

Phytochemical and antimicrobial study of *Alstonia scholaris* leaf extracts against multidrug resistant bacterial and fungal strains

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Abstract: In the present study, *Alstonia scholaris* leaves were explored for phytochemical constituents, antibacterial and antifungal potentials. Phytochemical screening of the extracts established the presence of glycosides, alkaloids, saponins, terpenoids, anthraquinones, reducing sugars and steroids which later on confirmed through fourier transform infrared spectroscopic analysis. The extract was applied against eight multidrug resistant bacterial and five fungal strains using standard protocols. Methanol and ethyl acetate extracts of the leaves showed highest diameter of inhibition zone (DIZ) of 28mm and 26mm respectively against *Enterobacter*. Ethanolic extract exhibited prominent DIZ of 26.33mm and 23.67mm against *Enterobacter* and *Pseudomonas* respectively. The n-Hexane extract showed DIZ of 23.67mm against *Enterobacter*. Aqueous extract showed 19.33mm DIZ against methicillin resistant *Staphylococcus aureus*. Similarly, the n-hexane extract showed highest DIZ of 20.33mm against *Aspergillus fumigatus* and this activity was highly effective than the control. Ethyl acetate extract showed 18.67mm DIZ against *Aspergillus niger* whereas methanolic extract showed marked inhibition against *Rhizopus* and *Acremonium* with a DIZ of 20mm and 17.03mm respectively. The current study on *A. scholaris* unveils about the presence of valuable phytochemicals in it having significant antimicrobial properties and further suggests to investigate for the minimum inhibitory concentration (MIC) value of the extracts in prospective research.

Keywords: *Alstonia scholaris*, antibacterial, antifungal, multidrug resistance, phytochemicals.

INTRODUCTION

Bacterial multidrug resistance (MDR) has developed a foremost stumbling block in the management of handling infectious diseases throughout the world (Mbaveng *et al.*, 2016). MDR is an emerging issue nowadays and is a major cause of morbidity and mortality in human beings. Development of MDR during the treatment of opportunistic infections caused by the microorganism is a serious problem and they can affect the immunocompromised persons similar to the patients receiving organ transplant and individuals receiving cytotoxic chemotherapy for the management of cancer. Knowing the molecular pathways for controlling MDR in microbes enables the scientists to develop innovative therapies that will be powerfully combating the serious infections (Thakur *et al.*, 2008).

Medicinal activities of the plants are due to the presence of phytochemicals which are mostly termed as secondary metabolites due to which they are used in traditional medicines (Savithramma and Rao, 2011). *Alstonia scholaris* is one of such medicinally important plant related to the family Apocynaceae usually known as white cheesewood. It is a tropical evergreen tree inhabitant to South and South East Asian countries having grayish rough bark and a milky sap (Singh *et al.*, 2012). Ethnomedicinally, *A. scholaris* has been used as antiperiodic, tonic and anthelmintic, in treatment of ulcers

(Kaushik *et al.*, 2011), fever, antidote to poison and wound (Mollik *et al.*, 2010) asthma and rheumatism (Vikneshwaran *et al.*, 2008), leucorrhoea (Bhandary *et al.*, 1995), dog bite (Prusti and Behera, 2007) and stomachic (Sharma and Kumar, 2011).

Antibacterial activities of *A. scholaris* against both Gram negative and Gram positive bacteria have been reported by numerous researchers (Bahadur *et al.*, 2012; Khyade and Vikos, 2009). Similarly the antifungal potential of *A. scholaris* has been reported by testing the plant extracts against various fungal strains (Antony *et al.*, 2012; Shafique *et al.*, 2014). Therefore the present study was intended for exploring the phytochemical constituents and antimicrobial potentials of *A. scholaris* against MDR bacterial and fungal strains.

MATERIALS AND METHODS

Collection, identification and extraction

A. scholaris leaves were collected from trees located in the University of Peshawar and recognized by the Botany Department, University of Peshawar. The leaves were dried under shade and grinded to powder form with the help of chopper in the Cidex herbal laboratory Peshawar (Sood and Sharma, 2010). One fifty grams of the powdered leaves were macerated independently in an adequate quantity of five different solvents (water, n-hexane, ethanol, methanol and ethyl acetate) and were left overnight at room temperature (Cseke *et al.*, 2006). The

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mixture was then passed through Whatman filter paper No 1 (Whatman Ltd, Maidstone, UK). This process was repeated in triplicate till sufficient quantity of the extracts was attained (Gracelin *et al.*, 2012). The crude extracts were then concentrated with the help of rotary evaporator at 40°C and the solvents were recovered. Water bath at a temperature of 60±5°C was used for drying the extract and the dried extracts were stored separately in air tight bottles at 4°C for further analysis.

Phytochemical scrutiny and fourier transform infrared spectroscopic (FTIR) study

Standard qualitative tests were executed in laboratory to confirm the presence of different phytochemicals (glycosides, flavonoids, alkaloids, saponins, tannins, terpenoids, anthraquinones, reducing sugar and steroids) in each extract (Khan *et al.*, 2011 and Gracelin *et al.*, 2013). Each extract in dried form was subjected to FTIR spectroscopy and the model used was IR Prestige-21 (Shimadzu, Japan) with an IR range of 400-4000cm⁻¹ (Liu *et al.*, 2006).

Isolation and identification of microbial strains

Pus and urine samples from 200 patients were collected from four tertiary care hospitals of Peshawar, Pakistan. All these samples were then processed for isolating the bacterial strains. The individual strain was later on confirmed by specific biochemical and morphological assessment in the Microbiology laboratory of Abasyn University Peshawar. The isolated strains of bacterial origin were *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Acinetobacter*, *Providencia*, *Methicillin-resistant Staphylococcus aureus* (MRSA), *Citrobacter* and *Enterobacter*). Similarly, pre-identified fungal strains (*Aspergillus fumigatus*, *Rhizopus*, *Aspergillus niger*, *Penicillium* and *Acremonium*) were obtained from the department of Soil chemistry, The University of Agriculture, Peshawar.

Culture sensitivity analysis

Prior to evaluate the antimicrobial activity of the leaves extracts, all the bacterial strains were screened for multi drug resistant profile. Muller Hinton Agar (MHA) was applied through disk diffusion method (Ushimaru *et al.*, 2007). The sensitivity pattern was tested by applying ten antibacterial and four antifungal standard discs. Every media plate was incubated at 37°C for 24 hours for bacterial strain and 25°C for 72 hours for fungal strain.

Antimicrobial activity of the extracts

For evaluating the antimicrobial potential of *A. scholaris* leaves extracts, agar well diffusion method as applied (Janovska *et al.*, 2003; Obeidat *et al.*, 2012). Ten milligram of each extract was dissolved in 1ml of dimethyl sulfoxide (DMSO) and pre-autoclaved Muller Hinton Agar (MHA) and Potato Dextrose Agar (PDA) plates were exposed to standard inoculums of 100 µL with

a 10⁻⁵ dilution (0.5 MacFarland turbidity standards, 10⁻⁶ CFU/ml) of bacterial and fungal cultures respectively. Sterile borers were used for creating the wells in the media plates, whereas the inoculum was spread uniformly over the surface of media plates using sterile cotton swabs. An amount of 100µL of each extract (10 mg/ml) i.e. distilled water extract (DWE), n-hexane extract (nHE), ethanolic extract (EE), methanolic extract (ME) and ethyl acetate extract (EAE) was introduced aseptically into the pre-marked wells in the agar plates via micropipettes. All the plates were then incubated at 37 ± 2°C for 18 hours for bacterial (Bobbarala *et al.*, 2009) and at 25±2°C for 72 hours for fungal strains (Mbaveng *et al.*, 2008). The procedure was repeated in triplicate and results were obtained as mean ± standard deviation.

RESULTS

Phytochemical and FTIR analysis of *A. scholaris*

In vitro phytochemical screening of *A. scholaris* leaves extract showed the presence of various constituents such as glycosides, alkaloids, saponins, terpenoids, reducing sugars and steroids, whereas tannins and flavonoids were not detected in each extract. Moreover, anthraquinones were detected in the aqueous extract while steroids were not found (table 1). The IR spectra of extracts showed functional groups for various compounds such as alcohol, ketones, aldehydes, carboxylic acid, amines, ethers, amides, phenol, peroxides, alkenes, oximes, imines, amino acids, alkanes, alkynes and esters as given in the Supplementary data (S1-S5).

Culture sensitivity for bacterial strains

Culture sensitivity was performed with ten antibiotic discs against the bacterial strains and DIZ were measured in triplicate. The organisms were classified as MDR's according to CLSI (2014) and NCCLS (2012) guidelines. *E. coli* was highly sensitive to SCF and resistant to TE, NA, SXT, CIP and CEC. *Pseudomonas* was highly sensitive to CN, SCF, CIP and CRO, moderately sensitive to MXF and AMC, while it was resistant to the remaining antibiotics. Similarly *Kelbsiella* was highly sensitive to SCF, CRO, CEC and AMC while it was found resistant to NA, SXT and CIP. Likewise, *Acinetobacter* was highly sensitive to SCF, SXT, MXF, CN and CEC and resistant to NA. *Providencia* was sensitive to SCF, AMC and CIP, while it was resistant to CRO, NA, SXT, CEC and CN. *MRSA* was sensitive to SCF, CN and CIP, whereas it was resistant to SXT. *Citrobacter* was found highly sensitive to SCF, CN and SXT, while it was resistant to NA. *Enterobacter* was sensitive to SCF and CN and resistant to NA, SXT and CEC (table 2) and (fig. 1).

Culture sensitivity for fungal strains

The culture sensitivity analysis for the fungal strains were carried out using disk diffusion method. *A. fumigatus* showed highest sensitivity to NS followed by AMPB,

VOR and FCA. *Rhizopus* was highly sensitive to NS, VOR and AMPB, whereas it was resistant to FCA. *A. niger* showed sensitivity to NS, AMPB and VOR, and showed resistance to FCA. Similarly *Penicillium* was sensitive to NS, AMPB and resistant to FCA and VOR. *Acromonium* was sensitive to NS, AMPB and VOR and resistant to FCA (table 3).

Antimicrobial activities of *A. scholaris* leaf extracts

Antimicrobial activity of each extract was evaluated against MDR bacterial strain using Cefoperazone-sulbactam as a positive control and their potency was quantitatively measured using DIZ (fig. 2). The aqueous extract showed 19.33 mm DIZ against *MRSA* followed by hexane and ethanol extracts with a DIZ of 18.33 mm respectively. Hexane extract showed DIZ of 23.67 mm against *Enterobacter*, whereas it showed no activity against *Acinetobacter* and *Providencia*. Similarly the

ethanol extract showed DIZ of 26.33 mm against *Enterobacter* and 23.67 mm against *Pseudomonas*. It showed less activity against *Providencia* (DIZ of 9.67 mm). Methanol extract showed DIZ of 28 mm and 21.67 mm against *Enterobacter* and *Pseudomonas*, whereas no activity was observed against *Acinetobacter*. Similarly the ethyl acetate extract showed highest activity of 26 mm DIZ against *Enterobacter* while it was least effective against *Acinetobacter* 12.67 mm).

Antifungal activity of each extract was also tested against the fungal strains using well diffusion method and Nystatin was used as a positive control in this case (fig. 3). The aqueous extract showed highest activity against *A. niger* (DIZ of 16.60 mm) which was almost equivalent with the standard disc used in experiment. Hexane extract showed DIZ of 20.33 mm against *A. fumigatus* followed by *Rhizopus* (DIZ of 17.67 mm), whereas it showed less

Table 1: Preliminary phytochemicals analysis of *A. scholaris*

Phytochemicals	Various solvent extracts				
	Aqueous	Hexane	Ethanol	Methanol	Ethyl acetate
Glycosides	+	+	+	+	+
Flavonoids	-	-	-	-	-
Alkaloids	+	+	+	+	+
Saponins	+	+	+	+	+
Tannins	-	-	-	-	-
Terpenoids	+	+	+	+	+
Anthraquinones	+	-	-	-	-
Reducing sugar	+	+	+	+	+
Steroids	-	+	+	+	+

+: Detected; -: Not detected

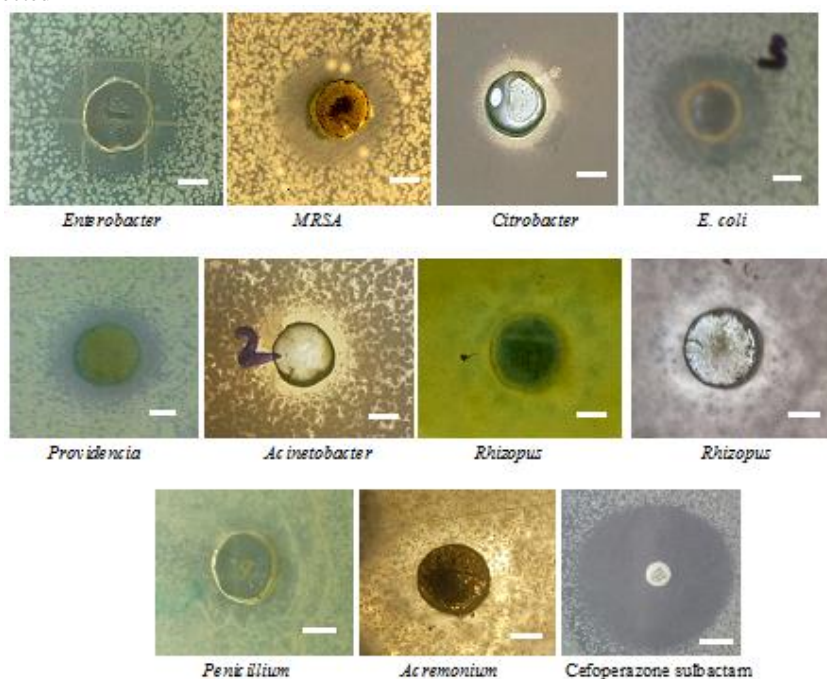


Fig. 1: DIZ shown by various extracts of *A. scholaris* against different pathogens. Scale bar= 1cm

activity against *Penicillium* (DIZ of 11.67 mm). The ethanol extract showed super activity against *A. niger* (DIZ of 17 mm) while it was not effective against *A. fumigatus*. Methanolic extract showed DIZ of 20 mm against *Rhizopus*, 17.03 mm against *Acremonium* while it showed no activity against *A. fumigatus*. Ethyl acetate extract showed inhibitory activity against *A.niger* (DIZ of 18.67 mm) while it was least effective against *A. fumigatus* by showing DIZ of 13.33 mm.

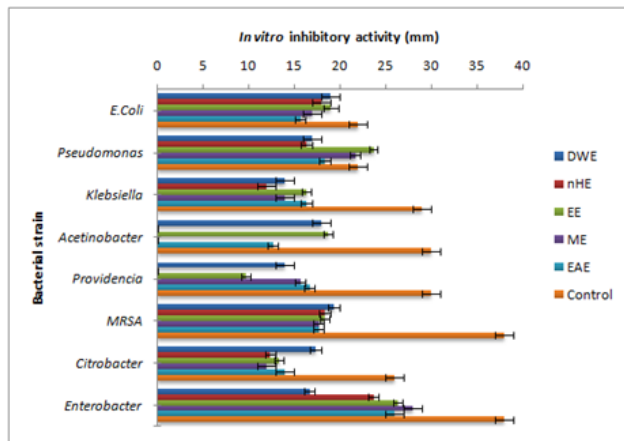


Fig. 2: Antibacterial assay by agar well diffusion method of five solvent leaf extracts of *A. scholaris* against MDR bacterial strain (diameter of inhibition zone in mm). DWE= Distilled water extract, nHE= n-hexane extract, EE= ethanolic extract, ME= methanolic extract, EAE= ethyl acetate extract and Control= Cefoperazone-sulbactam (75/30µg per disc).

DISCUSSION

Our results of phytochemical screening are in line with the study conducted by Kalaria *et al.*, (2012) and Shafique *et al.*, (2014). They reported the presence of alkaloids, phenols, tannins, saponins, terpenoids, anthraquinones and steroids. The present study results are supported by the FTIR analysis of *A. scholaris* performed by Thankamani *et al.*, (2011) and Shetty *et al.*, (2014) in which they reported the detection of various compounds such as phenols, alcohols, ethers, carboxylic acids and esters.

In the current study, it has been detected that most of the bacterial pathogens are resistant to the applied antibiotics. The culture sensitivity results exhibited that *E. coli* was 90% resistant; *Providencia* 80%, *Enterobacter* 70%, *Klebsiella* 60%, *MRSA* 50%, *Citrobacter* 30%, *Pseudomonas* 20% and *Acinetobacter* showed 10% resistance. The pattern of drug resistance of some of the bacterial strains has also been documented by Ishaq *et al.*, (2014). There was a slim variation in the resistance profile of the study conducted by Ishaq *et al.*, (2014) and our study. This deviation may be occurred due to the sample and nature of antibiotic discs used in the study. In the present study the DWE showed highest antibacterial

activity (DIZ of 19.33 mm) against *MRSA* and lowest activity (DIZ of 14.00 mm) against *Klebsiella*. The nHE showed highest activity against *Enterobacter* (DIZ of 23.67 mm) and lowest activity against *Klebsiella* (DIZ of 12.00 mm). *Acinetobacter* and *Providencia* were found resistant to the nHE. The EE showed highest activity against *Enterobacter* (DIZ of 26.33 mm) and lowest activity against *Providencia* (DIZ of 9.67 mm). Similarly the ME showed highest activity (DIZ of 28.00 mm) against *Enterobacter* and lowest activity against *Citrobacter* (DIZ of 12.00mm). *Acinetobacter* was completely resistant to the ME. Highest activity was showed by the EAE against *Enterobacter* (DIZ of 26.00 mm), while less activity was found against *Acinetobacter* (DIZ of 12.67 mm) as given in fig. 3. Previous reports of Misra *et al.*, (2011) and Mukherjee *et al.*, (2012) have also exhibited significant antibacterial activities of *A. scholaris* against *E. coli* and *Shigella dysenteriae*. In respect of comparison of antibiotics used and extracts applied it is obvious from our results that the extracts were more effective against MDR bacterial strains. According to our results, *E. coli* was resistant to 90% of antibiotics but it was highly sensitive (86.36% each) to the DWE and EE. It showed 81.8% sensitivity to the nHE, 77.2% sensitivity to ME and 70.9% sensitivity to EAE. *Providencia* which was 80% resistant showed sensitivity of 55.3% to EAE, 52% to ME, 46.6% to DWE and 32% to EE. It also showed 100% resistance to nHE. Strains of *Enterobacter* which were 70% resistance showed different sensitivities such as 73.68% to ME, 69.21% to EE, 68.42% to EAE, 62.10% to nHE and 43.68% to DWE. Similarly, *Klebsiella* was 60% resistant to antibiotics showed 56.20% sensitivities to both the EE and EAE, 48.27% sensitivities to DWE and ME while 41.37% sensitivity to nHE. Similarly, *MRSA* which was 50% resistant showed 50.78% sensitivity to DWE, 48.15% sensitivity to nHE and EE and 46.31% sensitivity to ME and EAE each. *Citrobacter* which was 30% resistant showed 66.53% sensitivity to DWE, 53.84% to EAE, 51.51% to EE, 47.30% to nHE and 46.15% to ME. *Pseudomonas* was 20% resistant while it showed high sensitivity of 107.27% to EE, 98.18% to ME, 83.18% to EAE, 77.27% to DWE and 74.09% to nHE. *Acinetobacter* which was the least resistant strain (10%) also showed 62% sensitivity to EE, 60% to DWE and 42% to EAE. It was completely resistant (100%) to the nHE and ME as shown in the table 2.

Before antifungal activity, culture sensitivity tests were performed. *Aspergillus fumigatus* showed highest sensitivity to NS (DIZ of 15.67 mm) while lowest DIZ of 10 mm against FCA. *Rhizopus* was highly sensitive to NS (22.37 mm DIZ) and least sensitive to AMPB (13.30 mm DIZ). Moreover, it was found completely resistant to FCA. *Aspergillus niger* was highly sensitivity to NS (16 mm DIZ) and least sensitive to VOR (11 mm DIZ). It showed absolute resistance to FCA. In the same way

Table 2: Antibiogram of the selected clinically isolated pathogenic organisms (diameter of inhibition zone in mm).

Bacterium	Susceptibility to prescribed antibiotics ($\mu\text{g}/\text{disk}$)									
	TE (30 μg)	CRO (30 μg)	NA (30 μg)	SXT (1.25/23.75 μg)	AMC (25 μg)	MXF (5 μg)	CIP (5 μg)	CEC (30 μg)	CN (10 μg)	SCF (75/30 μg)
<i>E. coli</i>	R	8.23 \pm 0.30	R	R	9.13 \pm 0.37	8.26 \pm 0.15	R	R	12.27 \pm 0.20	22.07 \pm 0.37
<i>Pseudomonas</i>	13.03 \pm 0.49	20.13 \pm 0.37	8.06 \pm 0.20	9.36 \pm 0.35	18.03 \pm 0.41	19.13 \pm 0.28	22.37 \pm 0.32	14.47 \pm 0.23	24.07 \pm 0.32	22.07 \pm 0.30
<i>Klebsiella</i>	9.03 \pm 0.55	28.33 \pm 0.30	R	R	20.13 \pm 0.25	10.33 \pm 0.15	R	23.33 \pm 0.20	10.13 \pm 0.25	29.13 \pm 0.15
<i>Acinetobacter</i>	19.06 \pm 0.32	15.26 \pm 0.55	R	24.27 \pm 0.35	20.13 \pm 0.40	23.07 \pm 0.11	19.27 \pm 0.25	22.13 \pm 0.25	22.17 \pm 0.35	30.03 \pm 0.41
<i>Providencia</i>	9.03 \pm 0.25	R	R	R	15.33 \pm 0.35	12.27 \pm 0.15	14.17 \pm 0.11	R	R	30.03 \pm 0.20
<i>MRSA</i>	14.06 \pm 0.40	12.26 \pm 0.37	10.33 \pm 0.15	R	12.43 \pm 0.15	15.47 \pm 0.20	16.27 \pm 0.15	9.17 \pm 0.15	20.17 \pm 0.32	38.07 \pm 0.15
<i>Citrobacter</i>	12.13 \pm 0.35	12.36 \pm 0.35	R	24.27 \pm 0.41	20.13 \pm 0.37	18.27 \pm 0.15	20.37 \pm 0.25	15.33 \pm 0.25	25.03 \pm 0.32	26.03 \pm 0.15
<i>Enterobacter</i>	10.16 \pm 0.40	13.06 \pm 0.20	R	R	10.26 \pm 0.15	19.17 \pm 0.20	18.27 \pm 0.20	R	24.07 \pm 0.23	38.07 \pm 0.30

Data is presented as Mean \pm Standard Deviation (n = 3).

TE = Tetracycline, CRO = Ceftriaxone, NA = Nalidixic acid, SXT = Trimethoprim-Sulfamethoxazole, AMC = Amoxicillin, CIP = Ciprofloxacin, CEC = Cefaclor, CN = Gentamicin, SCF = Cefoperazone-Sulbactam, MRSA = Methicillin resistant *Staphylococcus aureus*, R = Resistant.

Penicillium was highly sensitive to NS (16 mm DIZ) and moderately sensitive to AMPB (9.30 mm DIZ), while it was resistant to FCA and VOR. *Acremonium* showed high sensitivity to NS (DIZ of 20.37 mm) and less sensitivity

to VOR (DIZ of 13.00 mm). It showed resistance to FCA as shown in the table 3.

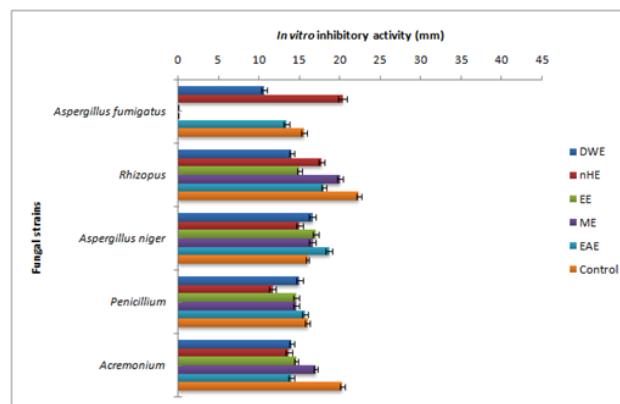


Fig. 3: Antifungal profile of the *A. scholaris* leaf extracts. DWE= Distilled water extract, nHE= n-hexane extract, EE= ethanolic extract, ME= methanolic extract, EAE= ethyl acetate extract and Control= Nystatin (100 $\mu\text{g}/\text{disc}$).

The leaves extracts of *A. scholaris* also showed significant antifungal activities. The DWE showed highest antifungal activity against *Aspergillus niger* (DIZ of 16.60 mm) and lowest activity against *Aspergillus fumigatus* (DIZ of 10.63 mm). The nHE showed highest activity against *Aspergillus fumigatus* (DIZ of 20.33 mm) and lowest activity against *Penicillium* (DIZ of 11.67 mm). Similarly, the EE showed highest activity against *Aspergillus niger* (DIZ of 17.00 mm) while it showed no activity against *Aspergillus fumigatus*. The ME showed highest DIZ of 20 mm against *Rhizopus*, while it showed no activity against *Aspergillus fumigatus*. Similarly, the EAE showed highest DIZ of 18.67 mm against *Aspergillus niger*, while it showed less activity against *Aspergillus fumigatus* (DIZ of 13.33 mm). Our study is also in line with the study conducted by Antony *et al.*, (2012). In their study the antifungal activities of *A. scholaris* was presented against different fungal strains. The extract showed highest DIZ (16mm) against the *Candida krusei*. Significant antifungal study of *A. scholaris* was also reported by Upadhyay *et al.*, (2014), in which numerous extracts of the stem bark of *A. scholaris* in a concentration of 200 $\mu\text{g}/\text{disc}$ was tested against *Alternaria alternata*, *Aspergillus niger*, *Trichoderma virens* and *Fusarium solani* species.

The current study also illustrates that *Aspergillus fumigatus* was highly sensitive to nHE and completely resistant to EE and ME. *Rhizopus* was more sensitive to ME while it was less sensitive to DWE. Similarly *Aspergillus niger* was highly sensitive to EAE and least sensitive to DWE and ME. *Penicillium* was more sensitive to EAE and less sensitive to nHE. Correspondingly *Acremonium* was more sensitive to ME and less sensitive to nHE. Moreover, the comparative analysis of table 3 and fig. 4 also confirms about the facts

Table 3: Culture sensitivity analysis of fungal strains (diameter of inhibition zone in mm)

Fungi	Pattern of susceptibility to antifungals ($\mu\text{g}/\text{disc}$)			
	FCA (25 μg)	VOR (1 μg)	NS (100 μg)	AMPB (100 μg)
<i>Aspergillus fumigatus</i>	10.00 \pm 1.00	11.63 \pm 0.26	15.67 \pm 0.32	13.30 \pm 0.27
<i>Rhizopus</i>	R	15.33 \pm 0.21	22.37 \pm 0.15	13.30 \pm 0.20
<i>Aspergillus niger</i>	R	11.00 \pm 0.50	16.00 \pm 0.30	12.67 \pm 0.35
<i>Penicillium</i>	R	R	16.00 \pm 0.45	9.30 \pm 0.36
<i>Acromonium</i>	R	13.00 \pm 0.30	20.37 \pm 0.25	16.30 \pm 0.20

Data is presented as Mean \pm Standard Deviation (n = 3).

FCA = Fluconazole, VOR = Voriconazole, NS = Nystatin, AMPB = Amphotericin B, R = Resistant

that the leaves extracts of *A. scholaris* applied in the study were more effective than the commercially available standard antifungal discs.

CONCLUSION

In the present study, phytochemical screening of various extracts of *A. scholaris* disclosed the existence of several chemical components like alkaloids, glycosides, saponins, reducing sugars, terpenoids and steroids. The antimicrobial potential of this plant is probably due to the presence of these phytochemicals. The experimental analysis for determining the antibacterial activities of *A. scholaris* declared the highest inhibitory potential against *enterobacter* specie by the methanol extract with a DIZ of 28mm, whereas as the n-Hexane extract showed significant antifungal activity against *A. fumigatus* with a DIZ of 20.33mm. The current study showed that crude extract of *A. scholaris* has valuable phytochemicals, on the basis of which it produces various antimicrobial activities. Natural medicines have a wider range of safety index and further efforts are needed to explore the commercial applications of *A. scholaris*.

ACKNOWLEDGMENTS

The authors are very much thankful to the Abasyn University Peshawar, Khyber Pakhtunkhwa, Pakistan for supporting the present research work. Remarkable thanks to the department of botany, University of Peshawar for the identification of plant and Pakistan Council of Scientific and Industrial Research (PCSIR) Labs, Peshawar for providing the FTIR facility. Support of the department of Soil Chemistry, The University of Agriculture, Peshawar is also acknowledged here for the identification of fungal strains.

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