Original Article

A Comparative Study on Antimicrobial Activity of Leaf Extract of Five Medicinal Plants and Commonly Used Antibiotics

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ABSTRACT

Now a day, multidrug resistance of pathogenic microbes throws a challenge to scientists to discover the source of alternative medicine. This study focuses on the antimicrobial properties of the leaf extracts of five commonly known medicinal plants–*Azadirachta indica, Alpine besseya*, *Mentha longifolia, Manilkara zapota, Bryophyllum pinnatum.* The methanolic and ethanolic extracts of the leaf of the above mentioned plants were prepared by distillation method. Then comparative analysis of antimicrobial effects between those extracts and available antibiotics in the market were tested in *Escherichia coli, Salmonella typhi and Pseudomonas sp.* culture. All of the leaf extracts have shown potent antimicrobial activity and the extract of *Bryophyllum pinnatum* have shown the most potent activity compare to others.

Keywords: Antimicrobial effect, multidrug resistance, pathogenic microbes, leaf extracts.

INTRODUCTION

The term *antibiotic* was first used in 1942 by Selman Waksman and his collaborators in journal articles to describe any substance produced by a microorganism that is antagonistic to the growth of other microorganisms in high dilution[1]. This definition excluded substances that kill bacteria but that are not produced by microorganisms such as gastric juices and hydrogen peroxide. It also excluded synthetic antibacterial compounds such as the sulfonamides. Many antibacterial compounds are relatively small molecules with a molecular weight of less than 2000 atomic mass units. In recent times, there have been increases in antibiotic resistant strains of clinically important pathogens, which have led to the emergence of new bacterial strains that are multidrug resistant[2,3]. The non-availability and high cost of new generation antibiotics with limited effective span have resulted in

increase in morbidity and mortality[4]. Therefore, there is a need to look for substances from other sources with proven antimicrobial activity. *Azadirachta indica, Alpine besseya*, *Mentha longifolia, Manilkara zapota and Bryophyllum pinnatum are* belong to the plant families- Meliaceae, Scrophulariaceae, Lamiaceae, Sapotaceae and Crassulaceae respectively. These plants generally used for the treatment of ear-ache, cough, diarrhoea, dysentery, abscesses, ulcers, insect bites, heart-troubles, epilepsy, arthritis, dysmenorrhea and whitlow[5]. However there is no information available of the antimicrobial activities of the leaf extract of those plants.

The present study is an attempt to find out the antimicrobial activities of above mentioned medicinal plants and also compared that activity with commonly available antibiotics.

MATERIALS & METHODS

Collection of plant materials

Soft and healthy leaves of *Azadirachta indica, Alpine besseya*, *Mentha longifolia, Manilkara zapota and Bryophyllum pinnatum* were collected from the different part of Eastern India.

Preparation of extract[6]

These leaves were washed well in fresh water and kept under sunlight for drying. After seven days the leaves became totally dry and then crushed well using mortar and pistil until the granules become totally power dust like. Cotton was plugged at the base of the extractor, in which 70 gm. of crashed materials was taken with 50ml methanol. After 10 minutes, crushed materials were pressed in the extractor with further addition of 20ml methanol. Again, 50ml of methanol was added. After rendering pressure on the extract, it could be stored in a beaker. The water flow was started in the condenser followed by the boiling of extracts for 8 hours at 50^{o}C - 65^{o}C. After full boiling, the extract was allowed to cool at room temperature for a while till the formation of yellow precipitate which was collected later by decanting. Then, wrapping it with aluminum foil, it was kept at room temperature for future use. Same process was followed for ethanolic extract preparation.

Collection of sample and identification of bacteria

Initially, the microbial samples were collected from the stool of diarrhea patients from the local hospital and serial dilutions of stool samples for 10 times at the ratio of 1:10 were performed. Various qualitative biochemical tests were performed in order to detect the presence of microbes in the collected stool samples, namely *Escherichia coli, Salmonella typhi, Pseudomonas sp.* by Methyl Red Test, Indole Test and VP Test accordingly[7].

Culture of pathogenic microbes with simultaneous application of antibiotics and extracts[7]

For the development of pure culture of identified bacteria, the Eosin-methylene blue (EMB) culture for *E.coli*, Xylose Lysine deoxycholate (XLD) media for *S.typhi*, Mannitol agar for *S.aureus*and Cetrimate agar for *Pseudomonous. sp* were prepared. Isolated colonies of bacteria from pure culture then poured into Lysogeny Broth (LB) and allowed them for incubation at 25° C for 48hs. After that, Mueller Hilton (MH) plates were prepared. Swabbing process of microbes was conducted from LB to the respective MH plates. Application of antibiotics (Penicillin, Ofloxacin, Ciprofloxacin, Kanamycin and Gentamycin, 30mg tablet for each) in order to observe the effects after incubation was carried out to detect the zone of clearance. Simultaneously, the herbal extracts (ethanolic and methanolic) were applied (1ml each) in the separate preparation of MH. Swabbing of

microbes from LB to MH plates along with the application of herbal extracts on the respective plates was performed for the purpose of detecting the zone of clearance by extracts. Then the results obtained from the effect of antibiotics and that of the herbal extracts on microbial growth were compared and evaluated.

RESULT AND DISCUSSION

It has been found that the methanolic and ethanolic extract of leaf of *Azadirachta indica, Alpine besseya*, *Mentha longifolia, Manilkara zapota and Bryophyllum pinnatum* have antimicrobial activity against *E. coli, Pseudomonas sp.* and *S.typhi*. Such antimicrobial activity was also again compared by the parallel study with five standard commonly available antibiotics like Norfloxacin, Ofloxacin, Ciprofloxacin, Kanamycin and Gentamycin (Table 2,3,4). Norfloxacin, Ofloxacin and Ciprofloxacin have shown potent antimicrobial properties by producing zone of inhibition. The methanolic extract of *Bryophylum* have shown nearly equivalent antimicrobial activity against *E.coli, S. typhi* and ethanolic extract of *Bryophylum* have shown the same property against *pseudomonas* compared to the antibiotics. The ethanolic & methanolic extract of *A. indica*, A. besseya, *M. zapota* have shown moderate effect against *Pseudomonas* but low against the other two types of microbes and the reverse is true for *M. longifolia*.

Different pathogenic microbes are becoming resistant to specific antibiotics day by day. As, *Pseudomonus* sp. is resistant to penicillin, *S.typhi* to chloramphenicol, ampicillin and trimethoprim, *E.coli* to tetracycline, ampicillin, sulfamethoxazole and trimethoprim etc[8-10]. The extract of *Bryophylum* have shown nearly equivalent antimicrobial activity compared to the antibiotics. The phytochemical analysis of leaf extract of those medicinal plants showed that it contains many secondary metabolites. *B. pinnatum* leaf extract contained flavonoid[11], saponin, flavonoid, tannin were present *M. longifolia* leaf extract[12], *A. indica* contained Seventeen limonoids[13], *A. besseya* leaf extract contained flavonol glycosides and other hydrocarbon products[14] and *M. zapota* contained flavonoids, tannins (mainly from unripe fruits) and triterpenes[15]. Recently we reported that alkaloid, saponin and terpenoid may have the antimicrobial properties[16]. Saponin has antibacterial activity either in combination with other secondary metabolites or alone against resistance pathogens[17,18]. Again terpenoid has also antimicrobial activity against *S. typhi*[17,18]. Even more, it is also reported that secondary metabolites of different medicinal plants from different geographic regions have potent antimicrobial activity[17,19]. The alkaloids exhibit the antimicrobial property possibly by intercalating into cell wall or DNA and Terpenoids by membrane disruption[17]. Further some modern studies support that herbs and their extracts are not only good antimicrobial agents but also maintain a good and healthy immune system as it contains good amount of carbohydrate, crude fiber, protein, calcium and potassium[19,20].

CONCLUSION

From this study it may be concluded that the leaf extract of *Azadirachta indica, Alpine besseya*, *Mentha longifolia, Manilkara zapota and Bryophyllum pinnatum* can be used as potent antimicrobial formulation in pharmaceutical and medicine industries in near future.

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Table 1. Results indicating the identification of microbes by different test methods

Test	*E.Coli*	*S.typhi*	*Psudomonas sp.*
Indole	+	+	+
Methyl Red	+	_	_
VP	_	+	_

Table 2. Comparative study of Zone of clearance between herbal extracts and commonly used antibiotics against *E.coli*

SL.	Antibiotics used		Herbal extracts applied(Methanolic)		Herbal extracts applied(Ethanolic)	
	Names	Zone of clearance in Cm	Names	Zone of clearance in Cm	Names	Zone of clearance in Cm
1	OFLOXACIN (Of 5mg/plate)	3.2	NEEM (5µl/plate)	1.8	NEEM (5µl/plate)	1.1
2	CIPROFLOXACIN (Cf 5mg/plate)	4.3	BAEL (5µl/plate)	2	BAEL (5µl/plate)	1.3
3	KANAMYCIN (K 5mg/plate)	1.8	MINT (5µl/plate)	2.5	MINT (5µl/plate)	2
4	GENTAMYCIN (G 5mg/plate)	2	BRYOPHYLLIUM (5µl/plate)	3	BRYOPHYLLIUM (5µl/plate)	2.3
5	NORFLOXACIN (N 5mg/plate)	4	SAPOTA(5µl/plate)	1.6	SAPOTA(5µl/plate)	1.4

Table 3. Comparative study of Zone of clearance between herbal extracts and commonly used antibiotics against *Pseudomonas sp.*

SL.	Antibiotics used		Herbal extracts applied(Methnolic)		Herbal extracts applied(Ehanolic)	
	Names	Zone of clearance in Cm	Names	Zone of clearance in Cm	Names	Zone of clearance in Cm
1	OFLOXACIN (Of 5mg/plate)	4	NEEM (5µl/plate)	2.5	NEEM (5µl/plate)	2.6
2	CIPROFLOXACIN(Cf 5mg/plate)	3	BAEL (5 µl/plate)	2.6	BAEL (5 µl/plate)	2.8
3	KANAMYCIN (K 5mg/plate)	2	MINT (5 µl/plate)	1.4	MINT (5 µl/plate)	1.5
4	GENTAMYCIN (G 5mg/plate)	2.2	BRYOPHYLLIUM (5 µl/plate)	3	BRYOPHYLLIUM (5 µl/plate)	3.2
5	NORFLOXACIN (N 5mg/plate)	3	SAPOTA (5 µl/plate)	2.3	SAPOTA (5 µl/plate)	2.5

Table 4. Comparative study of Zone of clearance between herbal extracts and commonly used antibiotics against *S.typhi.*

SL.	Antibiotics used		Herbal extracts applied(Methanolic)		Herbal extracts applied(Ethanolic)	
	Names	Zone of clearance in Cm	Names	Zone of clearance in Cm	Names	Zone of clearance in Cm
1	OFLOXACIN (Of 5mg/plate)	3.2	NEEM (5µl/plate)	1.8	NEEM (5µl/plate)	1.6
2	CIPROFLOXACIN (Cf 5mg/plate)	4.3	BAEL (5 µl/plate)	2	BAEL (5 µl/plate)	1.8
3	KANAMYCIN (K 5mg/plate)	1.8	MINT (5 µl/plate)	2.5	MINT (5 µl/plate)	2
4	GENTAMYCIN (G 5mg/plate)	2	BRYOPHYLLIUM (5 µl/plate)	3	BRYOPHYLLIUM (5 µl/plate)	2.5
5	NORFLOXACIN (N 5mg/plate)	4	SAPOTA (5 µl/plate)	1.6	SAPOTA (5 µl/plate)	1.4

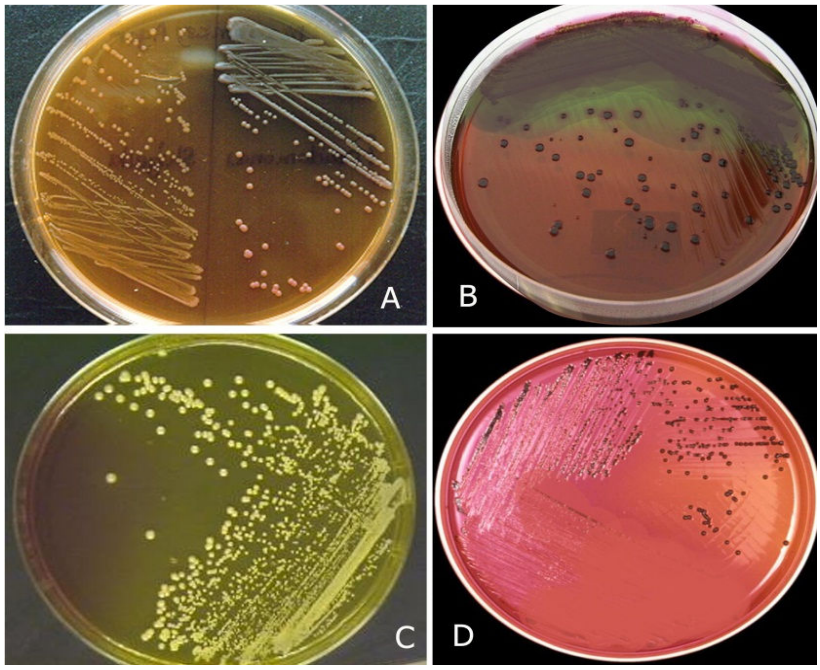

Figure 1. Specification of microbes on various agar plates: A- *Pseudomonas* sp. on Cetrimate Agar, B- *E.coli* on EMB, C- *E.coli*on Nutrient Agar, D- *S.typhi* on XLD**.**

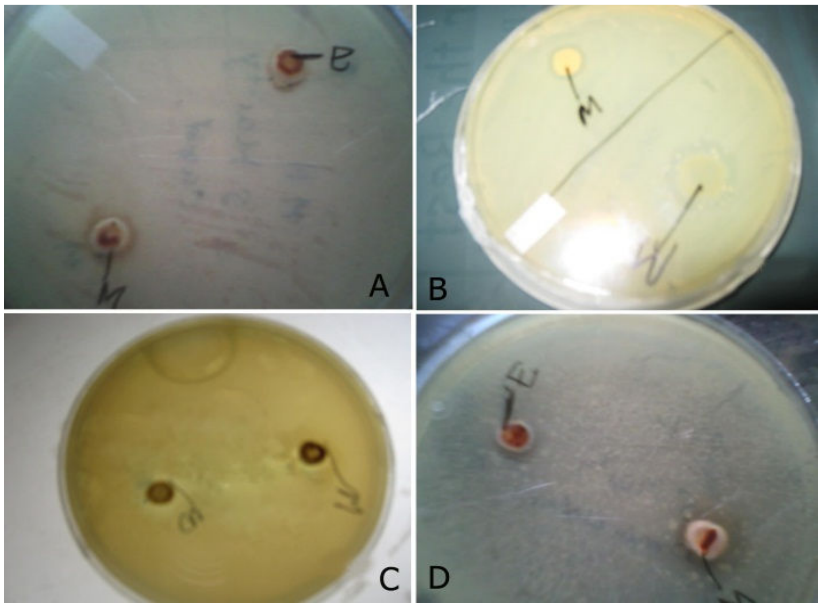

Figure 2. Figure showing the effect of *Bryophillium* leaf extract on different bacteria specified A- *S. typhi* B- *S. aureus* C. *Pseudomonas sp* D. *E. coli.*

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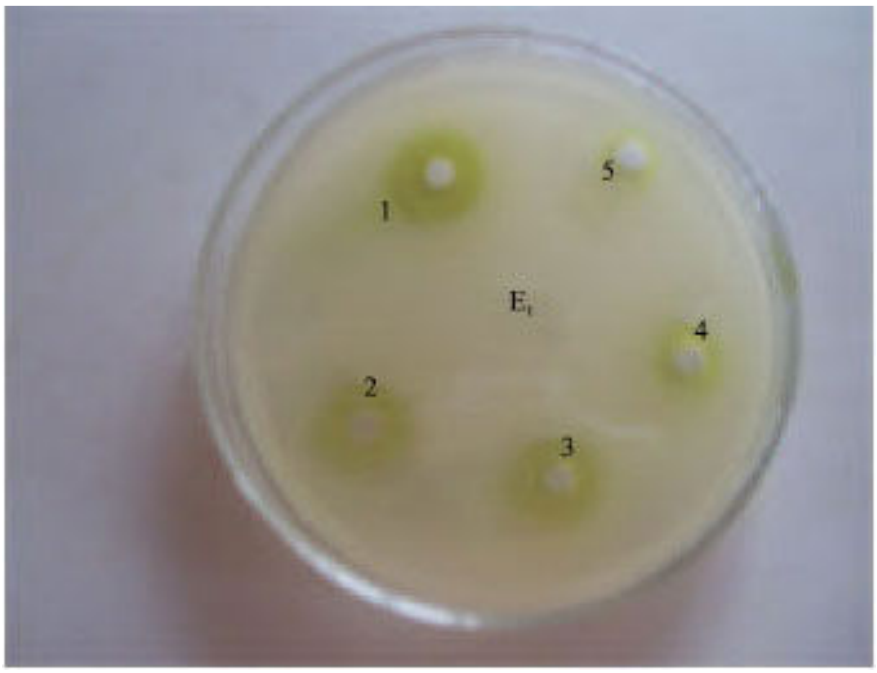

Figure 3. Application of different antibiotics.1-Norfloxacin, 2- Ofloxacin, 3-Ciprofloxacin, 4- Gentamycen, 5- Kanamycin. In the above experiment, kanamycin and gentamycin showed lower effect.