., 2014 Hussain et al., 2016). The current study was designed to evaluate the effect of extraction solvents on antibacterial and antifungal efficacy of *A. integrifolia* Wall ex. Benth against humans and plants pathogenic microorganisms.

Material and Method

Plant Material Collection and Identification

A. integrifolia was collected from the district Swat and taxonomically identified by Prof Dr. Zahid Khan University of Swat Khyber Pakhtunkhwa, Pakistan.

Chemicals and equipment used in experiments

All solvents used for extraction were analytical grade (Merck & Co., Inc., Kenilworth, NJ, USA). Nutrient agar and nutrient broth were purchased from Musaji Adam & Sons. Ciprofloxacin and Terbinafine were provided by Meditech Pharmaceuticals Peshawar. Whatman filter paper was used for filtration and a Rotary evaporator (Rotavapor R-R 210/R215; BUCHIL Labortechnik AG) for drying solutions.

Stem and Roots Crude Extract Preparation

Stem and roots were thoroughly washed and kept in the shade. Dry material is macerated to powder form. Five (5) and 2.5-liter methanol were added to the stem and root material respectively. The solution was filtered through Whatman filter paper No.1 and dried via a rotary evaporator and stored in a glass vial till used.

Stem and Roots Crude Extract Fractionation

The crude extract was weighted (52gm) and divided into two parts. One portion (10g) was kept in a glass vial and tested as a crude extract in antimicrobial efficacy while the other part (42 grams) was dissolved in three hundred mL of distilled water and poured into a Separatory funnel. The lower aqueous layer was collected first followed by hexane layers in separate flasks. The water fraction was re-extracted with 600mL more hexane. The same process was also applied to ethyl acetate and *n*-butanol. All the fractions were dried via a Rotary evaporator (Khan et al., 2017).

Preparation and Autoclaving of Culture Media

Nutrient agar and Nutrient broth were prepared according to standard procedure for antimicrobial bioassay. For fungal bioassay, potato dextrose media was prepared from 300g of potato extract, 20g of dextrose, 20g of agar, and 1L of water. Other accessories and media-containing bottles were autoclaved at 15psi for 15 minutes at 121°C. Media were poured into Petri plates under sterile conditions.

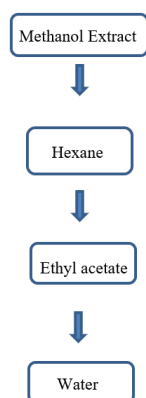


Figure 1. Solvents arranged in ascending order of polarity used in the fractionation of methanol extract

Anti-Fungal and Anti-Bacterial Bioassay

The anti-fungal and anti-bacterial bioassays were done by the well and disc diffusion method. Fungal species used in this study were *Alternaria alternata*, *Alternaria solani*, *Aspergillus niger* while the bacterial species were *Citrobacter feundii*, *xanthomonos oryzae*, *Agrobacterium tumefeciens* and *Bacillus subtilis*. The above strains were provided by the Department of Plant Pathology, the University of Agriculture Peshawar, and the Department of Microbiology University of Swat. Fungal strains were cultured on the potato dextrose agar and standardized on potato dextrose broth media while the bacteria were cultured on nutrient agar and standardized on nutrient broth media (Khan et al., 2016; Khan et al., 2017).

Statistical Analysis

The treatment applied to experimental units in triplicate and data were collected. The means and standard deviation of data were measured by the statistical software SPSS (v. 20.0 SPSS Inc., Chicago, IL, USA).

Results

Antibacterial Bioassay by Disk Diffusion Assay

Different solvent-extracted samples from the stem of *A. integrifolia* showed antibacterial potential against the tested microorganisms. Among the tested microorganisms the growth of *B. subtilis* was inhibited by crude methanol extracts and formed an equal zone of inhibition of 54% at 1000µg/disc, 2000µg/disc, and 3000µg/disc while *n*-butanol extracts showed 41% ZI at 1000µg/disc and produced equal ZI (48%) at 2000µg and 3000µg/disc. *n*-hexane fractions revealed an equal zone of inhibition of 33% at any concentration. Furthermore, ethyl acetate at 1000 and 2000µg concentration produced equal ZI (33%) while at 3000µg concentration revealed 48% ZI. Water stem extract showed 32%, 48%, and 51% ZI respectively at 1000, 2000, and 3000µg/disc. Hot-water stem extract did not show activity at any concentration level. Similarly, the root extracts of *A. integrifolia* also showed anti-*B. subtilis* activity. Crude methanol extract of roots produced 41%, *n*-butanol 63%, water fraction 48%, ethyl acetate 38%, and *n*-hexane 16% ZI at 1000µg/disc. Crude methanol and *n*-butanol produced an equal zone of inhibition (63% ZI) while *n*-hexane and water fraction revealed the same ZI 48% and ethyl acetate produced 41% ZI at 2000µg/disc. Methanol and *n*-butanol root extract showed equal ZI of 63% and *n*-hexane and water root extract showed the same zone of inhibition (54%) while ethyl acetate fraction showed 41% ZI at 3000µg/disc. The hot-water extract did not affect the growth of *B. subtilis* at any concentrations. The stem extracts showed antibacterial efficacy against *A. tumifacians*. Crude extract inhibited the growth of *A. tumifacian* by 59%, ethyl acetate extract by 45%, *n*-butanol extract by 43%, *n*-hexane extract by 45%, water extract by 59%, and hot-water stem extract by 32% (ZI) at 3000µg/disc. Furthermore, 2000µg/disc crude extract showed 57% ZI while ethyl acetate and *n*-hexane produced an equal zone of inhibition (45%). Similarly, *n*-butanol, water, and hot water exhibited equal ZI (32%) at 2000µg/disc. Crude extract showed 45% and *n*-hexane 48% ZI while ethyl acetate, *n*-butanol, hot water, and water

extracts produced equal zone inhibition of 32% at 1000µg/disc. Strong antibacterial potential against *A. tumifaciens* was also noted in root extracts of *A. integrifolia*. Maximum inhibitory activity was found in crude methanol extract (77% ZI). The *n*-hexane reduced the growth of the same bacterium by 65%, ethyl acetate by 52%, *n*-butanol by 42%, and water roots extract by 40% ZI at 3000µg/disc. The crude methanol extract produced 65% and water root extract 38% ZI while *n*-hexane, ethyl acetate, and *n*-butanol revealed equal ZI (32%) at 2000µg/disc. Crude methanol extract exhibited 42% ZI while ethyl acetate and water extract showed equal ZI (32%). On the other hand, *n*-butanol and *n*-hexane revealed the same ZI of 16% at 1000µg/disc. Hot-water root extracts did not show efficacy at any concentrations.

The extracts of both stem and roots showed antibacterial efficacy against *X.oryzae* (Table 1&2). Crude

methanol and *n*-hexane extracted fraction of stem produces an equal zone of inhibition of 63% while *n*-butanol produced 50% at 3000µg/disc. Furthermore, crude extract and *n*-hexane exhibited 63% and 53% ZI respectively while ethyl acetate and water roots extract revealed equal zone of inhibition (37.5%) at 2000µg/disc. Crude extract showed 50% and *n*-hexane 45 % ZI although, ethyl acetate and water roots extract produced similar ZI (38%) at 1000µg/disc. Hot-water root extract did not show efficacy at any concentration while *n*-butanol extract was inactive against *X.oryzae* at 1000 and 2000 µg/disc. On the other hand, the growth of *X.oryzae* was inhibited by crude methanol extracts of root (38% ZI), *n*-hexane (63% ZI), *n*-butanol(55% ZI), ethyl acetate (37.5% ZI) at 3000µg/disc. Similarly, crude extracted fraction revealed 38% ZI while *n*-hexane and *n*-butanol produced equal zone ZI (50%) at 2000µg/dis.

Table 1. Anti-bacterial potential of the stem extracts of *Ajuga integrifolia* against the different bacterial and fungal species

Bacterial /Fungi species	Extracts	% Zone of inhibition ±STDV		
		1000µg/disc	2000µg/disc	3000µg/disc
<i>Bacillus subtilis</i>	Methanol	54.4 ±1.1	54.5±1.4	54.6±1.1
	Ethyl acetate	32.7 ± 1.2	32.9± 1.6	49.2±1.3
	<i>n</i> -Hexane	32.5 ±1.3	32.6±1.2	32.6±1.2
	Butanol	40.8 ±1.4	49.1±1.3	49.2± 0.9
	Water	32.4± 1.1	49.2± 1.1	51.4±1.1
	Hot water	0	0	0
<i>Agrobacterium tumefaciens</i>	Methanol	42.4±1.3	58.4±1.2	59.5±1.3
	Ethyl acetate	30. 5 ±1.4	44.3± 1.0	44.5±1.6
	<i>n</i> -Hexane	44.8± 2.1	44. 5 ±1.1	44.7± 2.6
	Butanol	30.2±1.1	30.6± 1.1	40. 5 ±1.5
	Water	30. 5 ±1.2	30.8±1.3	59.3± 2.1
	Hot water	30.3±1.1	30.5± 2.1	30.6±1.6
<i>Xanthomonos oryzae</i>	Methanol	44.4±1.6	63.6± 1.1	63.8±1.6
	Ethyl acetate	39.5± 1.2	39.5±1.6	39. 5 ±1.5
	<i>n</i> -Hexane	42.4± 1.1	63.6± 1.6	63.8± 1.1
	Butanol	0	0	43.4±1.6
	Water	39. 5 ±1.5	39. 5 ±1.5	39.6± 1.1
	Hot water	0	0	0
<i>Citrobacter freundii</i>	Methanol	22.4±1.4	22.6± 1.4	63.2± 1.1
	Ethyl acetate	22.2± 1.2	22.6±1.7	38.2±1.3
	<i>n</i> -Hexane	24.8±1.1	50.3± 1.6	50. 5 ±1.4
	Butanol	24. 5 ±1.4	39.4±1.3	39.6± 1.1
	Water	22.4±1.6	22.6± 1.1	22.8±1.5
	Hot water	0	0	0
<i>Alternaria alternata</i>	Methanol	80.4± 1.1	82.6± 1.1	97.3± 1.1
	Ethyl acetate	74.2±1.5	76. 5 ±1.3	78.6± 1.2
	<i>n</i> -Hexane	76± 1.6	78.4± 1.4	80.6±1.3
	Butanol	60.2±1.7	80.6±1.6	83.4± 1.9
	Water	64.4±1.3	78.6± 1.1	83.6± 1.1
	Hot water	32.6±1.4	47.3± 1.1	50.4± 1.2
<i>Alternaria solani</i>	Methanol	66.4± 1.1	83.5± 1.1	97.2± 1.3
	Ethyl acetate	87.4±1.2	92.4±1.6	97.6± 1.1
	<i>n</i> -Hexane	80± 1.6	87.6± 1.1	97.8± 1.2
	Butanol	66.4±1.7	68.6±1.3	72.8± 1.1
	Water	86.6±1.5	90.4±1.2	97.6± 1.3
	Hot water	79.4± 1.1	83.8± 1.1	98.6± 1.2
<i>Aspergillus niger</i>	Methanol	71.3± 1.2	83.4± 2.1	98.4±1.4
	Ethyl acetate	67.2±1.3	78.5± 2.6	96.6±1.2
	<i>n</i> -Hexane	61.4±1.5	70.3±1.3	80.4±1.1
	Butanol	51.4±1.1	64.8±1.3	74.7±1.3
	Water	49.3± 1.1	60.6±1.6	70.8± 1.2
	Hot water	43.5± 1.2	49.6±1.3	73.4± 1.1

Table 2. Anti-bacterial potential of the root extracts of *Ajuga integrifolia* against the different bacterial and fungal species

Bacterial /Fungi species	Extracts	% Zone of inhibition \pm STDV		
		1000 μ g/disc	2000 μ g/disc	3000 μ g/disc
<i>Bacillus subtilis</i>	Methanol	41.4 \pm 1.1	62.8 \pm 1.5	62.9 \pm 1.3
	Ethyl acetate	38.4 \pm 1.2	39.8 \pm 2.1	39.9 \pm 1.3
	n-Hexane	16.2 \pm 1.4	48.8 \pm 1.3	51.4 \pm 1.5
	Butanol	62.9 \pm 3	62.9 \pm 1.3	62.9 \pm 2.1
	Water	48.6 \pm 2	48.6 \pm 1.5	51.4
	Hot water	0	0	0
<i>Agrobacterium tumefaciens</i>	Methanol	41.4 \pm 1.1	67.8 \pm 1.6	77.9 \pm 1.1
	Ethyl acetate	31.4 \pm 1.2	31.6 \pm 1.1	49.8 \pm 1.2
	n-Hexane	14.2 \pm 1.3	30.8 \pm 1.1	67.9 \pm 1.4
	Butanol	14.5 \pm 1.1	30.9 \pm 1.4	38.8 \pm 1.1
	Water	30.5 \pm 1.1	39.5 \pm 1.3	41.2 \pm 1.1
	Hot water	0	0	0
<i>Xanthomonas oryzae</i>	Methanol	39.5 \pm 1.2	39.6 \pm 1.1	39.8 \pm 1.1
	Ethyl acetate	28.8 \pm 1.1	39.6 \pm 1.1	39.8 \pm 1.5
	n-Hexane	51.6 \pm 1.4	51.8 \pm 2.1	63.5 \pm 1.4
	Butanol	19.6 \pm 1.1	51.6 \pm 1.1	53.5 \pm 1.1
	Water	22.5 \pm 1.1	39.8 \pm 1.2	41.2 \pm 1.1
	Hot water	0	0	0
<i>Citrobacter freundii</i>	Methanol	43.6 \pm 1.2	43.7 \pm 1.1	54.5 \pm 1.2
	Ethyl acetate	26.4 \pm 1.1	26.6 \pm 1.1	26.8 \pm 1.1
	n-Hexane	38.6 \pm 1.3	38.8 \pm 1.6	48.5 \pm 1.3
	Butanol	38.6 \pm 1.2	38.8 \pm 1.1	38.9 \pm 1.4
	Water	0	38.6 \pm 1.3	38.8 \pm 1.1
	Hot water	38.6 \pm 1.1	38.8 \pm 1.1	38.9 \pm 1.5
<i>Alternaria alternata</i>	Methanol	78.6 \pm 1.1	81.5 \pm 1.1	98.6 \pm 1.1
	Ethyl acetate	67.5 \pm 1.1	70.4 \pm 1.1	98.4 \pm 1.1
	n-Hexane	86.8 \pm 1.2	90.5 \pm 1.1	93.4 \pm 1.2
	Butanol	81.2 \pm 1.1	90.5 \pm 1.3	97.5 \pm 1.2
	Water	76.5 \pm 1.1	78.9 \pm 1.2	82.4 \pm 2.1
	Hot water	43.2 \pm 1.1	63.8 \pm 1.1	68.8 \pm 1.1
<i>Alternaria solani</i>	Methanol	83.5 \pm 2.1	90.4 \pm 1.3	98.5 \pm 1.5
	Ethyl acetate	83.6 \pm 1.3	91.5 \pm 2.1	98.5 \pm 1.5
	n-Hexane	68.8 \pm 1.5	78.5 \pm 1.3	81.2 \pm 2.1
	Butanol	0	0	0
	Water	68.9 \pm 1.3	71.4 \pm 2.1	80.5 \pm 1.3
	Hot water	80.5 \pm 1.3	86.5 \pm 1.5	92.8 \pm 2.1
<i>Aspergillus niger</i>	Methanol	49.8 \pm 1.3	68.5 \pm 1.1	90.4 \pm 1.1
	Ethyl acetate	46.5 \pm 1.3	49.8 \pm 1.1	84.5 \pm 1.2
	n-Hexane	49.6 \pm 1.1	51.4 \pm 1.2	83.8 \pm 1.6
	Butanol	49.5 \pm 1.2	51.5 \pm 1.3	79.8 \pm 1.1
	Water	37.5 \pm 1.1	49.8 \pm 1.1	49.8 \pm 1.2
	Hot water	44.5 \pm 1.2	49.8 \pm 1.3	49.8 \pm 1.1

Water and ethyl acetate produced similar ZI (38%) at the same concentration. Furthermore at 1000 μ g/disc *n*-hexane produced 50% and crude extract showed 38% ZI respectively while ethyl acetate, *n*-butanol and water stem extracted fractions revealed 30%, 20%, and 25% ZI respectively. Hot-water stem extracts revealed efficacy with 0% ZI at all concentration levels. The stem and root extracts of *A. integrifolia* also showed antimicrobial potential against *C. freundii*.

The crude extract of stem exhibited 63%, *n*-hexane 50%, and water extract 25% ZI while ethyl acetate and *n*-butanol produced equal ZI of 38% at 3000 μ g/disc. Furthermore, crude methanol, ethyl acetate, and water extract showed equal ZI of 25% while *n*-hexane exhibited 50% and *n*-butanol 38% ZI at 2000 μ g/disc. Further, the results of the study suggested that crude methanol, ethyl acetate, and water fraction exhibited equal ZI (25%) while *n*-butanol and *n*-hexane fraction produced similar ZI (28%)

at 1000 μ g/disc. Hot-water fraction did not show ZI at any concentration.

The crude extracts of the roots and *n*-hexane produced 55% and 50% ZI respectively. Ethyl acetate, *n*-butanol, water, and hot-water roots revealed equal ZI (25%) at 3000 μ g/disc. The crude extract and *n*-hexane extract exhibited 43% and 38% ZI while water, hot water, *n*-butanol, and ethyl acetate roots showed equal ZI (25%) at 2000 μ g/disc. Furthermore, methanol and *n*-hexane fractions exhibited 43% and 38% ZI while ethyl acetate, hot-water, and *n*-butanol roots fraction exhibited equal ZI (25%) at 1000 μ g/disc. The water roots extract sample was not active against *C. freundii* at 1000 μ g/disc.

Anti-Fungal Efficacy by Well Diffusion Method

The stem and root extracts of *A. integrifolia* were tested for anti-fungal efficacy. The strong antifungal potential was shown by crude methanol extract and formed 97%,

83%, and 80% zone of inhibition at 3000, 2000, and 1000µg/mL respectively. Ethyl acetate extracts revealed 77%, 73%, and 70% zone of inhibition at all three concentrations.

At 3000, 2000, and 1000µg/mL, *n*-hexane extract showed 83%, 77%, and 73% ZI while *n*-butanol extract formed 87%, 83%, and 61% ZI. Water and hot-water stem fractions indicated 83% and 50% ZI at 3000µg/mL while at 2000µg/mL showed 77% and 47% ZI respectively. Water and hot-water extract also revealed 67% and 33% ZI at 1000µg/mL. *A. integrifolia* root extracted fractions showed antifungal efficacy against *A. alternata*. The crude methanol extracts showed 97% 83% and 77%, ethyl acetate 97%, 73%, 67%, *n*-hexane 93%, 90%, 87%, *n*-butanol 97%, 93%, 83%, water fraction 83%, 77%, 73% and hot-water fraction 67% and 63% 43% zone of inhibition at 3000, 2000 and 1000µg/mL concentration.

Antifungal potential against *A. solani* was also shown by the stem and root extracts of *A. integrifolia*. Crude methanol, ethyl acetate, *n*-hexane, water, and hot-water stem extracted fractions revealed equal ZI (97%) while *n*-butanol produced 73% ZI at 3000µg/mL. The crude methanol extract reduced the growth of *A. solani* by 83%, ethyl acetate by 93% followed by *n*-hexane (87% ZI) *n*-butanol (70% ZI) at 2000µg/mL. Water fraction showed 90% ZI and hot water extract formed 83% zone of inhibition at 2000µg/mL. Furthermore, crude methanol, ethyl acetate, *n*-hexane, *n*-butanol, water, and hot-water extracted fractions revealed 67%, 87%, 80%, 67%, 83%, and 77% zone of inhibition respectively at 1000µg/mL concentration. Similarly, *A. integrifolia* root extracts were active against *A. solani*. Crude methanol and ethyl acetate extract exhibited equal ZI 97%, 93%, and 83% ZI at 3000, 2000, and 1000µg/mL. Although *n*-hexane extracts revealed 83%, 77%, 67%, water fraction 83%, 73%, 67%, and hot-water root extracts formed 93%, 87%, 83% ZI at 3000, 2000 and 1000µg/mL respectively. *n*-butanol root fraction did not reduce the growth of the same fungus at any concentrations. The findings of the study also revealed the anti-fungal efficacy of the stem and root of *A. integrifolia* against *A. niger*. Crude methanol and ethyl acetate extracts showed 97% and 93% ZI while *n*-hexane, *n*-butanol, water, and hot-water extracted fractions revealed 80%, 77%, 70%, and 73% ZI respectively at 1000µg/mL. Crude methanol, ethyl acetate, *n*-hexane, *n*-butanol, water and hot-water stem fractions exhibited 83%, 77%, 73%, 67%, 63% and 50% ZI respectively at 2000µg/mL. Data also showed that crude methanol, ethyl acetate, *n*-hexane, *n*-butanol, water, and hot-water fractions exhibited 73%, 67%, 63%, 53%, 50%, and 43% ZI respectively at 1000µg/mL. Similarly, crude methanol extract of root exhibited strong anti-*A. niger* potential by forming 90% ZI followed by ethyl acetate and *n*-hexane extracts (83% ZI). Butanol extracted fraction inhibited the growth of the same fungus by 80% 53%, 50% ZI at 2000 and 1000µg/mL. Water and hot-water root extracts showed equal zone of inhibition (50%) at 3000 and 2000µg/mL while at 1000µg/mL water fraction showed 37% and hot-water extracts produced 43% ZI respectively.

Discussion

The current study evaluated the antimicrobial efficacy of *A. integrifolia* wall ex. benth extracts against pathogenic bacteria and fungi species. The extracted samples of the *A. integrifolia* were effective in antimicrobial efficacy. Antifungal efficacy of the *A. integrifolia* (stem and roots) is more effective as compared to antibacterial efficacy. Five solvents were used for the extraction from two different parts of the *A. integrifolia* (stem and roots) included: methanol, ethyl acetate, *n*-hexane, *n*-butanol water, and hot water. Methanol-extracted fractions revealed broad and maximum efficacy against both groups of microorganisms (bacteria and fungi) followed by *n*-hexane and ethyl acetate. The yield of the bioactive compound/amalgam extraction depends on the polarities of solvents, time, and temperature as well as the physical status and chemical composition of the sample (Dai and Mumper, 2010). Methanol roots and stem extracts revealed their effectiveness against all the selected bacterial strains. The activity of the same extracted fraction was also reported by Vohra and Kaur (2011) against *S. aureus*. Stem methanol extract was also effective against *B. subtilis*, *S. aureus* *K. pneumonia* and *E. coli* (Shakoor et al., 2014). Hexane root extract showed high efficacy against *X. oryzae*. Rahman et al. (2015) reported that *n*-hexane extracts of the stem revealed great efficacy against *B. subtilis* and *E. coli*. The result of the study indicated that hot-water stem and root extracts were found less effective against the bacteria species. The decrease in the effectiveness of hot-water extract may be due to partial denaturation of the bioactive compound present in extracts. The data stated that methanol roots and stem extract were active against selected fungi followed by ethyl acetate. The strong antifungal activities in the leaves of *A. bracteosa* : were also reported by Iftikhar et al. (2014) against *A. niger* and *A. fumigatus* which further strengthen the findings of the current study. Furthermore, results also indicated that *n*-hexane, *n*-butanol, water, and hot water showed moderate activity against fungi species.

Solvent effects on the antimicrobial potential of extracts were also noted in the study. Among the tested samples, methanol was found best for the extraction of bioactive compounds followed by *n*-hexane. The same solvent was reported by Anwar et al. (2006) for the recovery of the highest amounts of bioactive compounds from the different plants. Similarly, Son et al. (2004) also reported methanol as the best solvent for the extraction of antioxidants compound from rice and other plants.

Conclusion

Stem and roots both parts were active against bacteria and fungi. Methanol was found best solvent for the extraction of bioactive compounds from different parts of the plants.

Declarations

Ethics Approval and Consent To Participate.

Not Applicable

Consent for Publication

Not applicable

Competing Interests

The authors stated that they have no competing interests.

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