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Research Article

Antioxidant, Antimicrobial and Cytotoxic Potential of *Abelmoschus esculentus*

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Abstract: *Abelmoschus esculentus* is an important medicinal plant belongs to family *Malvaceae*. It originates from Ethiopia and is widely spread all over tropical, subtropical and warm temperate regions of the world. This research work has been designed to evaluate the antioxidant, antimicrobial and toxicological potential of *A. esculentus* leaves and seeds. The antifungal and antioxidant components of *A. esculentus* leaves and seeds were extracted by using four solvent systems (80% methanol, 80% ethanol, 100% methanol and 100% ethanol) and leaves presented maximum extract yield (38.1 g/100g DW)in 80% methanolic solvent system. Phytochemical analysis of *A. esculentus* leaves and seeds extracts performed in terms of total phenolic and total flavonoid contents, showed that 80% methanolic leaves extract offered highest total phenolic contents (31.2 mg GAE/g DW), whereas 80% ethanolic leaves gave maximum total flavonoid contents (41.8 mg CE/g DW). Antioxidant activity was determined by DPPH radical scavenging activity and measure of reducing power. Results revealed that 80% methanolic leaves extract showed highest radical scavenging activity and reducing potential. Antimicrobial activity of *A. esculentus* leaves and seeds was investigated by Disc Diffusion Method and Minimum Inhibitory Concentration (MIC). Results showed that 80% methanolic extract of leaves exhibited highest antibacterial and antifungal potential against *P. multocida* (30 mm DIZ) and *A. paraciticus* (29 mm DIZ), respectively. Cytotoxicity analysis was performed on BHK-21 cell by adopting the MTT assay. The cytotoxicity activity of the 80% methanolic extract of leaves was evaluated by noticing the cell survival percentage (52.5%). Overall results of the present study showed that 80% methanolic leaves extracts of *A. esculentus* possesses very good antioxidant, antimicrobial and cytotoxic properties.

Keywords: Antioxidant, Antibacterial, Scavenging, Cytotoxicity, Potential

1. Introduction

Abelmoschus esculentus is an important medicinal plant belongs to family *Malvaceae*. It originates from Ethiopia and is widely spread all over tropical, subtropical and warm temperate regions of the world. This research work has been designed to evaluate the antioxidant, antimicrobial and toxicological potential of *A. esculentus* leaves and seeds.

The antifungal and antioxidant components of *A. esculentus* leaves and seeds were extracted by using four solvent systems (80% methanol, 80% ethanol, 100% methanol and 100% ethanol) and leaves presented maximum extract yield (38.1 g/100g DW) in 80% methanolic solvent system [1]. Phytochemical analysis of *A. esculentus* leaves and seeds extracts performed in terms of total phenolic and total flavonoid contents, showed that 80% methanolic leaves extract offered highest total phenolic contents (31.2 mg GAE/g DW), whereas80% ethanolic leaves gave maximum total flavonoid contents (41.8 mg CE/g DW) [2]. Antioxidant activity was determined by DPPH radical scavenging activity and measure

of reducing power [3, 4]. Results revealed that 80% methanolic leaves extract showed highest radical scavenging activity and reducing potential [5]. Antimicrobial activity of *A. esculentus* leaves and seeds were investigated by Disc Diffusion Method and Minimum Inhibitory Concentration (MIC). Results showed that 80% methanolic extract of leaves exhibited highest antibacterial and antifungal potential against *P. multocida* (30 mm DIZ) and *A. paraciticus* (29 mm DIZ), respectively. Cytotoxicity analysis was performed on BHK-21 cell by adopting the MTT assay. The cytotoxicity activity of the 80% methanolic extract of leaves was evaluated by noticing the cell survival percentage (52.5%). Overall results of the present study showed that 80% methanolic leaves extracts of *A. esculentus* possesses very good antioxidant, antimicrobial and cytotoxic properties [6].

2. Material and Method

Present research work was conducted in the laboratories of the Chemistry Department, Lahore Garrison University, Lahore, Pakistan; Toxicopathological Laboratory, University of Veterinary and Animal Sciences, Lahore, Pakistan.

2.1. Chemicals and Standard Compounds

Different chemicals were purchased from the Sigma Chemicals Co. (St, Louis, MO, USA). All standard antibiotic discs and culture media were purchased from Oxoid Ltd. (Hampshire, UK).

2.2. Collection of Plant Materials

Different parts of *Abelmoschus esculentus* i.e. leaves and seeds were obtained from the vicinity of Lahore Garrison University, Lahore, Pakistan.

2.3. Pretreatment of Plant Materials

The leaves and seeds of *Abelmoschus esculentus* were washed with tap water and then dried at 40°C in an oven (Memmert, Jarmany) to maintain constant weight. By using a commercial blender, dried leaves and seeds were grounded into a fine powder [17, 20]. Then the ground material was passed through 80-mesh sieve. The passed material was used for extraction purpose. Polythene bags were used to store the ground samples at 4°C until for further analysis [22, 23].

2.4. Antioxidant Activity of Plant Extracts

2.4.1. DPPH Radical Scavenging Assay

DDPH radical scavenging assay was applied to determine the free radical scavenging activity of medicinal plants. 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical was used to determine the scavenging activity as described by Suleman et al. with little amendments [7, 48]. Methanol solvent was used to determine the DPPH solution (33mg/L). Absorbance of the resulting solution was taken at 0 min. Then extract solutions (250µg/mL) were prepared. After that 5mL of methanolic solution of DPPH was added in 1mL of extract solution. The mixture was placed for 30 minutes in the dark place. Then by using a spectrophotometer, absorbance was measured at wavelength of 517nm with methanol was taken as a blank solution [8]. Free radical scavenging activity was expressed as percentage inhibition and calculation was done by using the following formula:

$$\text{Inhibition (\%)} = \frac{\text{Control Absorbance} - \text{Sample Absorbance}}{\text{Control Absorbance}} \times 100$$

Where control absorbance is absorbance of methanolic solution of DPPH taken at zero minute.

2.4.2. Determination of Reducing Power

The reducing power of the plants leaf and seed extracts was determined according to the procedure described by Hassan et al. by doing slight changing [22]. Different extracts having Concentration range (2.5-10.0 mg) were mixed with the buffer of sodium phosphate solution (5.0 mL, 0.2 M, pH 6.6) and potassium ferric cyanide (5.0 mL, 1.0%) solution. The incubation of resulting mixture was done at 50°C for 20 min. Then 5 mL of 10% tri-chloro acetic acid was added in the mixture and was centrifuged for 10 min at 980 g at 5°C in a refrigerated centrifuge (CHM-17; Kokusan Denki, Tokyo, Japan). Two layers were formed. The 5 mL of upper layer of the solution was poured in beaker and diluted it with 5.0 mL of distilled water and ferric chloride (1.0 mL, 0.1%) solution, and absorbance was measured at 700 nm using spectrophotometer (U-2001, Hitachi Instruments Inc., and Tokyo, Japan). Analysis was done thrice for each sample and results were averaged [23].

2.4.3. Evaluation of Antimicrobial Potential of Plant Extracts

The extracts of leaves and seeds were tested individually against a panel of microorganisms which may included five fungal strains (*Aspergillus parasiticus, Aspergillus flavus, Fusarium oryzae, Fusarium tritichum, Aspergillus oryzae*) and three bacterial strains (*Escherichia coli, Pasturella multocida* and *Staphylococcus aureus*) obtained from the Fungal Bank, University of Punjab, Lahore. Fungal strains were cultured overnight at 28°C in Potato Dextrose agar (Oxoid Hampshire, UK), however the bacterial strain were cultured at 37°C in nutrient agar (Oxoid Hampshire, UK). The slants of microbial strains were stored at 4°C. Antimicrobial potential of plants extracts were determined by using the disc diffusion and micro dilution broth assays [24].

2.4.4. Disc Diffusion Assay

Antimicrobial activity of leaves and seeds of *Abelmoschus esculentus* was tested against fungal strains (*Aspergillus flavus, Aspergillus paraciticus, Fusarium oryzae, Fusarium tritichum, Aspergillus oryzae*) and bacterial strains (*Escherichia coli, Pasturella multocida* and *Staphylococcus aureus*) by previously adopted method [31] with little modifications [34]. Potato dextrose agar (PDA) solution was prepared and autoclaved. About 20mL PDA solution was poured in sterilized petri plate under laminar air flow [21]. Sterilized discs (6mm) of wicks sheet impregnated with 50µL of particular plant extract were placed on the agar plates. To compare the activity with standard antibiotics, Fluconazol (30

µg/disc) (Oxoid) and Rifampicin (30 µg/disc) (Oxoid) were used as positive reference for fungal and bacterial strains respectively [10]. Disc without samples were used as a negative control. Test discs and standard disc were placed in separate petri dishes. The plates were incubated at 28°C for 48h for fungal growth and 37°C for 24h for bacterial growth. Antimicrobial activity was evaluated by measuring the diameter of inhibition zones (mm) by zone reader [9].

2.4.5. Micro Dilution Broth Method

MIC of plant extracts was evaluated by the method reported by Sherma et al. [46]. Briefly, 100µL of plant extract was transferred into the first row of the 96 well microtiter plates. To all other wells, 50µL of Sabarouraud dextrose broth and nutrient broth was added for fungal and bacterial strains, respectively. Two-fold serial dilutions were performed using a micropipette such that each well had 50µL of the test material in serially descending concentrations [17]. Finally, 10µL of microbial suspension was added to each well. Each plate had a row of negative control, a row of positive control of Fluconazol and Rifampicin for antifungal and antibacterial activities, respectively [41]. The plates were prepared in triplicate and incubated at 28°C for 48h for fungi and 37°C for 24h for bacteria. The absorbance was measured at 620 nm by ELISA reader. The lowest concentration at which there was no growth was taken as the MIC value [47].

2.5. Toxicological Analysis

2.5.1. Cytotoxicity Assay

Cytotoxicity of plant extracts was evaluated by adopting the MTT assay using baby hamster kidney cells (BHK-21) as described by Freshney and Frame (1982), while 10% DMSO was used as a positive control. Solutions of the tested materials were evaluated for cytotoxic potential. The BHK-21 cells were revived using DMEM (Sigma-Aldrich, Germany) media as described by Freshney (1998) and were then transferred into 40 ml cell culture flasks (Karrel Flasks, Corning, USA), which were then incubated for 72 h to get the confluent monolayer of cells. 100 µl of cell suspension (105 cells/ml) was dispensed into each well of 96-well plates (Corning, USA) and incubated at 37°C for 72 h. Media on the confluent monolayer of cells was regularly changed and of the respective sample concentrations was added in triplicate, which was then incubated at 37°C for 48 h [28-30]. Finally, the growth medium was removed, and wells were washed with PBS and replenished with fresh media. 100µL of 0.5% MTT solution was added to each well, and plates were incubated for ~4 h. The MTT solution was then removed, and plates were incubated at 37°C for 2 h after adding 5% DMSO to each well. Optical density was measured at 570 nm by an ELISA reader (Type355, Model 2005–05, Thermo, China) [8].

2.5.2. Statistical Analysis

All experiments were performed in triplicate (n=3) and the data was reported as mean ± SD. Data were analyzed using Minitab 2000 Version 13.2 statistical software (Minitab Inc. Pennsylvania, U.S.A) at 5% significant level. Antifungal activity data was presented as mean values at 95% confidence interval. Significant differences of mean were calculated by using LSD [9, 10].

3. Results and Discussion

The present research work was conducted to demonstrate the antioxidant and antimicrobial and cytotoxic potential of leaves and seeds of Abelmoschus esculentus.

3.1. Antioxidant Activity of Abelmoschus Esculentus

There are various numbers of medicinal plants that are being used due to their antioxidant properties. Active form of these chemical constituents is very helpful in order to prevent the destructive actions caused by their oxidative stress [43]. In the present study, two assays were used to find the antioxidant activity of *A. esculentus* leaves and seeds [49].

3.2. DPPH Radical Scavenging Activity

Natural constituents such as polyphenols, flavonoids, phenolics, terpenes and tannins possess antioxidant property to scavenge free radicals [20-22]. Antioxidant activity of these products can be evaluated by using DPPH radical scavenging assay. This assay has been widely used to test the scavenging ability of that compounds which act as free radical or hydrogen donors to DDPH [36, 38]. DPPH is a nitrogen centered free radical compound in stable form. Upon reduction, its colour changes from violet to yellow by hydrogen or electron donation [15]. Substances which have ability to perform such types of reactions are known as good antioxidants and better radical scavengers. It has been also found that with increasing the extract concentration, DDPH free radical scavenging ability also increases [16].

So, DPPH (1,1-diphenyl-2-picryl-hydrazyl-hydrate) free radical scavenging method is based on phenomenon of transfer of electron [41]. It is an antioxidant assay that produces a violet colouration in methanol solution. At room temperature, stability of this free radical is reduced due the presence of an antioxidant molecule which results in the formation of colourless solution [18]. It is an important mechanism that explains the oxidation process of proton radical scavenger. By decreasing the absorbance of DPPH solution to 517 nm, its reduction capability was evaluated suggesting that antioxidant activity of plant extract is due to its proton donating ability [13]. The antioxidant molecule has the hydrogen donating atom which contributes to its free radical scavenging nature which is an important quality of antioxidants [42]. DPPH radical assay has been used because it is a quick, reliable, easy and rapid method in order to investigate the general antioxidant activity of plants extracts as well as pure compounds. This method is also used for screening of many samples for radical scavenging potential and is independent on the polarity of sample [5, 45].

Antioxidant potential of medicinal plant *Abelmoschus esculentus* was evaluated by using the DDPH free radical

scavenging assay. This assay also explored its new potential sources for natural antioxidants. DPPH concentrations of medicinal plant leaves and seeds were tested and found to be reduced due to scavenging potential.

Table 1. *DPPH radical scavenging activity of the medicinal plant leaves and seeds.*

Sr. No	Solvent System	DPPH (%) radical scavenging activity	
		Leaves	Seeds
1	80%Methanol	$60.1\pm1.20^{a}{}_{a}$	$44.2\pm1.88^{b}{}_{b}$
2	80%Ethanol	$58.9\pm0.45^{b}{}_{a}$	$46.1\pm0.69^{a}{}_{b}$
3	100%Methanol	$57.3\pm2.48^{bc}{}_{a}$	$41.7\pm1.23^{d}{}_{b}$
4	100%Ethanol	$56.4\pm0.65^{c}{}_{a}$	$43.5\pm0.64^{c}{}_{b}$

Mean ± SD of two samples analyzed individually in triplicate at p <0.05. Superscripts alphabets within the column depicted significant difference among different solvent system. Superscripts alphabets within the rows depicted significant difference among different plant parts.

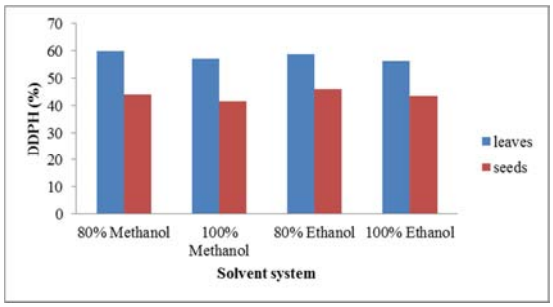

Figure 1. *DPPH radical scavenging activity of the medicinal plant leaves and seeds.*

Table 1 showed that there is significant difference of DPPH radical scavenging activities of *A. esculentus* extracts among different solvent system. The aqueous alcoholic extracts of leaves and seeds of *A. esculentus* exhibited satisfactory DDPH radical scavenging ability. The *A. esculentus* leaves exhibited highest DDPH radical scavenging potential significantly (p <0.05) in 80%methanolic extract followed by: 80% ethanol> absolute methanol> absolute ethanol. However, 80% ethanolic seeds extract showed the high DDPH radical scavenging potential followed by: 80% methanol> absolute ethanol> absolute methanol.

Present results are supported by the previous report of Liao et al. found that *A. esculentus* leaves exhibit higher free radical scavenging activity than seeds and fruits [27]. Zafar et al. also reported that methanolic extracts of pigeon pea also showed highest DDPH radical scavenging ability (54.5%) [56].

3.3. Reducing Power

Reductive abilities of the plant extracts can be an indication of their potential towards antioxidant activities [57]. Antioxidant ability of phenolic compound is generally due to their redox properties. These properties allow them to react as a reducing agent such as an oxygen quencher electron donor. Study on medicinal plants and vegetables revealed that plants are the great source of antioxidant properties. In biological systems, these plants are capable of applying the protection effects against certain oxidative stress [40, 51]. Electrons are donated to reactive radical specie due to the presence of antioxidants substances by the process in which these are neutralized into stable and nonreactive species [32].

In this assay, reduction of the $Fe^{3+}$to the ferrous form occurs due to presence of reducers which is also known as antioxidant. So reducing power is measured by donation of electron and reduction of $Fe^{3+}(CN^{-})_{6}$ to $Fe^{2+}(CN^{-})_{6.}$ Perl prussian blue colour product formation indicates the presence of Fe^{2+} concentration that can be monitored at the wavelength of 700 nm [7]. Reducing potential of medicinal plants was more at higher absorbance value [32]. Hence, activity of reducing power increases with increasing the concentration of extracts [44].

The reducing potential of Abelmoschus esculentus leaves and seeds extracts is presented in table 2. The reducing potential values of the examined extracts were observed at different concentration ranges from 2.5 to 10.0 mg/mL. It was observed that leaves extracts showed significantly (P< 0.05) higher reducing potential than seeds, irrespective to which type of solvent used. However, 80% methanolic leaves extract showed the highest reducing power. As reducing power is dependent on concentration so the given results in Figure 2 revealed that reducing power of Abelmoschus esculentus is increased with increase in concentration [33].

Results of present research work are supported by the previous analysis of Geng et al. investigated that the reducing power of Abelmoschus esculentus flower linearly increases with increasing the extract concentration [19].

Moreover, Chang et al. also described the reducing power of Phellinus merrillii extracts which also increased by concentration dependent manner and maximum reduction potential was observed at 2mg/mL concentration. In order to compare the reducing potential of leaves and seeds of medicinal plant with present results, there is no earlier report available [11, 12].

Table 2. *Reducing Power of A. esculentus leaves and seeds.*

Plant Parts	Solvent system	Concentration(mg/ml)			
		2.5^{a}	5.0^{b}	7.5^{c}	10^{d}
Leaves[a]	80%Methanol	0.121±0.03	0.127±0.02	0.135±0.01	0.148±0.06
	80%ethanol	0.118±0.01	0.121±0.04	0.132±0.03	0.140±0.05
	100%methanol	0.110±0.06	0.115±0.02	0.123±0.01	0.134±0.06
	100%ethanol	0.116±0.04	0.126±0.01	0.129±0.06	0.135±0.02
Seeds[b]	80%methanol	0.111±0.03	0.114±0.02	0.121±0.04	0.139±0.04
	80%ethanol	0.113±0.04	0.119±0.03	0.128±0.02	0.141±0.01
	100%methanol	0.108±0.05	0.117±0.01	0.124±0.04	0.131±0.06
	100%ethanol	0.105±0.02	0.118±0.04	0.122±0.03	0.129±0.01

Values are mean ± SD of two samples analyzed individually in triplicate at p <0.05. Superscripts alphabets within the column depicted significant difference among different plant parts. Superscripts alphabets within the rows depicted significant difference among different concentration.

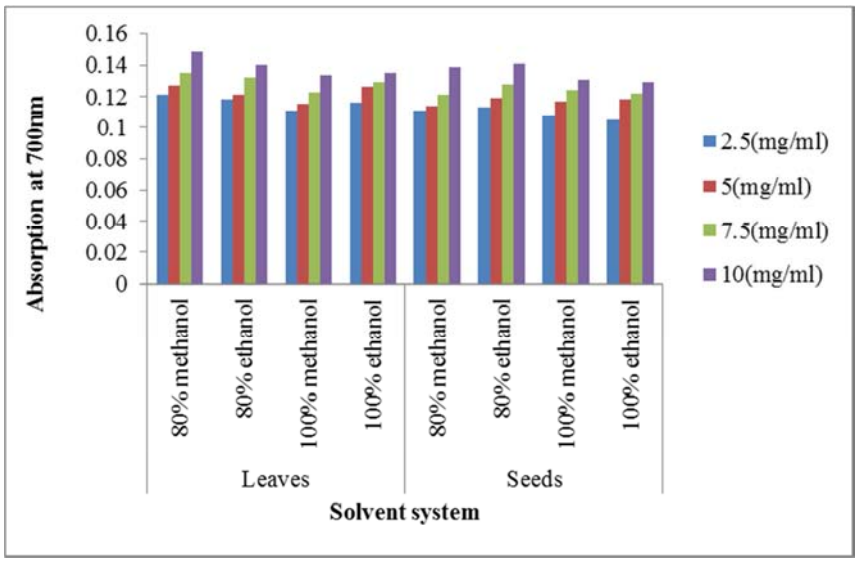

Figure 2. *Reducing Power of A. esculentus leaves and seed .*

4. Antibacterial Activity

Medicinal plants are rich source of antimicrobial bioactive constituents. Several infections can be treated by using the extensive range of medicinal plants extracts as they have potential antimicrobial activity [25]. The phytochemical analysis of the methanol extract of *A. esculentus* showed that certain Phytoconstituents such as alkaloids, saponins, cardenolides, anthraquinones and tennis are present in it. These plant metabolites have been described to have antimicrobial potential. So, antimicrobial properties of the medicinal plants might be ascribed due to the presence of these secondary metabolites [14].

4.1. Antibacterial Activity (Disc Diffusion Method)

The antibacterial components can be present in all plant parts like leaves, fruits, roots, flowers, pods, seeds and stems [26]. In the present investigation, disc diffusion method was used to determine the antibacterial activity of leaves and seeds extracts of *Abelmoschus esculentus* against three bacterial strains via *P. Multocida, E. coli* and *S. aureus*. Each extract was applied against bacterial strains individually and diameter of inhibition zone (DIZ) was measured by using a zone reader. From the given results, it was observed that among all the solvents, methanolic leaves extract exhibited significantly ($p < 0.05$) highest inhibitory activity against *P. Multocida* (30 mm DIZ). On the other hand, seed extracts showed no inhibitory potential against *S. aureus*. Overall, leaves extracts showed more antibacterial activity against all bacterial strains as compared to seed extracts [28].

Oloketuyi et al. reported that ethanolic extracts of *Abelmoschus esculentus* seed showed least inhibitory activity against *B. cereus* [35]. Dahham et al. also revealed that alcoholic extracts of *Abelmoschus esculentus* were found to be more sensitive to gram negative bacteria as compared to gram-positive bacteria which are in close agreement to our present findings in which *E. coli* is more sensitive to alcoholic extracts than *S. aureus* [14].

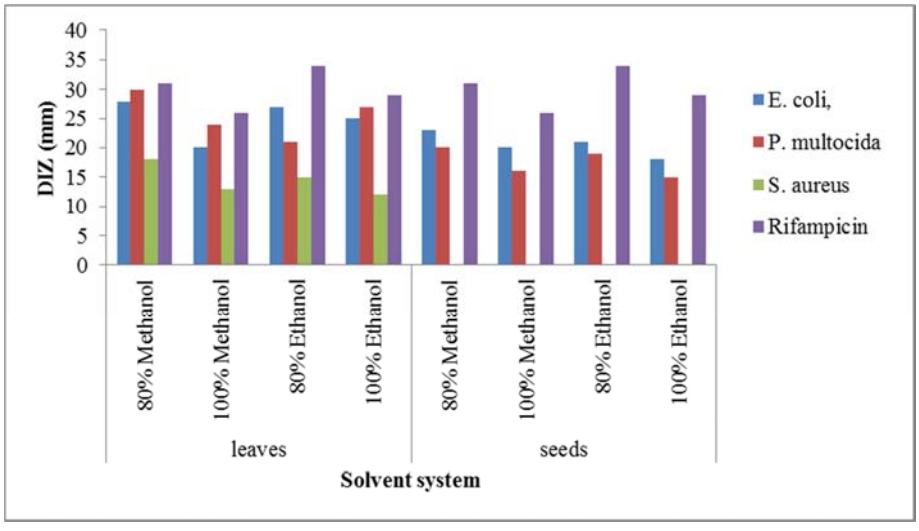

Figure 3. *Antibacterial activity of A. esculentus leaves and seeds.*

Table 3. *Antibacterial activity of Abelmoschus esculentus leaves and seeds.*

Plant parts	Microorganism	DIZ (mm)			
		80% Ethanol	Absolute Ethanol	80% Methanol	Absolute Methanol
Leaves	*E.coli*	$27\pm1.24^{b}_{ab}$	$25\pm1.28^{bc}_{c}$	$28\pm1.68^{bc}_{a}$	$20\pm2.49^{b}_{d}$
	P. multocida	$21\pm1.87^{c}_{d}$	$27\pm2.16^{b}_{b}$	$30\pm1.86^{b}_{a}$	$24\pm1.97^{ab}_{c}$
	S. aureus	$15\pm1.32^{d}_{b}$	$12\pm1.64^{e}_{c}$	$18\pm1.55^{e}_{a}$	$13\pm2.40^{d}_{bc}$
Seeds	*E. coli*	$21\pm1.67^{c}_{ab}$	$18\pm1.98^{c}_{c}$	$23\pm1.63^{cd}_{a}$	$20\pm1.77^{b}_{b}$
	P. multocida	$19\pm1.55^{cd}_{ab}$	$15\pm1.85^{d}_{c}$	$20\pm1.95^{de}_{a}$	$16\pm2.05^{c}_{bc}$
	S. aureus	Nil	Nil	Nil	Nil
	Rifampicin	$31\pm1.09^{a}_{b}$	$29\pm1.44^{a}_{c}$	$34\pm1.34^{a}_{a}$	$26\pm1.67^{a}_{d}$

Values are mean ± SD of two samples analyzed individually in triplicate at p <0.05. Superscriptswithin the same column depicted significant difference among different solvents, while subscripts within the same rowindicated significant difference (p < 0.05) between bacterial strains.

4.2. Antifungal Activity (Disc Diffusion Method)

The antifungal property of leaves and seeds of *Abelmoschus esculentus* was determined by disc diffusion method against a panel of five fungal strains such as *Aspergillus parasiticus,*

Aspergillus flavus, Fusarium oryzae, Fusarium tritichum, and *Aspergillus oryzae.* The diameter of inhibition zones is presented in the table 4. Given results showed that methanolic extracts of leaves of *Abelmoschus esculentus* exhibited significantly (p <0.05) strongest inhibitory activity (29 mm DIZ) against *A. parasiticus.* While ethanolic leaves extracts showed moderate activity against fungal strains. Given results also revealed that leaves and seed extract exhibited no antifungal activity against *Fusarium oryzae*. Results of present work are in accordance with the findings Oloketuyi et al. examined that methanolic extracts of *Punica granatum* possessed more efficacies against fungal strains that might be ascribed due to the presence of certain active Phytoconstituents including phenols, flavonoids and tannins in it [14, 29].

Table 4. *Antifungal activity of A. esculentus leaves and seeds.*

Plant parts	Microorganisms	DIZ(mm)			
		80%Methanol	80%Ethanol	100%Methanol	100%Ethanol
Leaves	*A. parasiticus*	29 ± 1.12^{ab}	21 ± 1.75^{b}	24 ± 1.43^{ab}	19 ± 2.09^{bc}
	A. flavus	25 ± 1.27^{bc}	22 ± 1.66^{b}	19 ± 2.04^{cd}	20 ± 1.83^{b}
	F. oryzae	Nil	Nil	Nil	Nil
	F. tritichum	19 ± 2.06^{de}	15 ± 1.88^{ef}	22 ± 2.03^{bc}	17 ± 2.07^{c}
	A. oryzae	20 ± 1.09^{de}	15 ± 1.53^{ef}	13 ± 1.57^{g}	13 ± 2.07^{de}
Seeds	*A. parasiticus*	23 ± 1.32^{cd}	12 ± 2.06^{f}	21 ± 1.39^{bc}	17 ± 2.00^{c}
	A. flavus	20 ± 1.09^{de}	15 ± 1.53^{ef}	13 ± 1.57^{g}	13 ± 2.07^{de}
	F. oryzae	Nil	Nil	Nil	Nil
	F. tritichum	17 ± 1.40^{de}	18 ± 2.09^{cd}	16 ± 1.52^{ef}	21 ± 1.67^{b}
	A. oryzae	11 ± 1.92^{f}	18 ± 1.76^{cd}	19 ± 2.02^{cd}	10 ± 1.83^{d}
	Fluconazol	34 ± 1.09^{a}	29 ± 1.44^{a}	31 ± 1.34^{a}	26 ± 1.67^{a}

Values are mean ± SD of two samples analyzed individually in triplicate at p <0.05. Superscripts x and y indicated significant difference (p < 0.05) between fungal strains while subscripts alphabets within the same column depicted significant difference among different solvents.

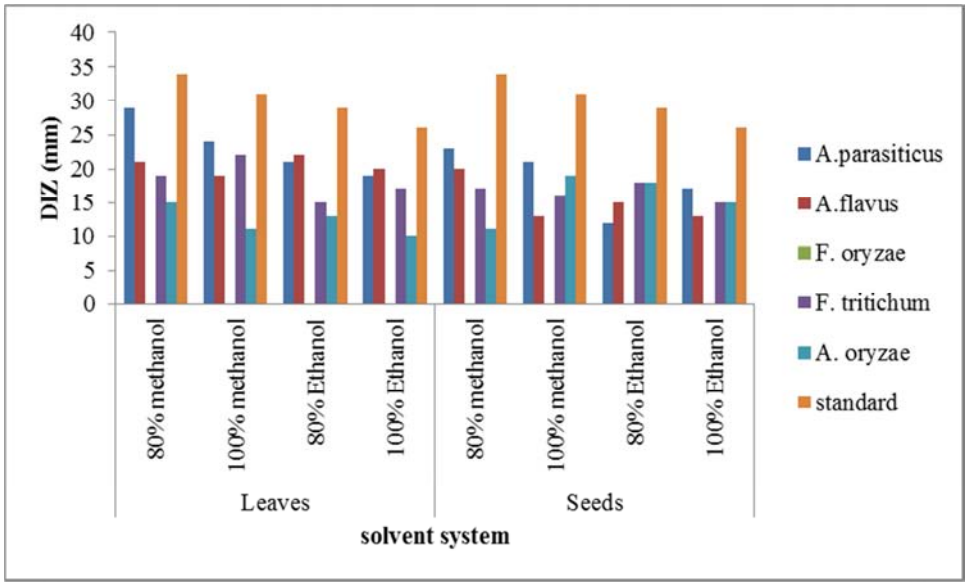

Figure 4. *Antifungal activity of A. esculentus leaves and seeds.*

4.3. Minimum Inhibitory Concentration (MIC) Against Bacterial Strains

The MIC is defined as the minimum concentration of drug that completely stops the observable progress of microorganism after overnight incubation. This period can be prolonged for an organism such as anerobes which required more incubation for growth [7]. Micro dilution broth method is used to determine the lowest concentration of the antimicrobial agent [53]. This assay was used to evaluate the minimum inhibitory concentrations of leaves and seeds extract of *Abelmoschus esculentus* as against bacterial strains. Its calculation was performed in µg/ml.

MIC values of the leave and seeds extract of medicinal plant *Abelmoschus esculentus* are given in the table 5. Here Rifampicin (standard) is used to compare the antibacterial activities of different extracts with it. Among all the leaves extracts, 80% methanolic leaves extract was found to be the most efficient and highest antibacterial activity with lowest MIC value (33µg/ml). Similarly 80% methanolic seeds extract showed appreciable antibacterial activity (48µg/ml) against bacterial strain *E. coli.* 80% ethanolic leave and seeds extract also showed moderate antibacterial activity against bacterial strain. Overall, 80% methanolic leaves extract of leaves exhibited potent antimicrobial activity.

Parekh et al. reported that the methanolic extracts of the medicinal plants showed less MIC value against Gram-negative bacteria [37]. Moreover, Pieme et al., (2008) also demonstrated that methanol extract of *E. hirta* was found to be more active against bacterial strains than that of chloroform extracts confirming that polar extract is more effective [54, 55].

Table 5. *Minimum inhibitory concentration (MIC) of A. Esculentus leaves and seeds against bacterial strains.*

Plant parts	Microorganism	MIC (µg/ml)			
		80% Methanol	80% Ethanol	100% Methanol	100% Ethanol
Leaves	*E.coli*	33 ± 1.3^{de}	46 ± 2.8^{ef}	58.3 ± 2.6^{de}	64 ± 2.9^{cd}
	P. multocida	40 ± 1.1^{cd}	52 ± 3.1^{de}	66 ± 3.4^{cd}	70 ± 2.3^{c}
	S. aureus	44 ± 1.6^{bc}	55 ± 2.2^{cd}	70 ± 2.5^{bc}	74 ± 1.9^{bc}
Seeds	*E.coli*	48 ± 1.9^{bc}	60 ± 2.4^{bc}	68 ± 3.3^{cd}	70.0 ± 2.7^{c}
	P. multocida	56 ± 2.0^{a}	68 ± 2.7^{a}	75 ± 2.8^{a}	84 ± 2.5^{a}
	S. aureus	Nil	Nil	Nil	Nil
	Rifampicin	26 ± 1.1^{e}	32 ± 2.1^{f}	38 ± 2.4^{e}	45 ± 1.8^{d}

Values are mean ± SD of two samples analyzed individually in triplicate at p <0.05. Superscripts x and y indicated significant difference (p < 0.05) between bacterial strains while subscripts alphabets within the same column depicted significant difference among different solvents.

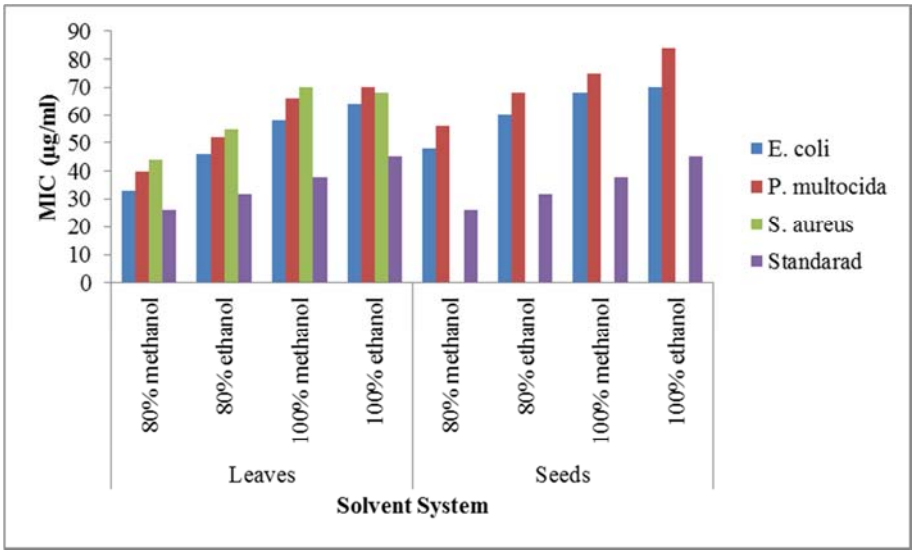

Figure 5. *Minimum inhibitory concentration (MIC) of A. esculentus leaves and seeds against bacterial strains.*

4.4. Minimum Inhibitory Concentration (MIC) Against Selected Fungal Strains

Minimum inhibitory concentration of the leaves and seeds extracts of *Abelmoschus esculentus* was investigated against five fungal strains via *A. parasiticus, A. flavus, F. oryzae, F. tritichum* and *A. oryzae.* In order to find the minimum inhibitory concentration, flucanozole was taken as the standard. MIC values of the leaves and seeds extracts of *Abelmoschus esculentus* are given in the table 6. These results revealed that each fungal strain showed different growth responses to different solvent extracts of the same plant species [41]. Here 80% methanolic leaves extract of *Abelmoschus esculentus* exhibited the highest antifungal activity by exhibiting lowest MIC value (32 µg/ml) against *A. paraciticus.* 80% methanolic seed extract also showed antifungal activity but lower as compare to that of

leaves. 80% ethanolic leaves and seeds extracts showed moderate activity but absolute extracts of methanol and ethanol exhibited the lowest antifungal activity against five fungal strains due to highest MIC values [49, 50].

The MIC values of medicinal plant extracts against fungal strains showed that fungi had inverse relationship in the level of their vulnerability to antifungal agents. This has been supported by the previous findings of Parekh et al. who revealed that antimicrobial additives having high activity against an organism have low MIC value [37]. while an antimicrobial additive have low activity against an organism possesses high MIC value [48, 52].

Table 6. *Minimum inhibitory concentration (MIC) of A. esculentus leaves and seeds against fungal strains.*

		MIC(µg/ml)			
Plant parts	**Microorganisms**	**80% methanol**	**80% ethanol**	**100% Methanol**	**100% Ethanol**
	A. parasiticus	33 ± 1.12^{fg}	38 ± 2.75^{fg}	42 ± 1.43^{fg}	46 ± 2.09^{ef}
	A. flavus	36 ± 1.27^{fg}	40 ± 2.66^{fg}	47 ± 2.04^{ef}	50 ± 1.83^{de}
Leaves	*F. oryzae*	Nil	Nil	Nil	Nil
	F. tritichum	45 ± 2.06^{ef}	51 ± 2.88^{ef}	65 ± 2.43^{de}	77 ± 2.07^{cd}
	A. oryzae	50 ± 1.65^{ef}	55 ± 2.23^{de}	70 ± 2.46^{cd}	80 ± 1.92^{cd}
	A. parasiticus	53 ± 1.32^{de}	62 ± 2.06^{cd}	75 ± 2.40^{bc}	87 ± 2.00^{bc}
	A. flavus	60 ± 1.09^{cd}	65 ± 2.53^{bc}	78 ± 2.57^{bc}	90 ± 2.07^{bc}
Seeds	*F. oryzae*	Nil	Nil	Nil	Nil
	F. tritichum	70 ± 1.40^{a}	73 ± 2.09^{ab}	88 ± 1.52^{ab}	99 ± 1.67^{ab}
	A. oryzae	67 ± 1.92^{ab}	77 ± 1.76^{a}	90 ± 2.02^{a}	104 ± 1.83^{a}
	Flucanozole (control)	26 ± 1.1^{g}	32 ± 2.1^{g}	38 ± 2.4^{g}	45 ± 1.8^{f}

Values are mean ± SD of two samples analyzed individually in triplicate at $p < 0.05$. Superscripts x and y indicated significant difference ($p < 0.05$) between bacterial strains while subscripts alphabets within the same column depicted significant difference among different solvents.

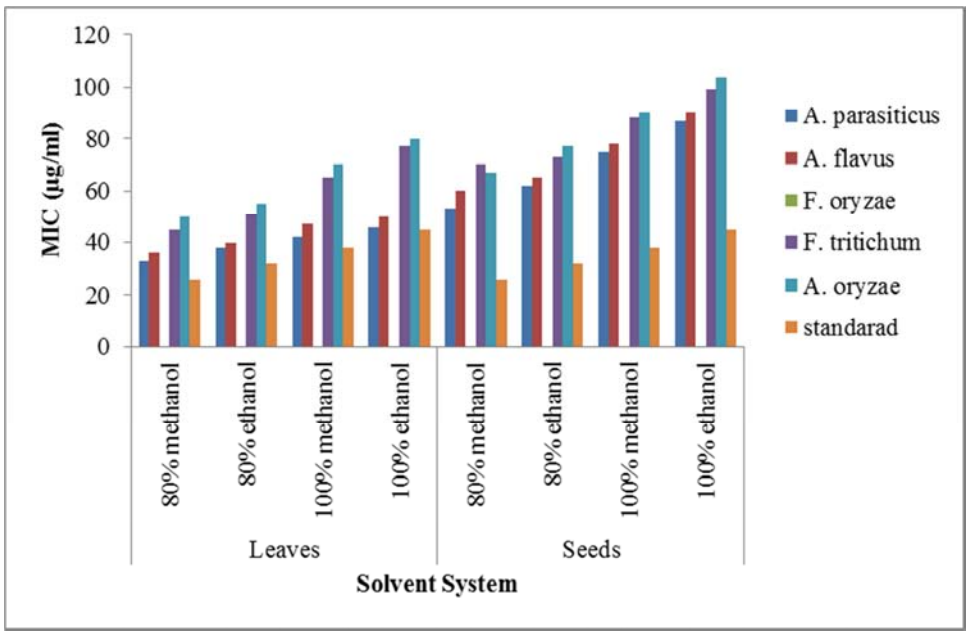

Figure 6. *Minimum inhibitory concentration (MIC) of A. esculentus leaves and seeds.*

4.5. Cytotoxicity Assay

The results of MTT technique showed that ethanolic leaves and seeds extracts of *A. esculentus* did not show any cytotoxicity on this cell while 80% methanolic leaves extract showed a concentration-dependent cytotoxic effect in proliferating BHK-21 cell line and determined by calculating their cell survival percentage [42]. Cells were exposed to different concentrations of plant leaves extract [43]. The data of the cell survival percentage (52.50%) for 80% methanolic leaves extract having concentration23810 µg/ml are presented in Table 7.

Hassan et al. reported that growth of cell line K562 by treating with methanolic leaves extract of *E. herbecta* showed inhibition by 66.3% [23]. Cytotoxicity of genus *A. esculentus* has been studied and attributed the anti-tumor activity of different species of this plant. It also showed that, it has a potential for cytotoxicity against different cell lines [50, 52].

Table 7. *Cytotoxic potential of Salvia hispanica leaves [39].*

Medicinal Plant extract	**Treatment (µg/ml)**	**Cell survival percentage (%)**
80% Methanolic leaves extract	23810	52.50±0.26

5. Conclusion

Results of present study demonstrate the pharmacological importance of medicinal plant A. esculentus which highlights the medicinal use of its different parts. This plant is medically important due to its antioxidant, antimicrobial and cytotoxic activity. Its wealth is in various metabolites because it is being used in prevention of many diseases related to oxidative stress, due to microbes. Present findings also revealed that 80% methanol is best solvent which showed more antioxidant, antimicrobial and cytotoxic potential of leaves of A. *esculentus*.

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