Antimicrobial Activities of Seed Extracts of Mango (*Mangifera indica L.);* **Jamun** *(Syzygium cumini)*, **or black plum; Karela** **(***Momordica charantia***)*or bitter gourd*** **and Neem** *(Azadirachta indica)* **or Margosa**

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Abstract- The use of and search for drugs and dietary supplements derived from plants have accelerated in recent years. Ethnopharmacologists, botanists, micro-biologists, and natural-products chemists are searching for new phytochemical entities which could be developed for treatment of infectious diseases. Traditional healers have long used plants to prevent or cure infectious conditions.

The present study aimed to investigate the *in vitro* antimicrobial activities of methanol and ethanol extracts of mango, Jamun, Neem and Karela seeds against gram positive, gram negative bacteria and *Candida albicans*. Seed were extracted by Soxhlet using methanol and ethanol as solvents. The extracts were tested against the microorganisms using disc diffusion method at different concentrations: 5 mg/mL, 4 mg/mL, 3 mg/mL, 2 mg/mL, 1 mg/mL and 0.5 mg/mL). *In vitro* antibacterial activities of methanol and ethanol extracts of all seeds showed inhibitions to tested organisms with variable inhibition zones. Resistance among the tested strains was shown in low concentration of the extract. The mean zone of inhibition produced ranged between 8 mm and 18 mm. *Staph aureus* showed the highest zone of inhibition (18 mm) followed by *Candida albicans* (16 mm). The methanol followed by ethanol extracts of Jamun seeds showed good inhibitory effects against almost all tested strains. The inhibition zones produced by seeds extract were less than those produced by standard positive control drug. This could be due to low diffusion rate of seeds extract in agar medium. These plant products can be a

potential new and promising antimicrobial therapy in infectious diseases.

Keywords: Antimicrobial Activities, Mango (*Mangifera indica* L.), Jamun *(Syzygium cumini)*, Karela (*Momordica charantia)*, Neem *(Azadirachta indica)*

Introduction

The use of and search for drugs and dietary supplements derived from plants have accelerated in recent years. Ethnopharmacologists, botanists, microbiologists, and natural-products chemists are combing the Earth for phytochemicals and "leads" which could be developed for treatment of infectious diseases. While 25 to 50% of current pharmaceuticals are derived from plants, none are used as antimicrobials. Traditional healers have long used plants to prevent or cure infectious conditions; Western medicine is trying to duplicate their successes. Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found *in vitro* to have antimicrobial properties.

Due to report of increasing developments of drug resistance in human pathogen as well as undesirable side effects of certain antimicrobial agents, it is necessary to search for new agents that are better, cheaper and without side effect for treating infectious diseases especially in developing countries. A wide variety of plant/natural products are used in the treatment of infections.

Phytoconstituents have been found to inhibit bacteria, fungi, viruses and pests[1]. There have been several reports on the pharmacological effects and suitability of medicinal plants as phytotherapies for diseases. Seeds extracts has been reported to possess anti-inflammatory, analgesic and immunoprotective effects[2,3].

Methanol and ethanol extract of different plant seeds was done against 4 bacterial strains. The presence of phytoconstituents in the seeds extracts may be responsible for the antibacterial activity of the plant[1]. No research has been carried out on antimicrobial activity of seeds extract of mango, jamun, karela and Neem In this study, the in vitro antimicrobial activity of methanol and ethanol extracts of the seeds of mango, jamun, karela and Neem was investigated.

Material and Methods

Plant seeds Collection and Identification

Fresh seeds of all plants were collected from the HWC Dehradun unit Uttarakhand, India and were identified and authenticated in the Department of Pharmacognosy. The collected seeds were kept at plastic bags at room temperature till use.

Extraction of Seeds

1000 grams of air dried and coarsely powdered of clean seeds were extracted in Soxhlet apparatus to obtain methanolic and ethanolic extracts. The extracts were filtered, and the filtrates were vaporized to dryness, and weighed in order to determine the % yield of the extracts, following the formula: % yield = (weight of extract/weight of ground plant material) × 100. The stock solutions of the crude methanolic and ethanolic extracts were prepared by dilution the dried extracts with 50% methanol and 50% ethanol to obtain the desired final concentrations of: 5 mg/mL, 4 mg/mL, 3mg/mL, 2 mg/mL, 1mg/mL and 0.5 mg/mL. These concentrations were used to impregnate filter paper disks (5.5 mm diameters). Disk impregnated into 50% methanol and 50% ethanol was used as control, while standard antimicrobial discs of ciprofloxacin was used as positive control.

Preparation of Extracts

The extraction was carried out using methanol and ethanol (separately). 400 gm each of dry seeds were extracted with 80% methanol till the color of the solvent returned colorless.

Solvent was evaporated under reduced pressure using Rotary evaporator apparatus (BUCHI Rotavapor R-200/ 20). Extracts were finally allowed to dry at air at room temperature till complete dryness. Extraction using ethanol followed the above procedures.

Assay for Antimicrobial Activity

The original extracts were subject to serial dilution was made as follows: 5 mg/mL, 4 mg/mL, 3mg/mL, 2 mg/mL, 1mg/mL and 0.5 mg/mL. Filter discs (5.5/mm) were made and impregnated into each of the above dilutions. The discs were dried at 37˚C for one hour. The dried discs were therefore having the following concentration: 5 mg/mL, 4 mg/mL, 3mg/mL, 2 mg/mL, 1mg/mL and 0.5 mg/mL**.**

Bacterial and Fungal Strains

Bacterial strains and *Candida albicans* recovered from frozen stocks at Department of Microbiology HWC Dehradun unit. One to three loopful of 24 h old cultures from each test strains were used to prepare 0.5 McFarland standard suspensions. Mueller Hinton's agar (Hi Media) plates were used the in vitro antimicrobial testing as recommended by clinical and Laboratory Standards Institute[13]. Then impregnated dried discs plus positive and negative control discs.

Results and Discussion

Antimicrobial Activity

The antimicrobial activity of all seeds extract against the test organism is shown in Table 1-4. The jamun seeds extract

showed potent antibacterial and anticandidal activity against all strains tested[4]. *Staphylococcus aureus* was the most susceptible strain amongst test bacteria followed by *Candida albicans* showed the diameter of zones of inhibition 18mm and 16 mm respectively (Table-2). After gram positive *Staph aureus E.coli* was the most susceptible test strain showed the zone size of 16 mm. Methanol was most strong solvent than ethanol. It is revealed from this study that *Syzygium cumini seed extract* possess remarkable antibacterial and anticandidal activity.

Table-1 Antimicrobial activity of Seeds extracts of *Mangifera indica*

Test organisms	ATCC No.	Extract concentrations						- ive control	+ tive control
		5mg/ml	4 mg/ml	3mg/ml	2 mg/ml	1mg/ml	0.5mg/ml		
		ME/EE	ME/EE	ME/EE	ME/EE	ME/EE	ME/EE		
Staph aureus		14/12	13/11	12/10	10/8	8/R	R	R	25
E.coli		12/10	11/10	10/8	8/R	8/R	R	R	23
Pseudomonas aeruginosa		13/12	12/10	10/R	8/R	R/R	R	R	23
Salmonella sp.		10/8	10/R	10/R	8/R	R/R	R	R	23
Candida albicans		13/11	12/11	10/10	10/8	10/8	R	R	26

***ME- Methanol, EE- Ethanol and R- Resistant**

Table-2 Antimicrobial activity of Seeds extracts of *Syzygium cumini*

Test organisms	ATCC No.	Extract concentrations						- ive control	+ tive control
		5mg/ml	4 mg/ml	3mg/ml	2 mg/ml	1mg/ml	0.5mg/ml		
		ME/EE	ME/EE	ME/EE	ME/EE	ME/EE	ME/EE		
Staph aureus		18/14	16/12	14/11	12/10	11/8	8/8	R	25
E.coli		16/14	15/13	13/13	11/8	10/R	R	R	23
Pseudomonas aeruginosa		15/12	14/10	12/8	11/8	10/R	R	R	23
Salmonella sp.		13/10	12/10	11/8	10/8	8/R	R	R	23
Candida albicans		16/14	14/12	13/10	13/8	12/8	10/R	R	26

***ME- Methanol, EE- Ethanol and R- Resistant**

Table-3: Antimicrobial activity of Seeds extracts of *Momordicha charantia*

Test organisms	ATCC No.	Extract concentrations						- ive control	+ tive control
		5mg/ml	4 mg/ml	3mg/ml	2 mg/ml	1mg/ml	0.5mg/ml		
		ME/EE	ME/EE	ME/EE	ME/EE	ME/EE	ME/EE		
Staph aureus		12/11	10/8	R/R	R/R	R/R	R/R	R	25
E.coli		10/8	8/R	R/R	R/R	R/R	R/R	R	23
Pseudomonas aeruginosa		8/R	8/R	R/R	R/R	R/R	R/R	R	23
Salmonella sp.		10/8	8/8	R/R	R/R	R/R	R/R	R	23
Candida albicans		11/8	10/8	R/R	R/R	R/R	R/R	R	26

***ME- Methanol, EE- Ethanol and R- Resistant**

Table-4: Antimicrobial activity of Seeds extracts of *Azadirachta indica*

Test organisms	ATCC No.	Extract concentrations						- ive control	+ tive control
		5mg/ml	4 mg/ml	3mg/ml	2 mg/ml	1mg/ml	0.5mg/ml		
		ME/EE	ME/EE	ME/EE	ME/EE	ME/EE	ME/EE		
Staph aureus		14/13	12/12	12/10	10/8	8/8	R/R	R	25
E.coli		12/10	11/9	11/8	10/R	8/R	R/R	R	23
Pseudomonas aeruginosa		12/11	11/10	10/8	10/R	8/R	R/R	R	23
Salmonella sp.		10/8	8/R	R/R	R/R	R/R	R/R	R	23
Candida albicans		14/12	12/10	12/8	10/8	8/R	R/R	R	26

***ME- Methanol, EE- Ethanol and R- Resistant**

Many sections in the rural population rely on medicinal plants and folklore healing methods for primary health care[1,8]. While 25% to 50% of current pharmaceuticals are derived from plants, none are used as antimicrobials.

Traditional healers have long used plants to prevent or cure infectious conditions[7]. Medicinal plants constitute an effective source of both traditional and modern medicines, but assessment of antimicrobial potential of these sources is essential. The present study found a very promising and readily available source for treating infections caused by bacteria and fungi. This is particularly significant because drug resistance to human pathogens has been increasing not only in the developing countries but throughout the world due to indiscriminate use of antibiotics[8]. The drug resistance bacterial and fungal pathogens have further complicated the treatment of infectious diseases in immunocompromised AIDS and cancer patients[9]. In the present scenario due to emergence of multiple drug resistance to human pathogenic bacteria and fungi, especially the antibiotic penicillins, cephalosporins and chloromphenicol types involve the enzymic inactivation of the antibiotic by hydrolysis or by the formation of an active derivative. This has opened a new vista for the search of new antimicrobial substance.

The results of the present study revealed wide ranges of inhibition zones from as low as 8 mm up to 18 mm. Both methanol and ethanol extract of the seeds extract showed zones of inhibitions in most strains tested. These levels of activities is promising. More application on these products will ensure its wide applicability and their bacteriostatic or bactericidal, fungistatic or fungicidal actions. It is clear that further research will be needed to cover a wide range of bacteria including multidrug (MDR) resistance ones. In this study *Staphylococcus aureus* was inhibited by

these extracts, this could open way for testing MDR organisms and to determine the MIC concentration of these extracts.

In addition in the present study we tested the methanolic extract of seeds and it showed potent antimicrobial activities. Ethanol extract is more likely to be selected for further pharmaceutical experimentation for human and animal use. This is because methanol is risky due to its high toxicity and not applicable for usage. It is noticed in this study, as expected, that inhibition of microorganisms is directly proportional with the concentration of the extract. The use of Mueller Hinton's agar along with a unified inoculums size (0.5 McFarland) follows the standard methods[6]. But is it expected that antimicrobial activity and inhibition zone diameter might have been affected by factor related to its diffusion in agars such as the Mueller Hinton's.

In the present study both ethanolic and methanolic were investigated. Both solvents are low molecular weight alcohols, polar compound and shows very little difference in their extractive abilities. But ethanol extraction is more convenient and non toxic for the biological purpose. Ethanol extraction shows little increase in the yield percentage, zone of inhibition of ethanol extract shows little increase than methanol extract when treated with different microorganisms and Methanol extraction which we have done for comparative studies for our knowledge.

Conclusion

In the present study encouraging results have been produced with both ethanolic and methanolic extracts.From the present screening, it could be concluded that the seed of *Syzygium cumini* are more potent antimicrobial agent than other extracts and could be compared to the known antibiotics. Further, the detailed phytochemical research is required to identify the active principal responsible for aforementioned activities and testing wider range of organisms would be encouraged.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the product.

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