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Study of Antibacterial Potentiality of Leaf and Fruit Extracts of *Syzygium jambos* (L.) Alston against five MDR Human Pathogens

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Abstract

The aim of the present study is to evaluate the antimicrobial potentiality of *Syzygium jambos* (L) Alston (Myrtaceae) against five different MDR bacterial strains namely*E.coli, Acinetobacter* spp., *Shigella sonnei, Klebsiella* spp. and *Pseudomonus* spp. collected from the icddr,b.S. jambos is a widespread medicinal plant traditionally used by tribal community in Bangladesh for different ailments. In this study methanol extracts of leaves and fruits of *S. jambos* were tested for antimicrobial activity in vitro by the agar well diffusion method in petri dishes. Both extracts were tested in dose dependent manner ranging from 500 -800µg and activity was measured in terms of zone of inhibition (mm). Both plant parts showed promising activity against all of the tested microorganisms and maximum zone of inhibition was observed at 800µg dose for both extracts. Between the two different parts, methanolic extracts of leaves have exhibited maximum zone of inhibition of 15 mm against *Shigella sonnei* and13.5 mm against *Klebsiella* spp. whereas fruit extract showed maximum zone of inhibition of 10mm against E. coli. This prominent efficacy of leaf extract seems to be related to the higher tannin content.The results of the extracts were compared with the commercial Kanamycin disc (30µg/disc) as standard antibiotic.

Keywords: *Syzygium jambos,* MDR, Zone of Inhibition, *E.coli, Shigella sonnei, Klebsiella* spp.

I. Introduction

Antimicrobial resistance is becoming increasingly important clinical issue in Bangladesh now a day as because several pathogens responsible for acute respiratory tract infections, acutewatery diarrhea, acute trauma and gastrointestinal symptoms have been progressively resistant to most of the commonly used antibacterial drugs once used to treat these infections. Study of isolates of *Pseudomonas aeruginosa* from a tertiary care center in Bangladesh suggested the decreased susceptibility to most of the commonly used antibacterial likecefixime, 93.3%; co-trimoxazole, 93.5%; ceftazidime, 86.8%; gentamycin, 77.3% and ciprofloxacin, 75.5% (A. Rashid *et al.*, 2007). Several enteric pathogens like *Shigella, Vibrio cholerae*, enterotoxigenic*Escherichia coli, Salmonella typhi*have progressively become resistant to most of the widely used and inexpensive antimicrobials. E. coli was resistant in 40% of casesto commonly used antibiotics ceftriaxone,levofloxacin, ciprofloxacin, amoxicillin andampicillin. *Klebsiella pneumoniae* also showed similarphenomena (M. M. Rahman *et al.*, 2004, R. B. Sack *et al.*, 1997). Emergence of *Shigella sonnei* becoming clinically important for infectious diarrhea and ciprofloxacin resistant S. sonnei are being increasingly isolated in Asia (H. C. The *et al.*, 2016, S. K. Das *et al.*, 2013).Theprevalence of *S. sonnei* increased from 7.2% in 2001 to 25% in 2011. *S. sonnei* isolates (biotype g strains) is predominant and resistance to commonly used antibiotics including trimethoprim-sulfamethoxazole, nalidixic acid, ciprofloxacin, mecillinam and ampicillin was 89.5%, 86.5%, 17%, 10.5%, and 9.5%, respectively (A. I. M. S. Ud-Din *et al.*, 2013). *Acinetobacter* isolates from critically ill patients from ICU at a tertiary care hospital in Dhaka shows 100% resistant to amoxicillin, ceftriaxone and gentamicin and 68.4% for amikacin and 66.7% in case of imipenem (A. Nahar *et al.*, 2012). Thus nosocomial and community-acquired infections caused by bacteria that are resistant to more than two classes of conventional antibiotics represent an increasingly important public health concern which necessitates the scientist to search for newantimicrobial substances from various sources including medicinalplants.

Natural products of plant origin have played significant role in the search of therapeutic drugs. Tannin content present in some plants has the ability

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to act against the microorganisms such as *S. aureus, Salmonella species, E. coli, Pseudomonas species* and so on (A. Scalbert, 1991). Several bactericidal plant peptidessuch asfabatin, circulin, cyclopsychotrideetc are discovered with activity toward human pathogens like *E. coli, P. aeruginosa, P. vulgaris* and *K. oxytoca* (E. S. Cândido *et al.*, 2011). A catechin (epigallocatechin-gallate) found in green tea extracts has shown to abolish tetracycline resistance in *staphylococcal* isolates expressing TetK, one of the effluxpumps primarily found in Gram positive bacteria and found to potentiate the activity of β-lactam antibiotics againstmethicillin-resistant *Staphylococcus aureus* (MRSA) (R. G.-Lamothe *et al.*, 2009). However several plants are used in Bangladesh in theform of crude extracts or infusions to treat infections like UTI, TB, pneumonia, and common cold, so on by Kavirajes without scientific validity of efficacy (N. Mahnoor *et al.*, 2015, M. S. Hossan *et al.*, 2010, M. Rahmatullah *et al.*, 2010).

Literaturereview reveals that besides fruits and bark of *Syzygium jambos* (L.) Alston, the most chemico-pharmacological studies were carried out on leave part. Orally administered aqueous, ethyl acetate and methanol extracts of *Syzygium jambos* leaves have been shown to possess anti-inflammatory activity in rat model (K. Slowing *et al.*, 1994). Hydro-alcoholic extracts of *Syzygium jambos* leaves was assessed in rats and remarkable analgesic effects on both cutaneous and deep muscle pain was found that is not mediated by opioid receptors (D. Ávila-Peña *et al.*, 2007). Acetone and aqueous extracts from bark, leaves and seeds of *Syzygium jambos* (L.) were investigated for antimicrobial properties on various microorganisms like *S. aureus, Y.enterocolitica, S. hominis, B. subtilis, E. coli, K. pneumoniae, P. vulgaris, P. aeruginosa, S. typhi* and prominent outcomes have been found with leaves and bark extracts on test microbes (S. Murugan *et al.*, 2011, C. D. Djipa *et al.*, 2000).

Syzygium jambos (L.) Alston is merely a shrub but is generally a perennial tree reaching 7.5-12 m in height, and has a dense crown of slender, wide-spreading branches. In Bangladesh it is naturalized all over the country and commonly known as Jamrul or Golapjam (C. Orwa *et al.*, 2009, A. Ghani, 2003). In Bangladesh leaves are traditionally used by tribal communities like Rakhain,Chakma, Tripura, and Marma (Bangladesh: Syzygium). Leaves are used as paste for pain relief, hot aqueous infusion is drunk for urinary problemsand is also used in sore-eyes, fruits are eaten as remedy for hepatic disorders and juice from bark is used as astringentto the bowels; used in asthma, fatigue and dysentery. The greenish fruits are eaten raw with salt. The ripe fruits are juicier and more flavorful and suitable for eating. The bark is astringent (A. Ghani, 2003, Bangladesh, E. D. of Taxonomy).

In spite of the studies made on antimicrobial properties of bark, leaves and seeds of this plant, noinvestigation was found to be carried on antimicrobial properties of fruit part. Taking into consideration of emerging MDR pathogens in Bangladesh, here the presentstudy was undertaken by undergraduate student of Southeast University, Dhaka, Bangladesh at Microbiology Laboratory to evaluate the efficacy ofantimicrobial activity of methanolic extracts of leaves and fruits of *S. jambos* against five MDR strains of human pathogen namely *E.coli, Acinetobacter* spp., *Shigella sonnei, Klebsiella* spp. and Pseudomonus spp. collected from icddr,b. Theefficacy was compared with the standard commercial antibiotic (Kanamycin) disc and the resultswere discussed.

II. Materials and Methods

A. Plant Materials

The fresh leaves & fruits of *Syzygium jambos* (L.) were collected in the month of July from the Botanical garden, Mirpur and were taxonomically identified from Bangladesh National Herberium, Mirpur, Dhaka, Bangladesh. After collecting fresh plant materials, rash leaves and fruits were first washed with water to remove adhering dirt, then the rough, death leaves and fruits were sorted, and finally these were cut into small pieces. The leaves and fruits were dried under sun for 10 days & finally dried at 45°C for 36 hours in an electric oven. After complete drying, the entire portion was pulverized into a coarse powder with the help of a grinding machine and was stored in an air tight container at room temperature ((30±2°C) until use.

B. Chemicals

All chemicals (analytical grade) including standard Kanamycin disc were purchased from local market.

C. Preparation of Methanolic Extracts

Methanolic extracts were prepared by utilizing maceration technique (N. N. Azwanida, 2015). To obtain the methanol extract, the dried and finely powdered leaf and fruit of *Syzygium jambos*were weighed about 500gm

and were soaked in sufficient amount of methanol in a stoppered flat bottom glass container, then were allowed to stand at room temperature for a period of 7 days with frequent agitation. The process was intended to soften and break the plant's cell wall to release the soluble phytochemicals. After 7 days, the mixture was pressed and strained by filtration with cotton plug and filter paper. Then the extracts were evaporated under reduced pressure using rotary evaporator at room temperature until solid residuesappeared. Residues were then collected in a clean beaker and were kept stand for few hours in ambient air for further evaporation. Residues of extracts were collected in an air tight glass vial and stored in the refrigerator.

D. Microorganisms used and their growth conditions

The test organisms included five MDR bacterial strains of *E.coli, Acinetobacter* spp., *Shigella sonnei, Klebsiella* spp. and Pseudomonus spp.and were collected from the International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b) located in Dhaka, Bangladesh. The pathogens were initially provided in sterile paraffin medium. The test organisms were transferred and grown in nutrient broth medium at 37°c for 18-24 hours and maintained on nutrient agar slants at 4°C under refrigerator for further study.

E. Antibacterial activity of plant extracts: Well diffusion method

Antibacterial activity of the methanolic extracts of leaves and fruits of S. jambos were tested using well diffusion method. A loopful of test organism was transferred from the subculture to a test tube containing 20ml autoclaved medium with the help of an inoculating loop under laminar air flow unit. The test tube was shaken to get a uniform suspension of the organism. The bacterial suspension was immediately transferred to a sterile petri-dish in an aseptic area and was rotated clockwise and then anticlockwise several times to ensure homogeneous dispersion of the organism into the medium. The depth of media into the petridish was approximately 4mm. In the same procedure allpetri-dishes were prepared for test organisms. Aqueous solution of the extracts of leaves and fruits of varying concentrations (500µg to 800µg) were applied on the sterile discs (5mm in diameter) with the help of a micropipette in an aseptic condition. These discs were left for a few minutes in aseptic condition for complete removal of solvent. The sample discs of both leaf and fruit extracts and standard antibiotic discs (Kanamycin, 30µg/disc) were placed gently on the solidified agar plates freshly seeded with the organism with the help of a sterile forceps to ensure complete contact with medium surface. The arrangements of the discs weresuch that the discs were no closer than 15 mm to the plate to prevent overlapping of the zone of inhibition.The plates were kept in a refrigerator in an inverted position for about 24 hours at 4°C. This was sufficient time for the material to diffuse to a considerable area of the medium. Finally, all the plates were incubated at 37°C for 24hours.The diameter of inhibition zones were measured in mm and theresults were recorded.

III. Results

Methanolic extracts of leaves and fruits of *S. jambos* were tested on five MDR human pathogens. The antibacterial activities of methanolic extracts of different plant parts are presented in table 1 and 2. Similarly, the zones of inhibition exhibited by standard antibiotic (Kanamycin) were represented. Both leaves and fruits extracts of *S. jambos* showed good to moderate activity against test microorganisms in a dose dependent manner and highest zone of inhibition was observed at 800µg for both plant parts.

IV. Discussions

The methanolic extracts of leaves and fruits of *S. jambos*, when tested have exhibited impressive antibacterial activity against test microorganisms. Between them, leaves of S. jambos were found to be effective against all test microorganisms with inhibition zone ranging from 9.5 to 15 mm at 800µg dose. When these results were compared it was found that leaf extracts exerts better effect against *Shigella sonnei* and *Klebsiella* spp. with zones of inhibition of 15 mm and 13.5 mm respectively compared to fruit extracts.When E. coli and *Acinetobacter* spp. were tested against both leaf and fruit extracts of *S. jambos*, both plant parts had shown moderate efficacy. E. coli exhibited inhibition zone of 12 mm against leaf extracts compared to 10 mm for fruit extracts. *Acinetobacter* spp. showed zone of inhibition of 10 mm against leaf extract compared to 9 mm against fruit. *Pseudomonas* spp. was also found to be more sensitized to leaf extracts showing inhibition zone 9.5 mm in contrast to 7 mmwith fruit extracts. The maximum sensitized test microorganism found against fruit extracts was *E. coli*.

The organic solvent methanol has the ability to extract the active aromatic orsaturated

organicphytochemicalcompounds especially tannins and other phenolics, saponins which are found to be active against microorganisms (K. Das *et al.*, 2010, N.S. Ncube *et al.*, 2008, M.M. Cowan, 1999, J. N. Eloff, 1998). S. jambos leaves are reported to contain tannins, oleoresins, several alkaloid and flavonoid group of phytochemicals like jambosine, quercetin and myricetin 3-O-β-d-xylopyranosyl (A. Ghani., 2003, K. Slowing *et al.*, 1994). From present study it is clearly evident that the leaf extract had exhibited better antibacterial activity than fruit extract. This may be due to the higher content of antimicrobial tannin in leaves. Tannins are well known factor responsible forantimicrobial activities (S. Murugan *et al.*, 2011). Tannins are quite resistant to microbial attack and are known to inhibit the growth of some microorganisms (M. S. Amiri *et al.*, 2014). On the other hand plant alkaloids and flavonoids were found to possess antibacterial activity to varying degrees (S. Garba *et al.*, 2012, T. P. T. Cushnie *et al.*, 2005). In a literature, ethanol extracts of *S. jambos* leaves was reported to possess antiviral activity on Herpes Simplex type I and inhibited the replication of vesicular stomatitis virus but had no effect on polio virus replication (M. J. Abad *et al.*, 1997). In this context, in present study we have found that methanolic extracts of leaves showed promising antibacterial activity against all test MDR pathogens especially against *Shigella sonnei* and *Klebsiella* spp. Similarly fruit also exhibited moderate antibacterial activity to all test microorganisms, more against *E. coli.*

Synergisticeffects resulting from the combination of extracts and antibiotics were documented where the association of anacardic acid and totarol with methicillinto inhibit strains of *S. aureus* resistant to methicillin (MRSA) was investigated (H. Muroi *et al.*, 1996). In present study the test organisms *Shigella sonnei* and *Klebsiella* spp. included in the study are attributed to be responsible for shigellosis and nosocomial infections respectively (L. B. Bourtchai *et al.*, 2008, R. Podschun *et al.*, 1998)and were susceptible toleaf extracts of S. jambos. The other microorganisms included in the study were also responsible for several life threatening infections. So this clearly indicates that both plant parts especially leaf extracts/powders can be used to treat patients with such infections in conjunction with antibiotics reported to be less sensitized against these microorganisms for synergistic effects.

V. Conclusions

Based on the results of the present study, we conclude that the methanolic extracts of leaves of *Syzygium jambos* exerts promising antimicrobial effect rather than fruit extracts against all test MDR microorganisms. The observed activities seem to be generally related to the total tannin content of leaves though all the strains do not seem to have the same sensitivity to the tannins present in the leaf. It is evident from the present study that both fruit and leaf extracts of *S. jambos* contains phytochemicals which are active against the test microorganisms. Further extensive phytochemical studies and isolation of individual compound are required to determine the type of compounds responsible for the antimicrobial effect of the plant and toxicity tests can be carried out before the drug development process using leaves and fruits of *S. jambos*.

Table 1: Antibacterial activity of methanolic extract of *Syzygium jambos* leaves

Test microorganisms	**Zone of inhibition (mm) in different dose of leave extracts (μg)**				
	500μg	**600μg**	**700μg**	**800μg**	**StandardKanamycin (30μg)**
E.coli	5	8	9	12	33
Acinetobacter spp.	4	7	8.5	10	31
Shigella sonnei	6	9	12	15	29
*Klebsiella*spp.	7	8	10	13.5	30
*Pseudomonas*spp.	6	7	8	9.5	28

Table 2: Antibacterial activity of methanolic extracts of *Syzygium jambos* fruits

Test microorganisms	Zone of inhibition (mm) in different dose of fruit extracts (μg)				
	500μg	600μg	700μg	800μg	Standard Kanamycin (30μg)
E.coli	5	7	8.5	10	28
Acinetobacter spp.	4.5	6	8	9	29
Shigella sonnei	4	7	8	9	32
Klebsiella spp.	6	8	7	8.5	27
Pseudomonas spp.	5.5	6	6.5	7	30

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