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Research Article

ANTIMICROBIAL ACTIVITY OF *EUPHORBIA THYMIFOLIA* (L.) AND *MANILKARA HEXANDRA* (ROXB.)

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A B S T R A C T

Plants are the richest source of natural antimicrobial agents. The aqueous extracts of *Euphorbia thymifolia* (whole plant) and *Manilkara hexandra* (leaves) were subjected to antimicrobial activity study. The present work was mainly focused on screening the antimicrobial activity of aqueous extracts against six bacterial strains including *Streptococcus mutans*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Salmonella bongori* and *Enterococcus faecalis* and two fungal strains *Candida albicans* and *Aspergillus niger* by well diffusion method by measuring the zone of inhibition. The results indicated that the tested microbes were sensitive to the aqueous extracts. The experiment showed that aqueous extracts of *Euphorbia thymifolia* (whole plant) and *Manilkara hexandra* (leaves) exhibited excellent antimicrobial activity against tested bacterial organisms as compared to the standards. The results demonstrated that aqueous extracts of these plant materials have concentration dependent antimicrobial activity against some of the tested organisms. The growth of most of the tested microbes was inhibited though to varying degree thus justifying the use of these plants in traditional medicine in treating enteric infections. Among the plants studied, aqueous extract of *Manilkara hexandra* (leaves) was found to possess greater activity than *Euphorbia thymifolia* (whole plant).

INTRODUCTION

Most of the pathogens causing enteric infections have developed resistance to the commonly prescribed antibiotics. Bacterial resistance to antibiotics increases mortality, likelihood of hospitalization and the length of stay in the hospital. For most bacteria, there is evidence that increased usage of a particular antimicrobial correlates with increased levels of bacterial resistance to that agent. There are several reports in the scientific literature describing the antimicrobial properties of crude extracts prepared from plants (Muhammad and Muhammad 2005; Falodun *et al*., 2006, El-Mahmood and Ameh, 2007) and such reports had attracted the attention of scientists worldwide. Medicinal plants have been used by human and their components are widely used since ages in traditional medicine due to their constituents of different medical products, in the food therapeutic potential (Derwich *et al*., 2010). The use of medicinal herbs in the treatment of infection is an age-old practice and several natural products are used as phytotherapic for treatment of many diseases. Human infections constitute a serious problem and most frequent pathogens are microorganisms such as bacteria and fungi.

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On the other hand, the development of resistant strains of pathogenic bacteria to antibiotics currently in use is a problem of continuing concern to public health (Cohen, 1992; Neu, 1992). Therefore, the search for discovery of new antimicrobial agents is necessary and stimulates the research of new antimicrobial agents in the medicinal plants.

Euphorbia thymifolia L. (*Euphorbiaceae*) is a small branched, hispidly pubescent, prostate annual herb, commonly known as *laghududhika* or *choti-dudhi*. The leaves, seeds and fresh juice of whole plant are used in worm infections, as stimulant, astringent. It is also used in bowel complaints and in many more diseases therapeutically. *E. thymifolia* is traditionally used as a blood purifier, sedative, haemostatic; aromatic, stimulant, astringent in diarrhea and dysentery, anthelminthic, demulcent, laxative; and also in cases of flatulence, constipation; in chronic cough; as an antiviral in bronchial asthma and paronychia (Mali and Panchal, 2013). *Mimusops hexandra* (Roxb.) is ethno medicinally important species of tropical deciduous forests of western and central India. It belongs to family Sapotaceae and it is native of South Asian region (Balfour, 1873; Vincken *et al*., 2007). *Mimusops hexandra* has been reported mostly in traditional medicinal system of India. Traditionally it is used in medicinal herbal drugs to cure various diseases such as jaundice, ulitis, odontopathy, fever, colic dyspepsia, helminthiasis, hyper dyspepsia and burning sensation (Joshi, 2000). It purifies the

blood and beneficial in swelling, abdominal colic, gout, rheumatism and toxicosis (Rao *et al.,* 1985). It contains a variety of components which possess various biological activities such as anti–inflammatory, diuretic, antiurolithiatic, analgesic, antipyretic and antimicrobial activity (Khare, 2007). However, only a small proportion has been investigated both phytochemically and pharmacologically. It is important to investigate the gaps in the studies, which may be further bridged in order to exploit the full medicinal potential of *Euphorbia thymifolia* and *M. hexandra*, as this plant has widespread use also with extraordinary medicinal potential which should be better explored to find new biological properties which may increase its importance as efficient medicinal plant in biodiversity. The present investigation was taken up to screen these medicinal plants for evaluating their antimicrobial activity.

MATERIALS AND METHODS

Collection of plant

Euphorbia thymifolia plant material was collected from the village, 'Nitardi', and *Manilkara hexandra* plant material was collected from 'Santosh nursery' located in Shajapur district, Madhya pradesh, during the month of December, 2016. Further plant materials were identified and voucher specimens were submitted in 'Herbarium', Department of Botany, Dr. Hari Singh Gour University, Sagar, M.P. and the registration numbers allotted to *Euphorbia thymifolia* and *Manilkara hexandra* specimens are Herbarium number P1 (bot/BG/201198) and P2 (bot//BG/201199) respectively. The plant materials were dried under shade at room temperature for about 15 days. The dried plant samples were powdered by mechanical grinder and sieved to give particle size 40- 100 mm. The powders were stored in polythene bags at room temperature before extraction.

Preparation of extract

Euphorbia thymifolia (whole plant) and *Manilkara hexandra* (leaves) dried and powdered materials (50 g each) were extracted with Hot continuous percolation method (Soxhlet extraction) separately. The temperature was maintained at 70°C. The extraction was carried out using water as a solvent. The extracts were filtered through a paper filter (Whatman, No.1) and evaporated to dryness under reduced pressure by the rotary evaporator. The obtained crude extract was stored in dark glass bottles for further processing.

Antimicrobial activity

Pathogenic antimicrobial used

The pathogenic bacteria and fungus used in the current study was obtained from Microbial Culture Collection, National Centre for Cell Science, Pune, Maharashtra, India.

Media preparation (broth and agar media)

Table 1 Composition of nutrient agar media

Composition	Weight
Agar	1.5 gms
Beef extract	0.3 gms
Peptone	0.5 gms
Sodium chloride	0.55 gms
Distilled water	to make 100 ml

Table 2 Composition of potato dextrose agar media

Composition	Weight
Potato infusion	20 gms
Dextrose	2 gms
Agar	1.5 gms
Distilled water	to make 100 ml

Method of preparation

This agar medium was dissolved in distilled water and boiled in conical flask of sufficient capacity. Dry ingredients were transferred to flask containing required quantity of distilled water and heated to dissolve the medium completely.

Sterilization culture media

The flask containing medium was cotton plugged and was placed in autoclave for sterilization at 15 lbs /inch^2 (121°C) for 15 minutes.

Preparation of plates

After sterilization, the media in flask was immediately poured (20 ml/ plate) into sterile petri dishes on plane surface. The poured plates were left at room temperature to solidify and incubate at 37 ºC overnight to check the sterility of plates. The plates were dried at 50 ºC for 30 minutes before use.

Revival of the microbial cultures

The microbial cultures used in the study were obtained in lyophilized form. With the help aseptic techniques the lyophilized cultures were inoculated in sterile nutrient and potato dextrose broth than incubated for 24 hrs at 37 ºC. After incubation the growth was observed in the form of turbidity. These broth cultures were further inoculated on to the nutrient and potato dextrose agar plates with loop full of microbes and further incubated for next 24 hours at 37 °C to obtain the pure culture and stored as stocks that are to be used in further research work.

Antimicrobial sensitivity

The antimicrobial sensitivity test was employed on to all the microbes used under present study with aqueous extracts obtained from *Euphorbia thymifolia* (Linn.) and *Manilkara hexandra* (Roxb.). For this experiment 6 mm diameter wells, stock of 100 mg/ml of extract separately applied on it. A nutrient and potato dextrose agar plate was seeded with particular microbes with the help of spread plate technique prior and left for 5 minutes then incubated for 24 hrs at 37 °C. After incubation, plates were observed to see the sensitivity of extracts towards test microbes at particular concentration in the form zone of inhibition.

Antibiogram studies

Broth cultures of the pure culture isolates of those test microorganisms which were sensitive towards the phytoextracts used in present study were prepared by transferring a loop of culture into sterile nutrient and potato dextrose broth and incubated at 37°C for 24-48 hrs. A loop full was taken from these broths and seeded onto sterile nutrient and potato dextrose agar plates through sterile cotton swab to develop diffused heavy lawn culture.

The well diffusion method was used to determine the antimicrobial activity of the extracts prepared from the plant material of *Euphorbia thymifolia* and *Manilkara hexandra* using standard procedure (Bauer *et al*., 1966). There were 3

concentrations used which are 25, 50 and 100 mg/ml for each extracted phytochemicals in antibiogram studies. It's essential feature is the placing of wells with the antibiotics on the surfaces of agar immediately after inoculation with the organism tested. Undiluted over night broth cultures should never be used as an inoculums. The plates were incubated at 37 ºC for 24 hrs and then examined for clear zones of inhibition around the wells impregnated with particular concentration of drug.

RESULTS AND DISCUSSION

The phytochemical extracts obtained from the *Euphorbia thymifolia* (whole plant) and *Manilkara hexandra* (leaves) were studied using well diffusion method against six bacterial strains including *Streptococcus mutans*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Salmonella* and *Enterococcus faecalis* and two fungal strain *Candida albicans* and *Aspergillus niger*. The fresh pure 100% extracts obtained from the plants was used to suitably dilute upto the concentrations of 25 µg/ml, 50 µg/ml and 100 µg/ml and applied on to the test organism using well diffusion method. Results of the experiment are being concluded in the Table 1, 2 & 3, which clearly shows the antimicrobial activity of extracts of *Euphorbia thymifolia* and *Manilkara hexandra.*

Table 1 Results of sensitivity of *Euphorbia thymifolia* and *Manilkara hexandra*

S. No.	Microbes Codes	Microbial Strains	*Euphorbia thymifolia*	*Manilkara hexandra*
1.	Bact-1	*Streptococcus mutans*	Yes	Yes
2.	Bact-2	*Bacillus subtilis*	No	Yes
3.	Bact-3	*Klebsiella pneumoniae*	No	No
4.	Bact-4	*Proteus mirabilis*	No	No
5.	Bact-5	*Salmonella bongori*	Yes	Yes
6.	Bact-6	*Enterococcus faecalis*	No	No
7.	Fungus-1	*Candida albicans*	Yes	Yes
8.	Fungus-1	*Aspergillus niger*	Yes	No

Table 2 Antimicrobial activity of standard drug on selected microbes

S.N	Name of drug	Microbes	Zone of inhibition(Diameter in mm)		
			10 µg/ml	20 µg/ml	30 µg/ml
1	Ofloxacin	*Streptococcus mutans*	12.37±0.13	15.33±0.09	17.33±0.09
2.	Ciprofloxacin	*Bacillus subtilis*	12.27±0.14	17.27±0.10	20.43±0.02
		Salmonella bongori	17.36±0.10	23.35±0.11	25.59±0.02
3.	Fluconazole	*Candida albicans*	16.30±0.02	20.48±0.12	28.38±0.13
		Aspergillus niger	8.27±0.02	10.35±0.05	14.52±0.03

Results are expressed as mean ± standard error of the mean (SEM), n=3.

Table 3 Antimicrobial activity of *Euphorbia thymifolia* and *Manilkara hexandra* on selected microbes

S. No.	Name of microbes	Zone of inhibition (Diameter in mm)		
		Euphorbia thymifolia		
		25 µg/ml	50 µg/ml	100 µg/ml
1.	*Streptococcus mutans*	8.26±0.08	11.30±0.07	14.26±0.03
2.	*Bacillus subtilis*	-	-	-
3.	*Salmonella bongori*	14.48±0.14	20.30±0.09	22.37±0.06
4.	*Candida albicans*	10.18±0.03	15.48±0.03	17.34±0.11
5.	*Aspergillus niger*	7.44±0.12	10.37±0.04	15.40±0.04
		Manilkara hexandra		
1.	*Streptococcus mutans*	10.40±0.08	14.31±0.05	15.45±0.11
2.	*Bacillus subtilis*	7.28±0.09	9.59±0.14	11.55±0.06
3.	*Salmonella bongori*	9.49±0.09	20.49±0.03	24.56±0.03
4.	*Candida albicans*	8.32±0.07	12.44±0.07	13.41±0.09
5.	*Aspergillus niger*	-	-	-

Results are expressed as standard error of the mean (SEM), n=3.

The high antimicrobial effect of the medicinal plant extracts may be due to secondary metabolites in the plant tissues and phytochemical studies indicates that the plants' antimicrobial activities are associated with compounds such as flavonoids, terpenes, alkaloids, tannins, hydroxyl group and phenol and essential oils such as yarrow, carvacrol, thymol, glycosides, tannins, saponins and steroids (Dorman and Deans, 2000; Choudhury *et al*., 2013; Jadhav, *et al*., 2013; Joshua *et al*., 2013).

The results indicated that the aqueous extracts of *Euphorbia thymifolia* and *Manilkara hexandra* exhibited significant antimicrobial activity against most of the tested microbes. This showed that each of the aqueous extracts of *Euphorbia thymifolia* and *Manilkara hexandra* was active against at least four microbes among all the tested bacterial and fungal strains with maximum zone of inhibition against *Salmonella bongori* at 100µg/ml. The results revealed that the aqueous extract of *Manilkara hexandra* leaves was very active antimicrobial and was comparable to that of the standard drug. Comparing the two aqueous extracts of *Euphorbia thymifolia* (whole plant) and *Manilkara hexandra* (leaves), it can be said that the *Manilkara hexandra* aqueous extract was more active than the *Euphorbia thymifolia* aqueous extract. Nevertheless, further studies are needed, including *in vitro* and *in vivo* investigations, toxicity evaluation as well as the purification of active antibacterial and antifungal constituents from *Euphorbia thymifolia* and *Manilkara hexandra* aqueous extracts looking towards a pharmaceutical employment.

CONCLUSION

Medicinal plants are very important to human beings in preserving our health. The use of antibiotics to control diseases produces adverse toxicity to the host organs, tissues and cells. The toxicity produced by the antimicrobial agents can be prevented by using herbs. In conclusion, the aqueous extracts of *Euphorbia thymifolia* and *Manilkara hexandra* offer potential antimicrobial property against bacterial and fungal strains. Further studies should be undertaken to elucidate the exact mechanism of action of antimicrobial effect to identify the active ingredients which can be used in drug development program.

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