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COMPARATIVE EVALUATION OF ANTIMICROBIAL ACTIVITY OF DIFFERENT PARTS OF *ABELMOSCHUS MOSCHATUS* AGAINST MULTI-RESISTANT PATHOGENS

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ABSTRACT: Now a day the exposure of multi-resistance human pathogens has challenged our existing defense to save human being. To minimize such alarming issue, identification of naturally-occurring antimicrobial agents from plant origin has become a realistic and powerful tool to the researchers. Therefore the aim of this study was to investigate the antimicrobial potential of the ethanolic extracts of different parts of *Abelmoschus moschatus* (*A. moschatus*) against some important human bacterial and fungal pathogens. Different parts (leaves, stem bark, fruits pulp and seeds) of *A. moschatus* were extracted with ethanol to get crude ethanolic leaves (LE), stem bark (SBE), fruits pulp (FPE) and seed extracts (SE). Antimicrobial activity of LE, SBE, FPE and SE were assessed by disk diffusion and serial dilution method. All the extracts showed moderate antimicrobial activity against all tested pathogens. Among the extracts LE was comparatively highly sensitive against all tested pathogens. In case of *S. dyesenteriae* and *S. sonnei* zone of inhibition of LE (26 and 28 mm diameter with MIC 31.25 and 62.5 µg/ml; respectively) was higher than the standard Gentamicin (24 and 26 mm diameter respectively). The MIC value of the extracts ranges from 31.25 to 125 µg/ml. All extracts except stem bark showed MIC=MBC against both *S. aureus* and *E. coli*. In conclusion, the spectrum of activity suggests that ethanolic extracts of *A. moschatus* could be a possible source to get noble and potential herbal medicines to treat infections, hence justified the ethnic uses of this plant against various infectious diseases.

INTRODUCTION: Antibiotic resistance has become an alarming issue globally due to exposure of new bacterial strains "Superbugs", which are multi-resistant [1, 2, 3]. Now a day's such multidrug resistance properties of a pathogen have threatened the clinical efficacy of many existing antibiotics [4].

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Due to increase the resistance of antibiotics, there is a continuous and urgent need to discover new antimicrobial agents with diverse chemical structures and noble mechanisms of action for new and re-emerging infectious diseases [5].

Among the potential sources of new agents, plants have long been investigated. Because, plants are rich in secondary plant metabolites (phytochemicals), such as tannins, terpenoids, alkaloids, flavonoids, phenols and quinines, which have been used globally in traditional medicine to treat several infectious diseases [6, 7, 8]. It has been reported that natural products, either as pure

compounds or as standardized plant extracts have multi-antimicrobial properties [9, 10]. While 25 to 50 % of current pharmaceuticals are derived from plants, none is used as antimicrobials [11]. Therefore, researchers have shown their eminence effort to medicinal plants, looking for new leads to develop better drugs against microbial infections.

Abelmoschus moschatus (*A. moschatus* commonly known as Mushkdana/Kasturi bhendi in Bangladesh) belongs to the family malvaceae is a delightful, soft, herbaceous trailing plant, which is 0.5- 2.5 meters high with soft hairy stems and a long slender tap root with triangular lobes leaves. Flowers are regular, bisexual, hibiscus-like, usually watermelon pink but sometimes white or cream, always with a dark center. The roots, leaves, and seeds of *A. moschatus* have valuable folkloric uses as traditional medicines.

The seeds of this plant are used to reduce thirst, cure stomatitis, dyspepsia, urinary discharge, gonorrhea, leucoderma and itch. The seeds are also used against venomous reptiles [12]. The leaf decoction is widely used to control vomiting and intestinal complications, where as tincture of leaf powder is applied for skin diseases. The roots and leaves are considered to cure gonorrhea [13, 14]. Leaves and seeds of *A. moschatus* have been reported to act as antioxidant and anti-proliferative properties [15]. Although, *A. moschatus* has folkloric reputations and has been used widely for several diseases, therefore we investigated several parts of this plant to evaluate the comparative antimicrobial activity against several pathogenic bacteria and fungus, which might be promising source of isolating new lead that can relief our human being from alarming issue "super bugs.

MATERIALS AND METHODS:

Plant collection: Leaves, fruits pulp, seeds and stem barks of *A. moschatus* were collected from Bangladesh Council of Scientific and Industrial Research (BCSIR), Rajshahi-6205, Bangladesh on January, 2016 and were identified by the Department of Botany, University of Rajshahi, where a voucher specimen (Voucher No. CN-15) was deposited. Plant materials were then washed separately with fresh water to remove dirt and other contaminants, and were shade-dried for several days with occasional sun drying. The dried materials were ground into coarse powder by a grinding machine and the materials were stored at room temperature for future use.

Preparation of the extract: The extraction was performed according to Alam *et al.,* (2002) [16]. About 250 g of each powdered plant materials was taken in four amber colored extraction bottles and soaked with 500 mL of 80 % ethanol. The sealed bottles were kept for 15 days with occasional shaking and stirring. The extracts were filtered separately through a fresh cotton plug and finally with Whatman No.1 filter papers. The filtrates were concentrated with a rotary evaporator (Bibby Sterlin Ltd, UK) under reduced pressure at 50°C to afford concentrated leaves extract (LE), fruits pulp extract (FPE), stem barks extract (SBE) and seeds extract (SE).

Test Micro-organisms: Antimicrobial activity of different parts of *A. moschatus* were determined against two Gram positive (*Staphylococcus aureus and Bacillus cereus*) *and* three Gram negative bacteria (*Escherichia coli, Shigella dysenteriae and Shigella sonnei*). Antifungal activity was determined against three fungi (*Aspergillus niger*, *Trichoderma viride* and *Trichoderma harizianum).* All the microorganisms were maintained at 4 °C on nutrient agar slants.

***In-vitro* antimicrobial screening:**

Test for antibacterial activity: *In-vitro* antibacterial activity was carried out on nutrient agar plate by disc diffusion method [17]. The crude extracts of different parts of *A. moschatus* were separately dissolved in 1 ml of ethanol solvent and the filter paper discs (6 mm diameter) were impregnated with known amounts of test substances and prepared as 250 and 500 µg/disc. Discs were placed on agar plate culture of test organisms by sterilized forceps. The inoculated agar plates were left in refrigerator for one hour for proper diffusion. The plates were then allowed to incubate at 37 °C for overnight. Standard disc of Gentamicin (GN) (10 µg/disc) were used as positive control. The antibacterial activity of the test agents was determined by measuring the diameter of zone of inhibition, expressed in mm.

Test for antifungal activity: *In-vitro* antifungal activity of crude ethanolic extract was carried out

on sabouraud dextrose agar plate by disc diffusion method against three pathogenic fungi at a concentration of 250 and 500 µg/disc as described in antibacterial screening section. Standard disk of Clotrimazole (CM) (10 µg/disc) was used as positive control.

Determination of Minimum Inhibitory Concentration (MIC): The minimum inhibitory concentrations (MIC) were performed by a serial dilution technique according to the NCCLS protocol [18]. The extracts were diluted to give the final concentrations of 1000, 500, 250, 125, 62.5, 31.25, 15.62, 7.81, 3.91 µg/ml. 10 µl of 10^7cell/ml of the tested microorganism was inoculated in tubes with equal volume of nutrient broth and plant extracts. MIC was measured in µg/ml after overnight incubation at 37 °C. Three control tubes were maintained for each strain (media control, organism control and extract control). The lowest concentration (highest dilution) of the extract that produced no visible growth (no turbidity) in comparison with control was defined as MIC.

Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentrations (MFC): MBC value was determined by sub culturing the test dilution (which showed no visible turbidity) on to freshly prepared nutrient agar media. The plates were incubated further for 18-42 h at 37 °C. The highest dilution that yielded no single bacterial colony on the nutrient agar plates was taken as MBC. If growth of bacteria is observed in the MIC tubes, it indicated the presence of bacteriostatic agents and in this case MBC>MIC. No growth of bacteria in the MIC tubes after dilution indicates the presence of bactericidal agent and in this case, MIC=MBC.

The minimum fungicidal concentrations (MFC) were determined by sub culturing the test dilution (which showed no visible turbidity) as well as MBC determination. The lowest concentration with no visible growth was defined as MFC indicating 99.5% killing of the original inoculums.

RESULTS: In the present antimicrobial screening, the inhibitory effects of ethanolic extracts of different parts (seeds, leaves, stem bark and fruits pulp) of *A. moschatus* were evaluated against both fungal and bacterial pathogens. **Table 1** and **2** summarizes the microbial growth inhibition of different part extracts of the experimental plant species.

Antibacterial activity: The antibacterial potential of plant extracts was evaluated according to their zone of inhibition against various pathogens and the results (zone of inhibition) were compared with the activity of the standards; GN (10 µg /disc). The results revealed that all the extracts showed potent antibacterial activity against both the Gram positive and Gram negative bacteria but their activity was varied according to different parts of the plant species. A considerable increase in inhibitory effect was found with increased concentration of the sample. All the parts showed increase in activity at 500 µg/disc than 250 µg/disc shown in **Table 1** and **2**. Among the four parts of *A. moschatus* the LE was found to have the comparatively maximum antimicrobial effect against both the tested Gram positive and negative bacterial strain.

The maximum inhibition zone diameter was obtained against *S. aureus* with diameter 20 mm at 250µg/disc and 26 mm at 500 µg/disc by LE in comparison to standard 26 mm at 10 µg/disc. Whereas the SE, SBE and FPE showed 20, 18 and 12 mm zone diameter respectively at 250µg/disc. In case of *B. cereus* SE exhibited maximum inhibition zone diameter 16 and 24 mm at 250µg/disc and 500µg/disc respectively, whereas the standard GN 26 mm at 10 µg/disc. The descending order of susceptibility of *B. cereus* against the extracts were GN> SE> SBE> LE> FPE. More specially, FPE showed very limited potentiality against *B. cereus* **Table 1**.

On the other hand, antimicrobial activity of different parts of *A. moschatus* against Gram negative bacteria was significantly better compare to the standard GN, where some parts showed higher zone of inhibition compared to standard. Maximum inhibition zone diameter 29 mm at 250µg/disc in *E. coli* was shown by SE than other parts of the plants, which also more than the standard GN (26 mm at 10 µg/disc). Among the Gram negative bacteria *S. dysenteriae* was highly susceptible to all parts of this plant species. The SBE and LE showed maximum zone of inhibition with diameter 27 mm and 26 mm at 250µg/disc than the standard GN 24 mm at10 µg/disc.

While the FPE and SE shows 24 mm and 22 mm diameter of zone inhibition at the same concentration. In case of *S. sonnei* at 250 µg/disc concentration, the antimicrobial activity of SE, LE, SBE, FPE and standard GN (10 µg/disc) was 16, 28, 19, 22 and 26 mm respectively. In this case maximum zone inhibition by LE was higher than the standard **Table 1** and **Fig. 1(A-B)**.

TABLE 1: ANTIBACTERIAL ACTIVITY (ZONE OF INHIBITION, MM) OF VARIOUS PARTS OF *ABELMOSCHUS MOSCHATUS* PLANT EXTRACTS AGAINST DIFFERENT MICROORGANISMS

Types of organism	Name of organisms	Zone of inhibition at 250 µg/disc				Zone of inhibition at 500 µg/disc				
		SE	LE	SBE	FPE	SE	LE	SBE	FPE	GN
Gram positive Bacteria	*Bacillus cereus*	16	10	12	8	24	22	20	14	26
	Staphylococcus aureus	20	22	18	12	26	25	23	18	26
Gram negative Bacteria	*Escherichia coli*	29	20	16	18	33	24	22	22	26
	Shigella dysenteriae	22	26	27	24	24	30	31	28	24
	Shigella sonnei	16	28	19	22	26	32	23	26	26

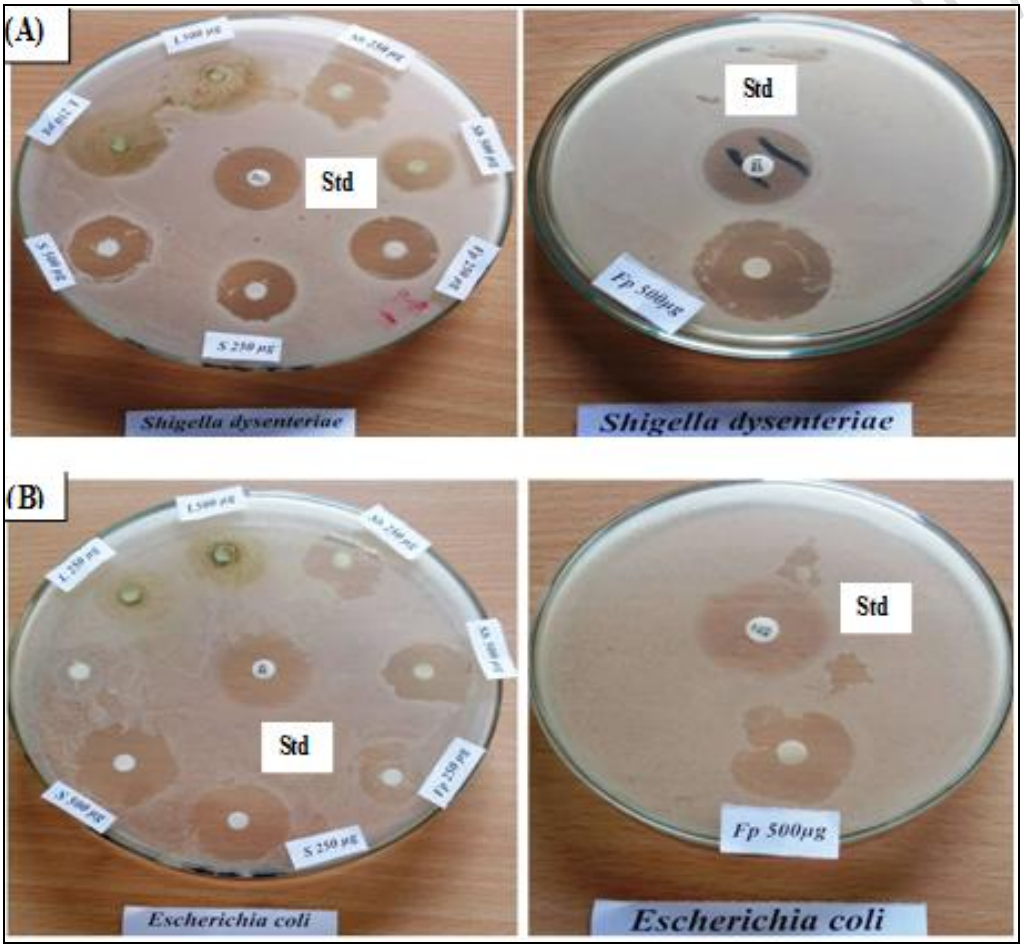

FIG. 1: ANTIMICROBIAL ACTIVITY OF DIFFERENT PARTS OF *A. MOSCHATUS* AGAINST: (A) S. DYSENTERIAE AND (B) *E. COLI*

Antifungal activity: Three fungi were used for antifungal activity test of the crude extracts. All tested fungi were promisingly inhibited by the different parts of *A. moschatus* except SBE which showed no inhibition against *A. niger* presented in **Table 2.** Among the tested fungi the *T. viride* was most susceptible against all parts of the mentioned plant. Where the activity of SE was remarkably comparable to standard and shows highest activity than other parts of the plant species the order of antimicrobial activity according to their zone of inhibition was followings: CM> SE> LE> SBE> FPE. In case of *T. harzianum* the SE, LE, SBE and FPE showed variant degree of zone of inhibition with diameter 18, 16, 20 and 10 mm at 250µg/disc in comparison to standard CM 36 mm at 10 µg/disc shown in **Table 2.**

TABLE 2: ANTIFUNGAL ACTIVITY (ZONE OF INHIBITION, MM) OF VARIOUS PARTS OF *ABELMOSCHUS MOSCHATUS* PLANT EXTRACTS AGAINST DIFFERENT MICROORGANISMS

Types of organism	Name of organisms	Zone of inhibition at 250 µg/disc				Zone of inhibition at 500 µg/disc				
		SE	LE	SBE	FPE	SE	LE	SBE	FPE	CM
Fungus	*Aspergillus niger*	14	22	NA	18	20	26	8	24	32
	Trichoderma viride	25	24	24	16	30	29	28	24	26
	Trichoderma harzianum	18	16	20	10	24	22	23	16	36

Determination of MIC, MBC and MFC values: The MIC, MBC and MFC values obtained for the ethanolic extracts against the tested pathogenic microorganisms was really remarkable as like their zone of inhibition activity. The results shown in **Table 3** and **Fig. 2** reported that ethanolic extract of different parts of *A. moschatus* posses potent antimicrobial agent. There was no remarkable variation of different parts of the plant species among their MIC values but their MBC and MFC values was characteristics in case of different micro-organisms. For instance, LE and FPE showed least MIC value 31.25 µg/ml against *B. cereus* and also the MBC value was higher than their MIC value in comparison to SE and SBE of the plant **(Table 3** and **Fig. 2)**. Interestingly, LE, SBE, FPE and SE exhibited equial MIC and MBC value except SBE of *A. moschatus*. MIC value equal to MBC value represents their bactericidal activity against respective organisms. In case of *S. dysenteriae* and *S. sonnei* the MIC value was 31.25 - 62.5 µg/ml, where MBC value was higher than MIC**.**

The extracts of *A. moschatus* also showed potent antifungal activity as compared to their antibacterial efficacy. The antifungal MIC value was the range between 31.25 to 62.5µg/ml where the extracts antifungal activity was varied against different microorganisms but SBE of *A. moschatus* was not responsive against *A. niger* as like their inhibitory effect. Among the tested fungus except SBE, all other parts of the plant species was highly effective against *T. harzianum* with their lowest MIC value of 31.25 µg/ml but MFC value was higher than their MIC value (**Table 3** and **Fig. 2).**

TABLE 3: MIC (µg/ml), MBC AND MFC PERFORMANCE OF DIFFERENT EXTRACTS OF *ABELMOSCHUS MOSCHATUS* AGAINST PATHOGENIC ORGANISMS

Types of Organisms	Name of organisms	SE		LE		SBE		FPE	
		MIC (µg/ml)	MBC/ MFC	MIC (µg/ml)	MBC/ MFC	MIC (µg/ml)	MBC/ MFC	MIC (µg/ml)	MBC/ MFC
G. positive Bacteria	*Bacillus cereus*	62.5	>62.5	31.25	>31.25	125	125	31.25	>31.25
	S. aureus	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5
G. negative Bacteria	*Escherichia coli*	62.5	62.5	62.5	62.5	62.5	>62.5	62.5	62.5
	S. dysenteriae	31.25	>31.25	31.25	>31.25	62.5	>62.5	62.5	>62.5
	Shigella sonnei	62.5	>62.5	62.5	>62.5	31.25	>31.25	31.25	>31.25
Fungus	*Aspergillus niger*	62.5	>62.5	31.25	>31.25	NA	NA	31.25	>31.25
	T. viride	62.5	>62.5	62.5	>62.5	31.25	>31.25	31.25	>31.25
	T. harzianum	31.25	>31.25	31.25	>31.25	62.5	>62.5	31.25	>31.25

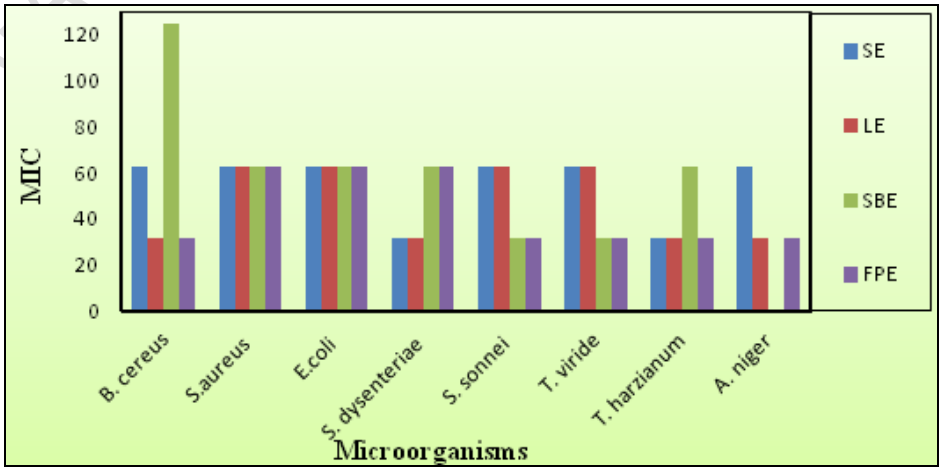

FIG. 2: MIC AGAINST VARIOUS MICROORGANISMS

DISCUSSION: Antimicrobial screening from natural sources has received immense attention of researchers and the antimicrobial activity attributed to some plants in treating diseases has been beyond belief. Now the scientists have directed their efforts to identify compounds that can act as suitable antimicrobial agents to replace synthetic ones. Plant derived photochemical serves as arsenal in controlling the growth of microorganisms due to their availability, fewer side effects and reduced toxicity. These compounds have significant therapeutic application against human pathogens including bacteria, fungi or virus [19]. Numerous studies have been conducted on the antimicrobial activity of different plant extracts. Many plants have been found to cure urinary tract infections, gastrointestinal disorders, respiratory diseases and cutaneous infections [20, 21].

In the present study, extracts of *A. moschatus* was evaluated for the exploration of their antimicrobial activity against certain Gram negative and Gram positive bacteria and fungus which was regarded as human pathogenic microorganism. In this study, for the first time we have attempted to compare antimicrobial activity of different parts of *A. moschatus*. Susceptibility of each part extracts was tested by serial microdilution method (MIC) and agar well diffusion method. Therefore, medicinal plants are finding their way into pharmaceuticals, neutralceuticals and food supplements.

Our preliminary findings showed that ethanolic extracts of different parts of *A. moschatus* were significantly active against some human pathogens like *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, *Shigella dysenteriae* and *Shigella sonnei* as well as few fungus. Previous studies only reported antimicrobial properties of methnolic extracts of flower, leaves and seed extract of *A. moschatus* [15, 22]. Though our results support the previous findings of the antimicrobial results but our findings may consider as a potential candidate for antimicrobial agents on the basis of their antimicrobial comparison.

Furthermore, it was difficult to recognize individual part as highly active principle against all tested pathogens. There was varying degree of antimicrobial susceptibility against various strains of tested pathogens. However, among the four parts mainly the leaf and seed part can be considered as potential extracts that showed significant antimicrobial activity against all tested pathogens. Though, the mechanism of action of these plant constituents is not yet fully known it is clear that the effectiveness of the extracts largely depends on the content and nature of their bioactive compounds that also depends on the type of solvent is used. It is reported that most of the antibiotic compounds already identified in plants are aromatic or saturated organic molecules which can easily solubilized in organic solvents [23, 24].

Our findings also indicate that the ethanolic extract of different parts mostly solubilizes the active compounds which was bacteriostatic and bacteriocidal in nature that shows significant antimicrobial properties. The MIC value of the plant extracts supports their zone of inhibition with the range of 31.25 to 125 µg/ml. In case of *S. aureus* and *E. coli* the MIC (62.5 µg/ml) value was equal to MBC (62.5) value of all parts except stem bark that suggests the presence of bacteriocidal effect. For other tested microorganisms the MIC value was lower than their MBC values **(Table 1, 2** and **3)** suggesting that the plant extracts were bacteriostatic at lower concentration but bactericidal at higher concentration against respective tested pathogens.

CONCLUSION: The current study reveals that ethanolic extracts of different parts of *A. moschatus* showed a broad spectrum and strong anti-bacterial as well as antifungal activity. Different parts were highly active against specific tested microorganisms. The reason of this varying antimicrobial activity was the difference in nature and content of active phytochemicals present in the extracts of *A. moschatus*. The antimicrobial effect was increased by increasing the concentration of the phytochemicals, which could be used as an alternative for existing antimicrobial agents. Therefore, further phytochemical and pharmacological study is necessary to isolate and characterize the bio-active compounds that can appeared as a miracle healing agent for infectious diseases caused by globally concerning multi-resistant human pathogens.

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