International Journal of Allied Medical Sciences and Clinical Research (IJAMSCR)

IJAMSCR |Volume 2 | Issue 3 | July-Sep - 2014
www.ijamscr.com

Research article

Comparative study of anti bacterial activity of barks, leaves and flesh extracts of *Moringa oleifera L*

S.Sundar*, L.Anusha, P.Hema, B.Thireesha, K.Sri Teja, V.Susannalatha kumara.
Department of Biotechnology, Vijaya Institute of Pharmaceutical Sciences for Women, Vijayawada, Andhra Pradesh-521108, India.

ABSTRACT

In the present study, comparative anti bacterial activity of barks, leaves and flesh extracts of *Moringa oleifera L* was evaluated with different pathogenic gram positive and gram negative microorganisms. Twelve extracts of barks, leaves and flesh of *Moringa oleifera L* were prepared with four solvent such as alcohol, ethyl acetate, benzene and acetone. The combinations of extracts were prepared in 1:1 ratio. Antimicrobial activity was evaluated by agar disc diffusion method. All the extracts showed the anti microbial activity against the microorganisms. According to the zone of inhibition observed in the entire agar plates, the ethyl acetate extracts of Moringa oleifera (Leaf and Bark), (Bark and Flesh) showed maximum zone of inhibitions 20 mm, 17 mm against Micrococcus luteus respectively. The alcoholic extract (Leaf and Flesh) showed zone of inhibition 19 mm against Bacillus Subtilis and (Bark and Flesh) extract showed zone of inhibition 18 mm against the pseudomonas aeuroginosa. The acetone extract of Moringa oleifera showed lowest antimicrobial activity when compare to the other solvents extracts. From these studies concluded that, ethyl acetate extract of Moringa oleifera showed good antimicrobial activity than other extracts. Further the analysis of active ingredients present in this plant responsible for antimicrobial activity is important for the further development of new drugs.

Keywords: Anti microbial activity, *Moringa oleifera L* extracts, Agar disc diffusion, Pathogens.

INTRODUCTION

Plants have played a significant role in maintaining human health and improving the quality of human life for thousands of years and have served humans well as valuable components of medicines, seasonings, beverages, cosmetics and dyes. Herbal medicine is based on the premise that plants contain natural substances that can promote health and alleviate illness. In recent times, focus on plant research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems[1]. A number of ancient cultures wrote on plants and their medical uses. In ancient Egypt, herbs are mentioned in Egyptian medical papyri, depicted in tomb illustrations, or on rare occasions found in medical jars containing trace amounts of herbs [2].

Reported that while 1,625 species of plants have been used by various Native American groups as food, 2,564 have found use as drugs. According to his calculations, this eliminates approximately 18,000 species of plants which were used for neither food nor drugs. Speculations as to how and why a selected number of plant species came into use for either food or drugs is fascinating but outside the scope of this review [3]. The documentation of herbs and their uses was a central part of both Western and Eastern medical scholarship through to the 1600s, and these works played an important role in the development of the science of botany [4].

Moringa oleifera has number of pharmaceutical applications such as Antioxidant[5], Antimicrobial[6], Anti-fertility[7], Anti-hepatotoxic[8], Antiulcer[9], and Antispasmodic[10]. As a consequence, this plant

***Corresponding author: S.Sundar**
E-mail address: **presundar@yahoo.co.in**

shows beneficial effects on asthma, pain, and other resultant symptoms[11]. Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen-substituted derivatives. Most are secondary metabolites, of which around 12,000 have been isolated, a number estimated to be less than 10% of the total. In many cases, these substances serve as plant defense mechanisms against predation by microorganisms, insects and herbivores. Eugenol is a well-characterized representative found in clove oil and is considered as bacteriostatic against both fungi and bacteria[12]. Similarly the number plant compounds possess the anti microbial activity such as Flavones, Catechin compounds, Tannins, terpenoids.

MATERIALS AND METHODS

Plant Material

Different parts of *Moringa oleifera L* including barks, leaves and flesh were collected from a locality in Vijayawada (Andhra Pradesh).

The Common Pathogenic Microorganisms

The Common pathogenic six microorganisms were used in the study, among these three gram -ve microorganisms namely Escherichia Coli (NCIM 2256), Pseudomonas aeruginosa (NCIM 2037), Serratia marcescens (NCIM 2078) and three gram +ve microorganisms namely *Micrococcus Luteus* (NCIM 2871), Bacillus Subtilis (NCIM 2710), Staphylococcus aureus (NCIM 2794), All the tested strains are reference strains, and were collected from National Collection of Industrial Microorganism (NCIM).

Preparation of Plant extraction

Freshly collected plant materials such as leaves, barks and flesh of *Moringa oleifera L* were thoroughly washed under tap water followed by sterile water separately. The washed plant materials were dried independently in shade followed by grinding in to a fine powder. The powdered plant materials of *Moringa oleifera L* were stored in air tight jars and refrigerated separately at 4ºC. For phytochemicals extraction, 15 gm of dry powdered leaves of *Moringa oleifera L* was extracted with 125 ml of ethyl acetate by soxhlet's apparatus for 6 hr (or) till the plant material get colourless.

The solvent was removed using a rotary vacuum evaporator to give a concentrated extract. In the same method followed for the extraction of *Moringa oleifera L* leaves with alcohol, acetone, and benzene solvents also. Totally twelve extracts of *Moringa oleifera L* were prepared with different solvents. Then the combinations of extracts were prepared by mixing the extracts in 1:1 ratio.

SCREENING OF ANTIMICROBIAL ACTIVITY

Media for test organisms

15.2 g of Mueller Hinton Agar was added to 400 ml of sterile distilled water and autoclaved at 121^0C for 15 mins at 15 lbs. 1.0 g of dextrose was added to 10 ml of sterile distilled water and steam sterilized for 15 mins. After cooling both the contents were mixed and poured into sterile petriplates upto approximately 4 mm and allowed to set at ambient temperature and used.

Preparation of Inoculum

To prepare bacterial inoculum, pure culture of test organism was inoculated into 5 ml of sterile nutrient broth and incubated at 37°C for 2 to 8 hr till moderate turbidity developed. The inoculum was standardized by matching with 0.5 McFarland turbidity standards, which corresponds to cell density approximately 10^8 CFU/ml.

Antimicrobial activity by agar disc diffusion method[13]

Antimicrobial activity of each plant extracts was determined by using a modified Kirby Bauer disc diffusion method. Briefly, broth cultures of test bacteria were spreaded on the Mueller Hinton Agar media in petriplates and microbes broth culture were applied on media by swabbing, under lab condition. The extracts were tested using 5mm sterilized filter paper discs which impregnated with the test samples (ethyl acetate, alcohol, acetone and benzene) allowed to dry for few minutes at room temperature, plates were incubated at 37^0C about 24 hr. Then, the diameters of the Inhibition zones were measured in mm and results were recorded.

RESULTS AND DISCUSSION

All the extracts showed varying degrees of antimicrobial activity. The results were expressed as mean ± standard deviation. Ethyl acetate extract showed maximum zone of inhibition against micrococcus luteus and acetone extract showed lowest activity against pseudomonas aeruginosa.

Benzene extract of leaves, barks, flesh of Moringa oleifera against Escherichia coli ranges from 5 mm to 12 mm and staphylococcus aureus ranges from 5 mm to 19 mm, Serratia marcescens ranges from 5 mm to 14 mm, and pseudomonas aeruginosa ranges

from 5 mm to 11 mm whereas against bacillus subtilis shows 5 mm to 12 mm at 100 mg.

Table- 1 Antimicrobial Activity of Ethyl Acetate Extracts of Different Parts of *Moringa oleifera L*

S.No	Plant parts	Micrococcus luteus (mm)	E.coli (mm)	S. aureus (mm)	Serratia marcescens (mm)	Pseudomonas aeruginosa (mm)	Bacillus subtilis (mm)
1	Leaf + Bark	20	13	5	17	5	6
2	Bark +Flesh	17	13	5	12	5	5
3	Leaf +Flesh	12	17	5	13	5	8
4	Drug	22	8	18	12	9	19
5	Solvent	5	5	5	5	5	5

Figure - 1. The zone of inhibition of ethyl acetate Extract of Moringa oleifera

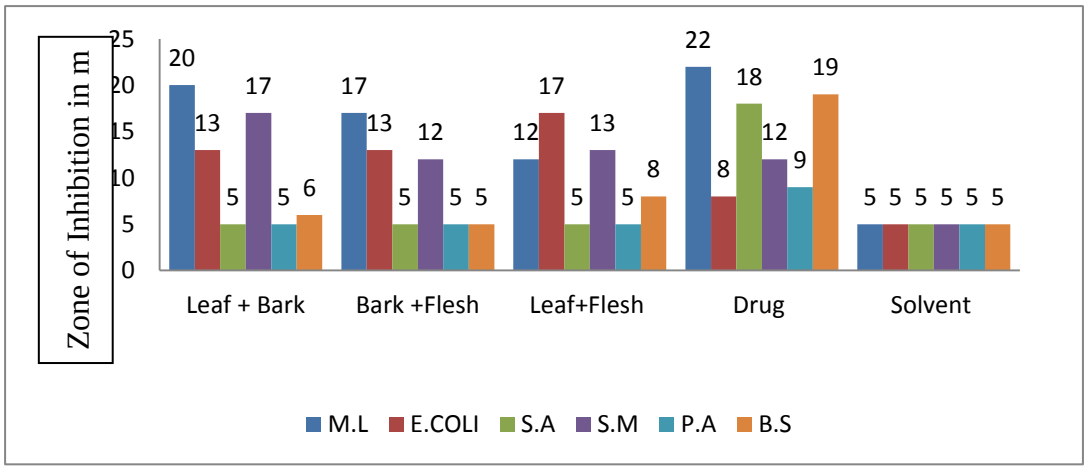


Table -2 Antimicrobial Activity of Alcoholic Extracts of Different Parts of *Moringa oleifera L*

S.No	Plant parts	Micrococcus luteus (mm)	E.coli (mm)	S. aureus (mm)	Serratia marcescens (mm)	Pseudomonas aeruginosa (mm)	Bacillus subtilis (mm)
1	Leaf + Bark	13	12	5	8	12	5
2	Bark +Flesh	7	11	12	9	18	18
3	Leaf +Flesh	14	9	5	9	11	19
4	Drug	29	12	21	2	12	2
5	Solvent	5	19	5	14	8	5

Figure–2. The zone of inhibition of alcohol extract of Moringa oleifera

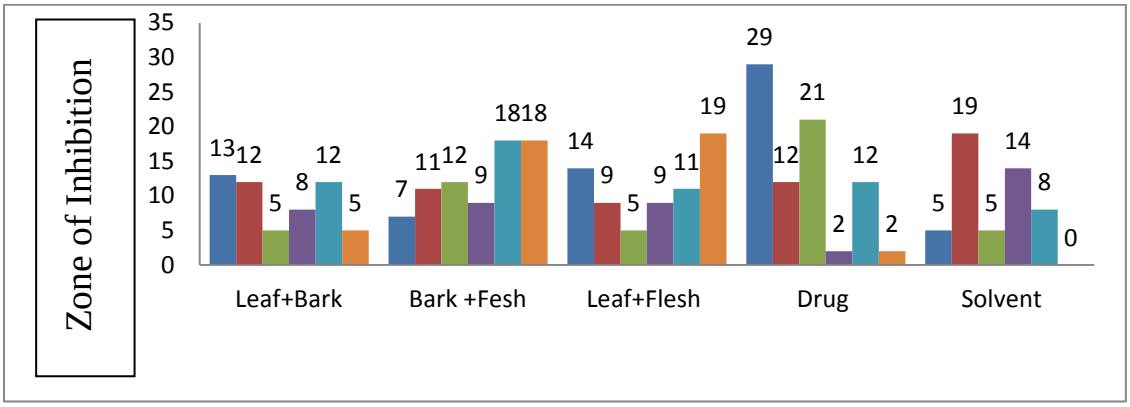


Table -3 Antimicrobial Activity of Acetone Extract of Different Parts of Moringa oleifera

S.No	Plant parts	Micrococcus luteus (mm)	E.coli (mm)	S. aureus (mm)	S.Marcescens (mm)	Pseudomonas aeruginosa (mm)	Bacillus subtilis (mm)
1	Leaf+Bark	13	12	5	5	5	7
2	Bark+Flesh	9	5	5	5	5	7
3	Leaf +Flesh	14	5	5	5	5	11
4	Drug	23	18	8	16	16	13
5	Solvent	5	5	5	5	5	11

Figure - 3. The zone of inhibition of acetone extract of Moringa oleifera. L

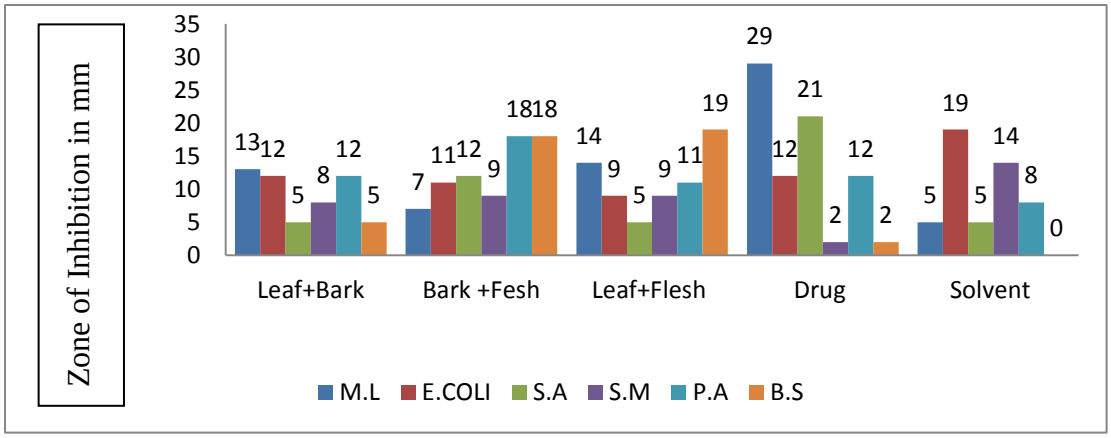


Table -4 Antimicrobial Activity of Benzene Extract of Different Parts of *Moringa oleifera L*

S.No	Plant parts	M.luteus (mm)	E.coli (mm)	S. aureus (mm)	serratia marcescens (mm)	Pseudomonas aeruginosa (mm)	Bacillus subtilis (mm)\
1	Leaf+Bark	15	5	5	5	5	12
2	Bark +Flesh	14	5	5	12	5	13
3	Leaf+Flesh	12	5	5	12	5	5
4	Drug	23	12	11	14	19	22
5	Solvent	5	5	5	5	5	5

Figure -4. The zone of inhibition of benzene extract of Moringa oleifera

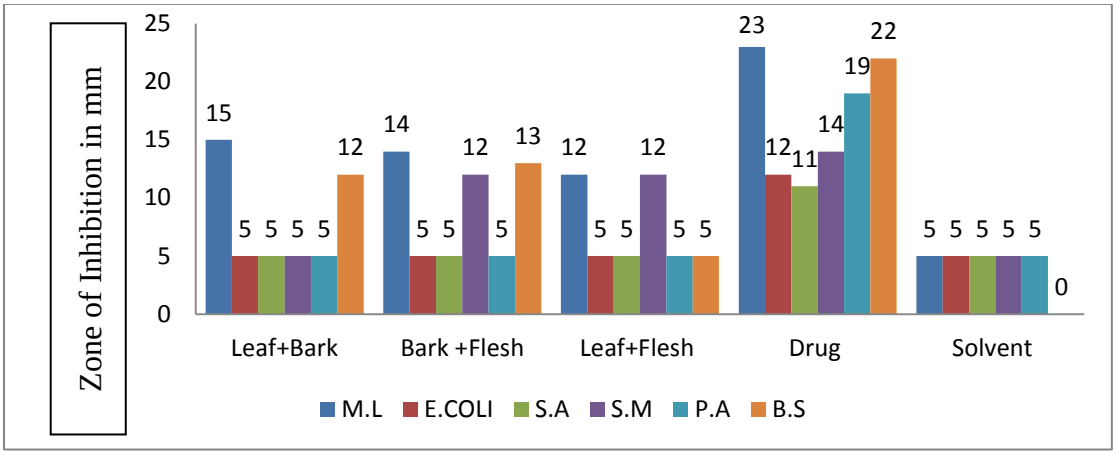


Figure -5. The zone of inhibition of ethyl acetate extract of Moringa oleifera against E.coli

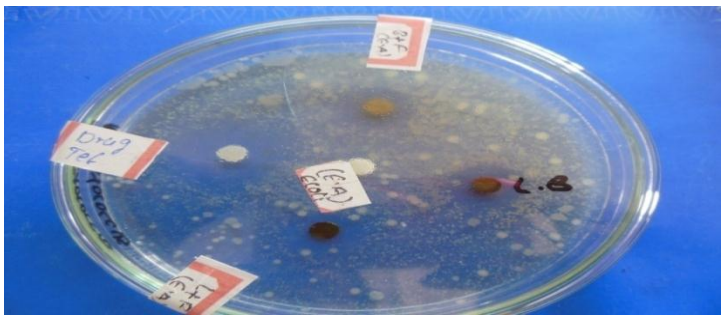
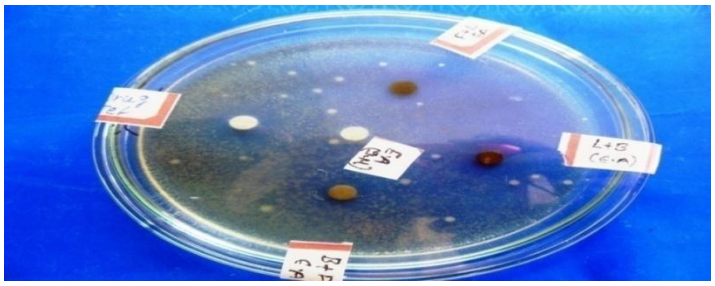


Figure - 6. The zone of inhibition of ethyl acetate extract of Moringa oleifera against Serratia marcescens



All the solvents extracts showed varying degree of antibiotic activity on the microbial activity on the tested gram +ve, gram -ve microorganisms.

- According to the zone of inhibition observed in the entire agar plates, the ethyl acetate extract of Moringa oleifera (L+B), (B+F) showed maximum zone of inhibitions 20 mm,17 mm against Micrococcus luteus (Table 1)
- The ethyl acetate extract of Moringa oleifera of (L+F) showed zone of inhibition 17 mm against E.coli and (L+B) extraction showed zone of inhibition 17 mm against the serratia marcescens. (Table 1)
- The alcoholic extract of Moringa oleifera (L+F) showed zone of inhibition (19 mm) against Bacillus Subtilis and (B+F) extract showed zone of inhibition 18 mm against the Bacillus Subtilis and pseudomonas aeuroginosa. (Table 2)
- The acetone extract of Moringa oleifera showed lowest antimicrobial activity when compare to the other solvents extracts of *Moringa oleifera L*. (Table 3)

The present study was conducted to observe the comparative study of antimicrobial activity of *Moringa oleifera* Leaves, barks, flesh of *Moringa oleifera*. The disc diffusion method was applied to be used in this study. The ethyl acetate extract

of Moringa oleifera (L+B) showed highest antimicrobial activity against M.luteus and we observed that ethyl acetate extract active against the most of gram -ve bacteria tested along with employed gram +ve bacteria.

Previous study reported that ethanolic extract of *Moringa oleifera L*eaf extract showed maximum zone of inhibition against S.aureus (15mm) and E.coli showed from 7 mm to 12 mm for zone of inhibition at 200 mg/ml. The ethanol extract of *Moringa oleifera L*eaves showed less zone of inhibition (1mm to 1.5 mm) against E.coli, S.aureas at 50 to75 mg/ml [14].

From the previous studies it is clearly indicated that the leaf extract demonstrated weak antibacterial activity. However by combinations of leaf, bark, flesh of Moringa oleifera it gives synergistic activity and the zone of inhibition is ranges from17 mm to 20 mm. These consequences suggest that Moringa oleifera L of leaf, bark, flesh combinations contain more bio-components whose anti bacterial potential are highly comparable with the antibiotic tetracycline gram +ve, gram –ve bacteria tested.

CONCLUSION

Today, most pathogenic organisms are becoming resistant to antibiotic. The *Moringa oleifera L*am. could become promising natural antimicrobial agents with potential applications in pharmaceutical industry for controlling the pathogenic bacteria. However, if plant extracts are to be used for medicinal purposes, issues of safety and toxicity will always need to be considered. Further investigation is required for phytochemical studies of *Moringa oleifera L* to achieve lead molecule in search of novel and herbal drugs.

ACKNOWLEDGEMENT

The presenting authors are thankful to Vijaya institute of pharmaceutical sciences for women, Vijayawada for their valuable support in carrying out this work.

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