J. Appl. Environ. Biol. Sci., 4(8S)47-54, 2014

ISSN: 2090-4274
Journal of Applied Environmental and Biological Sciences
www.textroad.com

Extracts From *Asparagus adscendens* Exhibit Potential Antifungal Activity

Arif Ali Khattak[1,2,#], Ayaz Ahmad[1,#1,*], Rehan Naeem[2], Muhammad Sohaib[1], Muhammad Bilal[3], Amjad Iqbal[4], and Farooq Shah[4]

[1] Department of Biotechnology, Abdul Wali Khan University Mardan, Pakistan
[2] Department of Biotechnolgy and Genetic Engineering, KUST, Pakistan
[3] Department of Chemistry, Islamia College University Peshawar, Pakistan
[4] Department of Agriculture, Abdul Wali Khan University Mardan, Pakistan

Received: September 1, 2014
Accepted: November 13, 2014

ABSTRACT

Asparagus adscendens (*A. adscendens*) has been used as traditional herbal medicine for centuries. In this study, methanolic root and leaves extracts of *A. adscendens* were evaluated for antifungal activity. Antifungal activity and percentage inhibition of the methanolic root and leaves extracts of *A. adscendens* and antibiotic Fluconozol was analyzed at the concentration of 1000 µg/mL, 500 µg/mL, 250 µg/mL, 125 µg/mL and 62.5 µg/mL against four different strains of fungi including *Aspergillus flavus* (*A. flavus*), *Aspergillus terreus* (*A. terreus*), *Aspergillus niger* (*A. niger*) and *Alterneria spp.* The results showed that linear fungal inhibition for root and leaves extract was maximum at 1000 µg/mL and minimum at 62.5.5 µg/mL. Area of inhibition of fungal strain for different concentration of methanolic root and leaves extracts of *A. adscendens* were also analyzed. Extracts at 1000 µg/mL concentration showed activity against all fungal strains ranging from 3.45 cm for *A. niger* to 1.8 cm for *Alterneria spp.* Mean of mycelial inhibition percentage of fungal strains of methanolic root and leaves extract of *A. adscendens* showed activity against all fungal strains ranging from 115 % for *A. niger* to 78.6 % for *A. flavus.* Minimum Inhibitory Concentration (MIC) of methanolic root extract was 1000 µg/mL each for *A. flavus, A. niger* and *Alterneria spp,* while MIC of methanolic leaves extract was 62.5 µg/mL for *A. flavus,* 250 µg/mL for *A. niger* and 1000 µg/mL for *Alterneria spp.* In the same way, MIC for antibiotic fluconozol was 125 µg/mL for *A. terreus and Alternaria spp.* while it was 62.5 µg/mL each for *A. flavus* and *A. niger.* Our results show that the methanolic extract of *A. adscendens* have significant antifungal activity and can be used as herbal medicine to treat different infections caused by the tested organisms.

KEYWORDS: *Asparagus adscendens*, Antifungal activity, methanolic extract, medicinal plant, minimum inhibitory concentration.

INTRODUCTION

Medicinal plants, since times immemorial, have been used in virtually all cultures as a source of medicine [1]. Traditionally, thousands of herbs have been investigated and suggested for therapeutic and medicinal purpose all around the world[2]. The practice of traditional medicine is widespread in China, India, Japan, Pakistan, Sri Lanka and Thailand [1]. Antimicrobial and antifungal activity of variety of herbal extracts has been noticed and found effective in different ailments [3, 4]. About 3000 species of *Asparagus* are known to occur in world. The genus *Asparagus* has been recently moved from the family Liliaceae to newly created family *Asparagaceae* [5].

Asparagus adscendens is a member of the genus *Asparagus* belonging to the family Liliaceae[6]. Asparagus adscendens is commonly referred to as Shweta musali in India and Sufaid musk in Pakistan[7]. *A. adscendens* is distributed in Punjab plains and foothill regions of Pakistan Kashmir and India[8, 9]. *A. adscendens* is used as rejuvenative herb and is also very powerful aphrodisiac and is very beneficial herb for treating low sperm count and male infertility with a very high demand in the market[7]. *A. adscendens* provides a rich source of potential antidiabetic agents for diabetic persons [4, 7]. The roots of *A. adscendens,* showed a significant antitumor action in skin and fore stomach papillomagenesis [4]. In Ayurveda, *Asparagus*

[1] [#] These authors contributed equally to this work

***Corresponding author:** Ayaz Ahmad, Department of Biotechnology, Abdul Wali Khan University Mardan, Pakistan
Phone # 0092-937-542046, E-mail: ahdayazb5@awkum.edu.pk

adscendens is also used in making health. It is also used in curing problems like leucorrhea, menorrhagia, arthritis, pre-natal and post-natal sufferings [4, 7].

Asparagus genus is considered to be of medicinal importance because of the presence of steroidal saponin and sapogenins in various parts of the plant [10]. Other species of this genus possess potential antimicrobial ability against various microbes. Mshelia *et al*. (2008) evaluated the phytochemistry and antimicrobial effect of the stem, bark and leave of *Asparagus flagellaris* [11].

Uma *et al*. (2009) reported the antifungal activity of *Asparagus racemosus* roots and tubers extract against *Candida albicans, Candida tropicalis, Candida krusei, Candida guillermondii, Candida parapsilosis* and *Candida stellatoida*. The extract of *Asparagus racemosus* showed high degree of activity against all the *Candida* strains. The inhibitory effect of the extract against all the *Candida* tested was found comparable with that of standard antibiotics used [12].

Sangvikar (2012) investigated the root extracts of five plants namely *Asparagus racemosus*, *Chlorophytum tuberosum*, *Hemidesmus indicus*, *Withania somnifera*, *Rauwolfia tetraphylla* against two seed borne pathogenic fungi *Alternaria solani*, *Fusarium moniliforme*. Out of the five root extracts, two showed strong antifungal activity. The extract of *Hemidesmus indicus* showed maximum activity while minimum activity was observed by *Rauwolfia tetraphylla* [13].

The screening of plant extracts and their product for antifungal activity has shown that higher plants represent a potential source of novel antibiotic prototypes. Thus, this study was conducted with aim to evaluate antifungal activity of the methanolic root and leaves extract of *Asparagus adscendens* on various fungal strains.

MATERIALS AND METHODS

Collection of plant material

The fresh plants of *Asparagus adscendens* were collected from different area of Hangu including Alizai, Marai, KaamSaam, and Usterzai. The taxonomic identification of *Asparagus adscendens* was confirmed by the taxonomic key and flora of Pakistan.

Fungal Strains

The fungal strains evaluated in the present study were *Aspergillus flavus*, *Aspergillus terreus*, *Aspergillus niger* and *Alternaria spp.*

Antibiotics Dilution Method

10 mg reference antibiotic, Fluconozol (Glaxo Smith Kline Beecham, Pakistan) was prepared in Dimethyl Sulfoxide (DMSO) at the concentration of 10,000 µg/mL, 1000 µg/mL, 500 µg/mL, 250 µg/mL, 125 µg/mL and 62.5 µg/mL in 1.5 mL Eppendorf tubes and used for antifungal activities.

Methanolic Root and Leaves Extract Preparation

The leaves and roots of *A. adscendens* were washed with distilled water and shade dried under room temperature. After drying, plants were powdered using electrical grinder. About 120 g of leaves powder and 90 gram of the roots powder was suspended in 200 mL (95 %w/w) of absolute methanol in 1000 mL flat bottom flask and kept at room temperature for maceration at 37 °C for about 15-20 days. The mixture was filtered through Whattman filter and a viscous mass was obtained by evaporating in rotatory evaporator which was then dried under reduced pressure at 40-50 °C. The dried crude extracts obtained this way were stored at 4 °C and used for antifungal activities.

Screening of Antifungal Activities

In order to check the antifungal activities, sterilized 5 mL media was poured to each test tube and kept in slant position for solidification in aseptic and sterilized environment of biosafety cabinet. The inoculum from the fungal strain was taken for inoculation on the medium in test tubes.

The stock solution of both roots and leaves extract at the concentration of 10,000 µg/mL was prepared by taking 1 mg of dried extract both from roots and leaves and dissolved in 1 mL of DMSO (Dimethyl Sulfoxide). Then from the stock solution of 10,000 µg/mL, further dilutions of concentration of 1000 µg/mL, 500 µg/mL, 250 µg/mL, 125 µg/mL and 62.5 µg/mL were prepared. The control antibiotic was also diluted from the stock solution at same concentrations.

About 500 mL of each concentration of roots and leaves extract of *Asparagus adscendens* and standard control (antibiotic) was poured into the 5 mL pre solidified SDA media in test tubes. After 30 minutes the SDA media was solidified and streaked from standard fungal strain under sterilized conditions. The test tubes were then kept in refrigerator at 4 ℃ in slant position in order to allow the medium to diffuse effectively. The test tubes were incubated at 37 ℃ for 3 night period of time.

Statistical Analysis

After incubation of the test tubes at 37 ℃ for 3 nights, different concentrations of methanolic root and leaves extracts and standard control (antibiotic) were analyzed for the antifungal activities by measuring the Fungal Inhibition, Mycelial Inhibition Percentage and Minimum Inhibitory Concentration (MIC).

RESULTS AND DISCUSSION

Finding the *in vitro* antimicrobial activity of plant extracts is first step in the development of new drugs. Antifungal activity and percentage inhibition of the methanolic root and leaves extracts of *A. adscendens* and antibiotic Fluconozol was analyzed at the concentrations of 1000 µg/mL, 500 µg/mL, 250 µg/mL, 125 µg/mL and 62.5 µg/mL against four different strains of fungi including *A. flavus*, *A. terreus*, *A. niger* and *Alternaria spp.* In the present study, Linear Fungal inhibition of methanolic leaves and root extract of *Asparagus adscendens* was analyzed at the concentration of 1000 µg/mL, 500 µg/mL, 250 µg/mL, 125 µg/mL and 62.5 µg/mL. Tables 1 and 2 show linear inhibition of antibiotic and plant roots and leaves extract, respectively. Figures 1.A, 1.B, 1.C and 1.D show the antifungal activity of antibiotic Fluconozol and *A. adscendens* roots and leaves methanolic extracts against *A. flavus*, *A. terreus*, *Alternaria spp* and *A. niger,* respectively.

Table 1: Linear Inhibition of Root Extract and Antibiotic (Fluconozol)

Fungal Strains	Linear Inhibition of Root Extract and Antibiotic (cm)									
	1000 µg/mL		500 µg/mL		250 µg/mL		125 µg/mL		62.5 µg/mL	
	Antibiotic	Root Extract	Antibiotic	Root Extract	Antibiotic	Root Extract	Antibiotic	Root Extract	Antibiotic	Root Extract
Aspergillus flavus	3.5	2.2	3.2	2	3.1	1	2.8	1	2.6	0.5
Aspergillus terreus	1.6	1.3	1.45	1	1.2	1	1.3	0.9	1.15	0.9
Aspergillus niger	3.1	2.3	2.7	1.8	2.6	1.8	2.5	1.7	2.3	1.1
Alternaria spp	1.45	1.2	1.4	1	1.2	0.9	1.2	0.7	0.9	0.4

Table 2: Linear Inhibition of Leaves Extract and Antibiotic (Fluconozol)

Fungal Strains	The Linear Inhibition of Leaves Extract and Antibiotic (cm)									
	1000 µg/mL		500 µg/mL		250 µg/mL		125 µg/mL		62.5 µg/mL	
	Antibiotic	Leaves Extract	Antibiotic	Leaves Extract	Antibiotic	Leaves Extract	Antibiotic	Leaves Extract	Antibiotic	Leaves Extract
Aspergillus flavus	3.5	3	3.2	2	3.1	1	2.8	1	2.6	0.5
Aspergillus terreus	1.6	1.3	1.45	1	1.2	1	1.3	0.9	1.15	0.9
Aspergillus niger	3.1	2.3	2.7	1.8	2.6	1.8	2.5	1.7	2.3	1.1
Alternaria spp	1.45	1.2	1.4	1	1.2	0.9	1.2	0.7	0.9	0.4

The results showed that linear fungal inhibition for root and leaves extract was maximum at 1000 µg/mL and minimum at 62.5.5 µg/mL. Similar results were also obtained by Naz and Bano (2012) who investigated the antifungal activity of methanolic and aqueous leaf extracts of *Ricinus communis* against selected fungal strains including *Aspergillus fumigatus* and *Aspergillus flavus*. They noticed that methanolic and water extracts of *R. communis* were able to show broad spectrum antifungal activity against the selected fungal strains at 12 mg/mL final concentration. Leaf extracts in methanolic and water from *R. communis* inhibited the growth of *A. fumigatus* and *A. flavus* by 59.5 % and 56.3 %, respectively [14].

Table 3: Area of Inhibition of fungal strains for different concentrations of root extract of *Asparagus adscendens*

Fungal strains	Area Of Inhibition Of Fungal Strains For Root Extract (cm^2)				
	1000 µg/mL	500 µg/mL	250 µg/mL	125 µg/mL	62.5 µg/mL
Aspergillus flavus	3.3	3	1.5	1.5	0.75
Aspergillus terreus	1.95	1.5	1.5	1.35	1.35
Aspergillus niger	3.45	2.7	2.7	2.55	1.65
Alternaria spp.	1.8	1.5	1.35	1.05	0.6

Table 4: Area of Inhibition of fungal strains for different concentrations of root extract of *Asparagus adscendens*

Fungal Strains	Area Of Inhibition Of Fungal Strains For Leaves Extract (cm^2)				
	1000 µg/mL	500 µg/mL	250 µg/mL	125 µg/mL	62.5 µg/mL
Aspergillus flavus	4.5	3	1.5	1.5	0.75
Aspergillus terreus	1.95	1.5	1.5	1.35	1.35
Aspergillus niger	3.45	2.7	2.7	2.55	1.65
Alternaria spp.	1.8	1.5	1.35	1.05	0.6

Area of inhibition of fungal strain for different concentration of methanolic root extract and leaves extract of *A. adscendens* were also analyzed. Extracts at the concentration of 1000 µg/mL showed activity against all fungal strains ranging from 3.45 cm^2 for *A. niger* to 1.8 cm^2 for *Alternaria spp*. The maximum area of inhibition was observed at the concentration of 1000 µg/ml that was 3.45 cm^2 for *A. niger*, 3.3 cm^2 for *A. flavus*, 1.95 cm^2 for *A. terreus* and 1.8 cm^2 for *Alternaria spp.* While at 62.5 µg/ml, area of inhibition was 1.65 cm^2 for *A. niger*, 1.35 cm^2 for *A. terreus*, 0.75 cm^2 for *A. flavus* and 0.6 cm^2 for *Alternaria spp.*

Table 5: Area of Inhibition of Fungal Strains for Different Concentrations of Fluconozol

Fungal Strains	Area Of Inhibition Of Fungal Strains For Fluconozol (cm^2)				
	1000 µg/mL	500 µg/mL	250 µg/mL	125 µg/mL	62.5 µg/mL
Aspergillus flavus	5.25	4.8	4.65	4.2	3.9
Aspergillus terreus	2.4	2.17	1.8	1.95	1.69
Aspergillus niger	4.57	4.2	4.05	3.75	3.45
Alternaria spp	2.17	2.14	1.8	1.8	1.35

Table 6: Mean of Mycelial Inhibition percentage of fungal strain for different concentration of methanolic root extracts of *Asparagus adscendens*

Fungal Strains	Means Of Mycelial Inhibition Percentage Of Fungal Strains (%)				
	1000 µg/mL	500 µg/mL	250 µg/mL	125 µg/mL	62.5 µg/mL
Aspergillus flavus	78.6 ± 0.7	66.6± 0.8	25.0 ± 1.41	25.0 ± 0.7	11.1± 0.14
Aspergillus terreus	86.6 ± 0.84	55.5± 0.7	55.5 ± 0.7	47.4 ± 0.42	47.4 ±0.14
Aspergillus niger	115.0 ± 2.82	72.0 ±1,41	72.0 ± 2.82	65.4 ± 0.99	34.4 ±1.84
Alternaria spp	80.0 ± 1.41	58.8 ±3.96	50.0 ± 4.2	35.0 ± 1.41	17.4 ± 0.7

Mean of Mycelial inhibition percentage of fungal strains of methanolic root and leaves extract of *Asparagus adscendens* was analyzed at the concentration of 1000 µg/mL, 500 µg/mL, 250 µg/mL, 125 µg/mL and 62.5 µg/mL against four different strains of fungi including *A. flavus*, *A. terreus*, *A. niger* and *Alternaria spp.* Methanolic root extracts of *Asparagus adscendens* at 1000 µg/mL concentration showed activity against all fungal strains ranging from 115% for *A. niger* to 78.6% for *A. flavus*. The maximum Mycelial inhibition percentage at 1000 µg/mL concentration was 115% for *A. niger*, 86.6% for *A. terreus*, 80.0% for *Alternaria spp* and 78.6% for *A. flavus.* Similarly, methanolic leaves extract of *Asparagus adscendens* at the concentration of 1000 µg/mL showed activity against all fungal strains ranging from 188.8% for *A. niger* to 78.6% for *A. flavus*. The maximum Mycelial inhibition percentage at the concentration of 1000 µg/mL was found 188.8% for *A. niger*, 115.5% for *A. terreus*, 92.8% for *Alternaria spp* and 78.6% for *A. flavus*.

Table 7: Mean of Mycelial inhibition percentage of fungal strain for different concentration of methanolic leaves extract of *Asparagus adscendens*

Fungal Strains	Means Of Mycelial Inhibition Percentage Of Fungal Strains				
	1000 µg/mL	500 µg/mL	250 µg/mL	125 µg/mL	62.5 µg/mL
Aspergillus flavus	78.6 ± 0.7	66.6± 1.5	11.1± 0.28	11.1 ± 0.7	8.7 ± 0.42
Aspergillus terreus	115.5 ± 2.54	100.0 ± 2.82	133.3± 0.42	100.0 ± 4.24	122.2 ±2.2
Aspergillus niger	188.8± 9.3	115.0 ±4.24	87.0 ± 1.27	48.8 ± 0.98	48.8 ±1.6
Alternaria spp	92.8 ± 0.56	58.8 ±1.69	35.0 ± 1.41	35.0 ± 2.82	35.0 ± 0.98

Table 8: Mean of Mycelial inhibition percentage of fungal strain for different concentration of Antibiotic Fluconozol

Fungal strains	Means of Mycelial inhibition percentage of fungal strains				
	1000 µg/mL	500 µg/mL	250 µg/mL	125 µg/mL	62.5 µg/mL
Aspergillus flavus	233.3 ± 0.42	177.7± 0.42	163.1± 0.42	127.3 ± 0.28	108.3 ± 2.4
Aspergillus terreus	133.3 ± 2.4	107.4 ± 1.13	75.0 ± 2.82	86.6 ± 0.56	67.6 ±0.93
Aspergillus niger	244.0 ± 2.82	186.6 ±7.5	168.7 ±7.49	138.8 ± 1.13	115.0 ±2.8
Alternaria spp	116.0 ± 2.8	112.6 ±0.7	80.0 ± 2.8	80.0 ± 1.4	50.0 ± 0.7

Table 9: Minimum Inhibitory Concentration (MIC) of Methanolic leaves, root extracts of *Asparagus adscendens* and Antibiotic Fluconozol against fungal strains

Fungal Strains	Minimum Inhibitory Concentration (MIC) µg/ml		
	Asparagus adscendens root extract	*Asparagus adscendens* leaves extract	Antibiotic Fluconozol
Aspergillus flavus	n.d.	n.d.	62.5
Aspergillus terreus	1000	62.5	125
Aspergillus niger	1000	250	62.5
Alternaria spp.	1000	1000	125

* n.d. = Not determined

Minimum Inhibitory Concentration (MIC) of methanolic root extract, leaves extract of *Asparagus adscendens* and antibiotic Fluconozol was evaluated. Minimum Inhibitory Concentration (MIC) of methanolic root extract was 1000 µg/mL each for *A. flavus, A. niger* and *Alternaria spp.* While MIC of methanolic leaves extract was 62.5 µg/mL for *A. flavus,* 250 µg/mL for *A. niger* and 1000 µg/mL for *Alternaria spp.* In the same way, MIC for antibiotic Fluconozol was 125 µg/mL for *A. terreus and Alternaria spp.* while it was 62.5 µg/mL each for *A. flavus* and *A. niger.*

Fungi are responsible for many skin diseases. Many fungal species fungi also cause several plant infections. *A. adscendens* was evaluated for antifungal activity and considerable activity was noticed. Root and leaves extract at the concentration of 1000 µg/mL inhibited mycelial growth up to 115.0 % for *A. niger*, 86.6 % for *A. terreus*, 80.0 % for *Alternaria* spp. and 78.8 % for *A. flavus*. With the development of resistance against antibiotics, medicinal plants should be considered as alternative to get all the possible antimicrobial benefits of the useful compounds contained in medicinal plants. Thus these studies indicated that this plant can be used as an herbal medicine to treat the infections caused by these tested organisms.

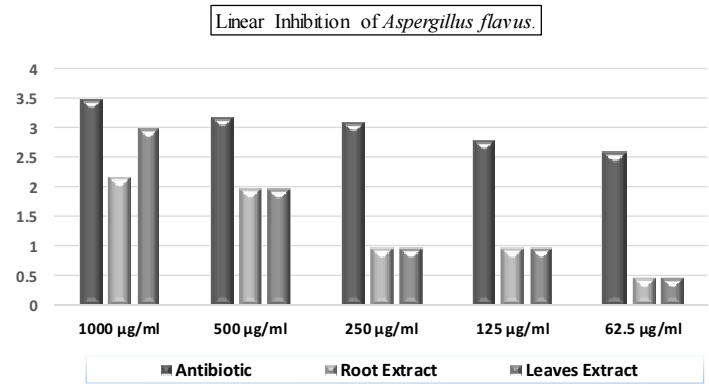

Figure 1.A: Linear Inhibition of *Aspergillus flavus* by Root and Leaves extracts of *Asparagus adscendens* and Fluconozol.

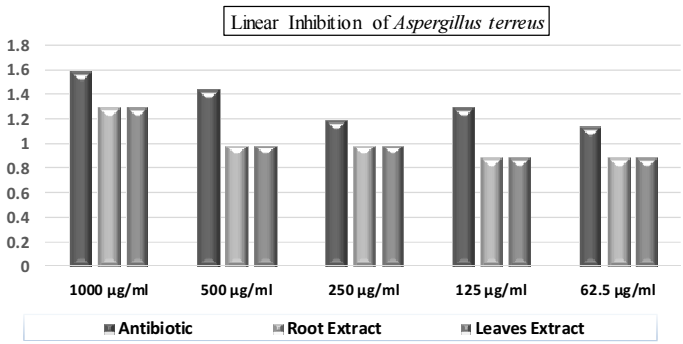

Figure 1.B: Linear Inhibition of *Aspergillus terreus* by Root and Leaves extracts of *Asparagus adscendens* and Fluconozol.

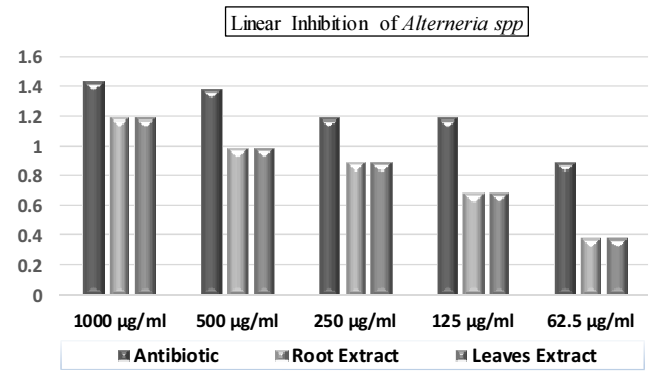

Figure 1.C: Linear Inhibition of *Alternaria spp.* by Root and Leaves extracts of *Asparagus adscendens* and Fluconozol.

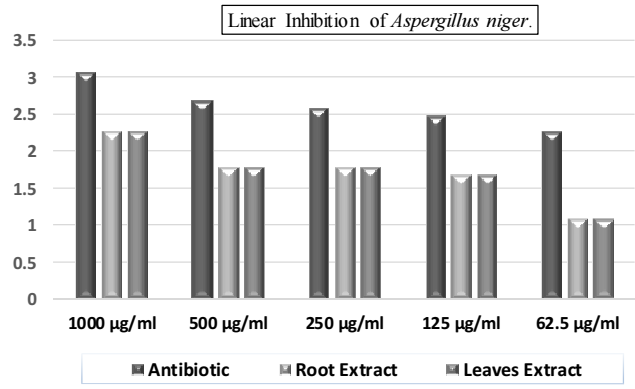

Figure 1.D: Linear Inhibition of *Aspergillus niger* by Root and Leaves extracts of *Asparagus adscendens* and Fluconozol.

CONCLUSION

The present study revealed that the methanolic root and leaves extracts of *Asparagus adscendens* exhibit promising antifungal activity against fungal strains tested. Methanolic root and leaves extracts of *A. adscendens* may be used as useful medicine against diseases caused by the test fungal strains.

ACKNOWLEDGEMENTS

The authors would like to thank Abdul Wali Khan University Mardan (AWKUM), Kohat University of Science and Technology (KUST) and Higher Education Commission of Pakistan for the financial support.

REFERENCES

1. Hoareau, L. and E.J. DaSilva, *Medicinal plants: a re-emerging health aid.* Electronic Journal of Biotechnology, 1999. **2**(2): p. 3-4.
2. Ali, N., et al., *In Vitro Antibacterial, Antifungal and Phytotoxic Activities of Ficus Carica Methanolic Leaves Extracts.* International Journal of Current Biotechnology 2014. **2**(2): p. 11-15.
3. Chung, P., et al., *Antimicrobial activities of Malaysian plant species.* Pharmaceutical biology, 2004. **42**(4-5): p. 292-300.
4. Umashanker, M. and S. Shruti, *Traditional Indian herbal medicine used as antipyretic, antiulcer, anti-diabetic and anticancer: A review.* International Journal of Research in Pharmacy and Chemistry, 2011. **1**(4): p. 1152-1159.
5. Goyal, R., J. Singh, and H. Lal, *Asparagus racemosus--an update.* Indian journal of medical sciences, 2003. **57**(9): p. 408.
6. Gautam, M., et al., *Immunoadjuvant potential of< i> Asparagus racemosus</i> aqueous extract in experimental system.* Journal of ethnopharmacology, 2004. **91**(2): p. 251-255.
7. Mathews, J.N., P.R. Flatt, and Y.H. Abdel-Wahab, *Asparagus adscendens (Shweta musali) stimulates insulin secretion, insulin action and inhibits starch digestion.* British journal of nutrition, 2006. **95**(03): p. 576-581.
8. Mehta, S.R. and R. Subramanian, *Direct in vitro propagation of Asparagus adscendens Roxb.* Plant Tissue Culture, 2005. **15**(1): p. 25-32.
9. Chen, S., et al., *Liliaceae.* Flora of China, 2000. **2**: p. 73-263.

10. Sharma, S., R. Chand, and O. Sati, *Steroidal saponins of< i> Asparagus adscendens</i>*. Phytochemistry, 1982. **21**(**8**): p. 2075-2078.
11. Mshelia, E., et al., *Phytochemical Analysis and Antibacterial Screening of Asparagus Flagellaris (Kunth) Bak used in The Traditional Treatment of Sexually Transmitted Diseases and Urinary Infections.* Ethiopian Journal of Environmental Studies and Management, 2008. **1**(2): p. 44-48.
12. Uma, B., K. Prabhakar, and S. Rajendran, *Anticandidal activity of Asparagus racemosus.* Indian journal of pharmaceutical sciences, 2009. **71**(3): p. 342.
13. Sangvikar, R.V., *Screening of some plant root extracts for their antifungal activity against seed borne pathogenic fungi.*
14. Naz, R., A. Bano, and N. Ilyas, *Antimicrobial potential of Ricinus communis leaf extracts in different solvents against pathogenic bacterial and fungal strains.* Asian Pac J Trop Biomed, 2012. **2**(12): p. 944-7.