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Screening of Antibacterial Activity of *Syzygium Jambolanum* (Jamun) Seed Extract

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Abstract—*Medicinal use of Syzygium jambolanum is known to mankind since antiquity. This species, from the myrtle family (Myrtaceae), has been used to treat illnesses caused by bacterial, fungal and viral pathogens, ulcers in genitourinary tract caused by* Candida albicans*, as well as cold, cough, fever and skin problems such as rashes in the mouth, throat and intestines. It is also used, in a mix with honey or milk, to treat diabetes and digestive diseases and the fresh fruits has been taken orally to treat stomach ache. Syzygium jambolanum has been found to possess antibacterial properties. The purpose of this study was to investigate the antibacterial activity of Methanol, ethyl acetate and aqueous extract of Syzygium jambolanum seeds against dominating bacteria isolated from herbal drugs which was Escherichia coli & Bacillus cereus by Disc diffusion method, Minimum Inhibitory Concentration, Minimum Bactericidal Concentration. It was found that Methanolic extract showed the highest zone diameter (18.6mm) followed by ethyl acetate (7.6mm) and aqueous extract (5mm) respectively. In case of E. coli, highest activity was exhibited by methanolic extract (10mm) followed by ethyl acetate (6mm). Aqueous extract showed no activity. Streptomycin and distilled water were used as positive and negative control. Hence these extract can be add into herbal drugs where they can work as preservative and antibacterial agent in herbal drugs.*

Keywords: *Syzygium jambolanum, Antibacterial property, herbal drugs, disc diffusion method, Minimum Inhibitory Concentration, Minimum Bactericidal Concentration.*

1. INTRODUCTION

Medicinal plants serve as important therapeutic agents as well as valuable raw materials for manufacturing numerous traditional and modern medicines. These plants naturally synthesize and accumulate some secondary metabolites, like alkaloids, sterols, terpenes, flavonoids, tannins, quinines and volatile oils. *Syzygium jambolanum* DC (Holland) is widespread in India, Ceylon, Malaysia and Australia. *S. jambolanum* seeds has been used to treat illnesses caused by bacterial, fungal and viral ulcers in genitourinary tract caused by *Candida albicans*, as well as cold, cough, fever and skin problems such as rashes and the mouth, throat and intestines. In India, it has been used, in a mixture with honey or milk, to treat diabetes and digestive diseases and the fresh fruits has been taken orally to treat stomachache. Present studies was planned to evaluate the antibacterial activity of seeds of *S. jambolanum.*

2. MATERIALS AND METHODS

2.1 Collection of plant materials

Seeds were collected from tree located in Haridwar and its adjoining areas. Plant materials were identified with the help of taxonomic literature, standard flora and herbarium available in the library of Gurukul Kangri University, Haridwar. These plant materials were washed with water, then surface sterilised and were air dried at room temperature. The samples were ground into a fine powder and then used for evaluation of their antimicrobial potential against selected contaminants found in herbal drugs[3]

2.2 Preparation of plant extracts

2.2.1 Aqueous extract

The plant materials (40g) with distilled water (500ml) were placed in orbital shaker for 36 hours. After which it was filtered with buchner funnel with whatman no. 1 filter paper. The filtrate was concentrated using a rotary evaporator upto 40ml [5]. It was cooled and stored in a glass vials with screw capped at 4°C for further analysis.

2.2.2 Soxhlet extraction

Air dried powder (40g) of the respective plant was thoroughly mixed with 500 ml organic solvent (methanol, ethyl acetate) and subjected in soxhlet apparatus and left for 36 hours. After 36 hr, the extract was further evaporated using rotatory evaporator up to 40 ml. It was cooled and stored in a glass vials with screw capped at 4°C for further analysis.

2.2.3 Extraction of essential oil

The components were extracted for the essential oil as follows. Two hundred and fifty grams of the powdered leaves was put in a round bottom flask, 1000 ml of distilled water

was added and then subjected to hydro distillation in a modified Clevenger apparatus for 8 hours. The oil recovered was dried over anhydrous sodium sulphate. Extracted oil was stored in glass bottle covered with aluminium foil to prevent the effect of sunlight and kept in the refrigerator at 4°C before use [1].

2.3 Microorganisms used

Two Bacteria ***Escherichia coli*** **strain KR4.11(**GenBank Accession Number: **JQ912539.1**) and ***Bacillus cereus*** **strain Fd**(GenBank Accession Number: **KC510288.1)** were used**.** Bacteria were isolated from herbal drugs and preparation through serial dilution method and were identified by 16SrRNA sequencing from **Xcelris Labs Ltd., Ahmedabad.** *In vitro* antibacterial activity was determined by using Mueller Hinton Agar and Mueller Hinton Broth.

2.4 Antibacterial activity

2.4.1 Preparation of bacterial inoculum

Muller Hinton Broth (5ml) was inoculated with 4-5 similar colonies, broth was incubated at 35-37°C for 2-8 hours until its turbidity matched with that of standard 0.5McFarland (0.5ml of 1.175% barium chloride and 99.5ml of .36Nsulfuric acid)[4].

2.4.2 Disc diffusion method

1 100µl of actively growing culture (0.5 McFarland standard) was transferred to Muller Hinton plate. Culture was inoculated by spread plate technique with the help of spreader, allowed it to dry for 5-15 minutes. Sterile paper discs (6 mm diameter) prepared from Whatman Number 1 filter paper were impregnated with the plant extracts. Now disc was placed on seeded agar plate. Now plates were incubated for 24hours at 37°C. After incubation zone of inhibition was measured. Streptomycin (10mg/ml) was taken as positive control. Distilled water was used as negative control [2].

2.5 Micro Broth Dilution Assay

2.5.1 Minimum Inhibitory Concentration (MIC)

Broth dilution assay was done in 96 wells microtiter plate. Broth was added in each well in same amount (150µl). Now two fold serial dilutions of selected plant extract was made in broth ranging from 0.15 to 80mg/ml. 50µl of inoculum was added to each well. The final volume in wells remained 200µl. Plate was incubated at 37°C. After incubation, lowest concentration of plant extract (MIC) that inhibit the growth of microorganism was determined.

2.5.2 Minimum bactericidal concentration (MBC)

All the wells used in the MIC study which did not show any growth of bacteria were then subcultured on to the surface of the freshly prepared Mueller Hinton Agar plates and incubated in incubators at 37°C for 24 h. The MBC were recorded as the lowest concentration of the extract that did not permit any visible bacterial growth on the agar plate after the period of incubation.

3. RESULTS

Results showed that the methanol and aqueous extracts of *S. jambolanum* seeds possessed antibacterial activities against the microorganisms isolated from herbal drugs. The extracts were assayed against the test organisms by disc diffusion methods (Table 1). **Methanolic** extract showed the highest zone diameter (**18.6mm**) followed by **ethyl acetate** (**7.6mm**) and **aqueous** extract (**5mm**) against *B. cereus*. Essential oil showed least activity (**1.3mm**) i.e. *B. cereus* was resistant to essential oil. In case of ***E. coli,*** highest activity was exhibited by **methanolic** extract (**10mm**) followed by **ethyl acetate (6mm**). Aqueous extract and essential oil showed no activity. Negative control (water) did not inhibit any of the microorganisms tested.

Bacillus cereus, methanolic extract of *S. jambolanum* exhibited lowest MIC (**2.5 mg/ml**) and MBC (**5mg/ml**). For *E. coli*, methanolic extract of *S. jambolanum* exhibited lowest MIC (**5mg/ml**) and MBC (**10mg/ml**). Hence it is found that Methanolic extract of *S. jambolanum* exhibited lowest MIC against both the pathogens *Bacillus cereus* and *E. coli* which were **2.5mg/ml** and **5mg/ml** respectively. MBC against both the pathogens were **5mg/ml** and **10mg/ml**(Table 2).

4. DISCUSSION AND CONCLUSION

Herbal medicine has a long history and is quite popular among many people, particularly Asians and Northern Europeans. The consumption of medicinal plants and herbal products has been somewhat surprising in recent years, constituting a promising pharmaceutical segment. This fact is primarily noted as a result of easy access, low cost and its popular or cultural compatibility (Tyler, 1996). Present study was aimed to find out the occurrence of contamination in herbal drugs and preparations and to determining the antimicrobial activity of selected medicinal plant extract and oil. Results of antibacterial activity showed that best activity was given by **methanolic** extract of ***S. jambolanum*** against *E. coli* (10mm), *Bacillus cereus* (18.6mm). This study revealed that tested plants have antimicrobial potential and can be added in liver tonic without having any side effect. *S. jambolanum* is famous as an antidiabetic agent which has direct connection with liver. *S. jambolanum* can be added in liver tonic, so in this way these plants can solve patient's problem and improve quality of herbal drug and preparation by increasing the

shelflife and reducing the chance of contamination. Hence **plant extracts of *S. jambolanum* can be added to herbal drugs as antimicrobial agent and as preservative.**

Table 1: Antibacterial activity of *S. jambolanum* seeds against tested bacteria

Bacteria	Mean zone of inhibition (mm)a				
	Me	Aq	Et	Eo	Pc
E. coli	10± 1	-	6 ± 2	-	14±1.5
B. cereus	18.6±1.1	5±1	7.6±0.5	1.3±0.5	9±1

*** a – average of three replicates, Me- methanol, Aq- Aqueous, Et- Etyl acetate, Eo- Essential oil, Pc- Positive Control (streptomycin), ±- Standard deviation**

Table 2: MIC and MBC of methanol extract of *S. jambolanum* seeds tested bacteria

-	MIC(mg/ml)	MBC(mg/ml)
E. coli	5.0	10
B. cereus	2.5	5

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