RESEARCH ARTICLE

Antibacterial and Antifungal Activities from Leaf Extracts of *Mimusops elengi* Linn.

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Received: 17th March, 2020; Revised: 22nd April, 2020; Accepted: 26th May, 2020; Available Online: 25th June, 2020

ABSTRACT

This study was carried out with an objective to investigate the antibacterial and antifungal potentials of leaves of *Mimusops elengi* Linn. The aim of the study is to assess the antimicrobial activity and to determine the zone of inhibition of extracts on some bacterial and fungal strains. In the present study, the microbial activity of different extracts of leaves of *M. elengi* Linn. was evaluated for potential antimicrobial activity against medically important bacterial and fungal strains. The antimicrobial activity was determined in the extracts using agar disc diffusion method. The antibacterial and antifungal activities of different extracts of *M. elengi* Linn. were tested against two gram-positive *Staphylococcus aureus*, *Bacillus*, and two gram-negative *Escherichia coli*, *Xanthomonas* human pathogenic bacteria, and one fungal strain—*Candida albicans*. Zone of inhibition of different extracts were compared with that of standards like ampicillin for antibacterial activity and clotrimazole for antifungal activity. The results showed that the remarkable inhibition of bacterial growth was shown against the tested organisms. The phytochemical analyses of the plants were carried out. The microbial activity of the *M. elengi* Linn. was due to the presence of various secondary metabolites. Hence, these plants can be used to discover bioactive natural products that may serve as leads in the development of new pharmaceuticals research activities.

Keywords: *In vitro* antibacterial and antifungal activity, *Mimusops elengi* Linn., Phytochemical screening.

International Journal of Pharmacognosy and Phytochemical Research (2020); DOI: 10.25258/phyto.12.2.8

How to cite this article: Ansari A, Ahmad S, Husain M, Usman MRM, Akhtar R, Salunkhe SD. Antibacterial and antifungal activities from leaf extracts of *Mimusops elengi* Linn. International Journal of Pharmacognosy and Phytochemical Research. 2020;12(2):118-122.

Source of support: Nil

Conflict of interest: None

INTRODUCTION

An infectious disease has become a serious problem for mankind, particularly in developing countries. It is the second-largest cause of death after cardiovascular diseases. The treatment of infectious diseases often fail because of the rise of drug-resistant microbes. Therefore, it is necessary to discover new antimicrobial drugs, especially from natural sources. Plants have a place and play an important role in therapy. This is evident by the fact that a number of drugs used today is derived from plant sources, which was initially used as medicinal herbs.[1]

Many medicinal plants are considered to be potential antimicrobial crude drugs as well as a source for novel compounds with antimicrobial activity, with possibly new modes of action. This expectation that some naturally occurring plant compounds can kill antibiotic-resistant strains of bacteria such as *Bacillus cereus*, *E. coli*, *Micrococcus luteus*, and *S. aureus* has been confirmed.[2]

Due to indiscriminate use of antimicrobial drugs, microorganisms have developed resistance to many antibiotics, and that has created immense clinical problems in the treatment of infectious disease strains of beta-lactam resistant *S. aureus*, methicillin-resistant *S. aureus* (MRSA) is posing a serious problem to hospitalized patients and their care providers. In addition, antibiotics are sometimes associated with adverse effect on host, which include depletion of beneficial gut and mucosal microorganism, immune-suppression, hypersensitivity, and allergic reaction. The drug-resistant bacteria have further complicated the treatment of infectious disease in immune-compromised, aids, and cancer patients, specially in the case of nosocomial infection. There is not only the loss of effect of antibiotic against multi drug-resistant

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bacteria, but also global problem for the loss of budget for treating infectious disease. In the present scenario of emergence of drug resistance in human pathogenic organisms, there is a need to develop alternative antimicrobial drug for the treatment of infectious disease. One approach is to screen new, inexpensive, and effective drug from other sources including plants from possible antimicrobial properties that will be able to act for longer periods before resistance set in recent years antimicrobial properties of medicinal plant have been reported.[3]

The plant *M. elengi* is an annual or perennial *Ayurvedic* plant that is widely distributed in India. It is used in traditional medicine, especially for skin disease, disease of the gum and teeth, astringent, diuretic, etc.

There have been reports of some pharmacological activity on different parts of the plant *M. elengi* Linn. Cardio-tonic and anthelmintic activity on bark, analgesic, and antipyretic activity on leaf part and aphrodisiac, diuretic and astringent activity on root and ripe fruit of the plant *M. elengi*.

The leaves of the plant contain quercitol, hentriacontane, beta-carotene, and glucose D-mannitol, beta-sitosterol, beta-D-glucoside, and quercetin. Bark of *M. elengi* contains tannin, some caoutchouc, wax, coloring matter, starch, and ash forming inorganic salts. Flowers contain volatile oil seeds contain a fixed fatty oil. Pulp of the fruit contains a large proportion of sugar and saponins.[4-6]

MATERIAL AND METHODS

Collection of Plant Material

The leaves of *M. elengi* was collected from local area of Dhule district, Maharashtra, India, in July 2012, and authenticated by Dr. J. Jayanthi, scientist "C," HOD, Deputy Director, Botanical Survey of India, Koregaon Road, Pune, by comparing morphological features and a sample voucher specimen of plant was deposited for future reference (voucher specimen number ANSMIE2). After authentication, the leaves are cleaned and dried at room temperature in shade and away from direct sunlight. The dried aerial part was coarsely powdered in grinder. The powdered material was sieved through 60 to 120 mesh to remove fine, and the powder was subjected for further study.

Preparation of Crude Plant Extract

The leaves of *M. elengi* was collected and dried in the shade and then, pulverized in a grinder. The powder material was passed through 60 to 120 meshes to remove fine powders and coarse powder was used for extraction. The extraction was carried out in Soxhlet extract by using different solvents in increasing order of polarity, petroleum ether (60–80), chloroform, and then, methanol.

Aqueous extraction was carried out by maceration. About 500 grams of fresh powder was subjected to cold maceration with chloroform:water (1%) in a 2 liters round bottom flask for about 7 days at room temperature. The flask was securely plugged with absorbent cotton and was shaken periodically till complete maceration. After maceration, the marc was then pressed in a muslin cloth and the filtrate was concentrated to residue at low temp.[7]

Preliminary Phytochemical Screening

The different extracts were subjected to preliminary phytochemical testing to detect the presence of different chemical groups of compounds. Air-dried and powdered plant materials were screened for the presence of alkaloids, glycosides, amino acids, tannins, flavonoids, phenolic compounds, steroids, triterpenoids, and saponins in the petroleum ether, chloroform, methanol, and aqueous extract of leaves of the plant were carried out.[8]

Test Microorganisms and Growth Media

S. aureus (MTCC 96), *E. coli* (MTCC 443), Xanthomonas (MTCC 2286) *Basillus subtilis* (MTCC 441), and fungal strains *C. albicans* (MTCC 227), were chosen based on their clinical and pharmacological importance.[9] The bacterial strains obtained from Jai Hind Senior College, Dhule, Maharashtra, were used for evaluating antimicrobial activity. The bacterial and fungal stock cultures were incubated for 24 hours at 37°C on nutrient agar and potato dextrose agar (PDA) medium, respectively, following refrigeration storage at 4°C. The bacterial strains were grown in Mueller-Hinton agar (MHA) plates at 37°C (the bacteria were grown in the nutrient broth at 37°C and maintained on nutrient agar slants at 4°C), whereas the yeasts and molds were grown in Sabouraud dextrose agar and PDA media, respectively, at 28°C. The stock cultures were maintained at 4°C.

ANTIMICROBIAL ACTIVITY

Determination of Zone of Inhibition Method

In vitro antibacterial and antifungal activities were examined for different extracts (petroleum ether ext, chloroform ext, methanol ext, ethyl acetate fraction, and aqueous extract of *M. elengi*). Antibacterial and antifungal activities of plant part extracts against four pathogenic bacteria (two gram-positive and two gram-negative) and one pathogenic fungus were investigated by the agar disk diffusion method.[10-12] Antimicrobial activity testing was carried out by using agar cup method. Each purified extracts were dissolved in dimethyl sulfoxide, sterilized by filtration using sintered glass filter, and stored at 4°C. For the determination of zone of inhibition, pure gram-positive, gram-negative, and fungal strains were taken as a standard antibiotic for comparison of the results. All the extracts were screened for their antibacterial and antifungal activities against *E. coli, Xanthomonas, S. aureus, Bacillus,* and the fungi *C. albicans*. All extracts of *M. elengi* (10 mg/mL) and standard drugs (1 mg/mL) were prepared in double-distilled water using nutrient agar tubes. Mueller-Hinton sterile agar plates were seeded with indicator bacterial strains (10^8 CFU), and allowed to stay at 37°C for 3 hours. Control experiments were carried out under similar condition by using ampicillin for antibacterial activity and clotrimazole for antifungal activity as standard drugs. The zones of growth inhibition around the disks were measured after 18 to 24 hours of incubation at 37°C for bacteria and 48 to 96 hours for fungi at 28°C. The

sensitivities of the microorganism species to the plant extracts were determined by measuring the sizes of inhibitory zones (including the diameter of the disk) on the agar surface around the disks, and values <8 mm were considered as not active against microorganisms.[13-18]

RESULTS AND DISCUSSION

Preliminary Phytochemical Screening

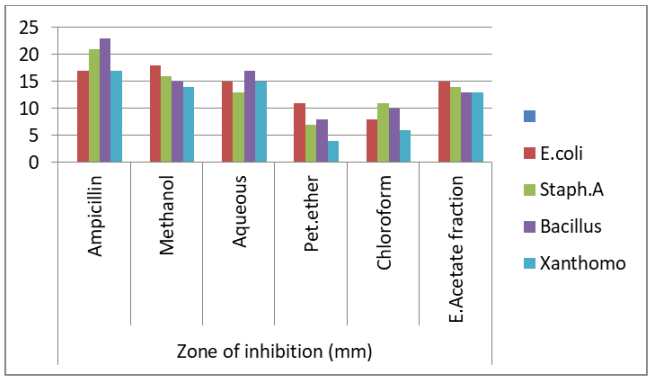
It was found that petroleum ether extracts of *M. elengi* leaves contained steroids, glycosides. Chloroform extract contains carbohydrates, steroids, flavonoids, and tannins. Methanol extract contains carbohydrates, amino acid, saponins, steroids, glycosides, alkaloids, flavonoids, tannins, and aqueous extract contains carbohydrates, amino acids, saponins, steroids, glycosides, alkaloids, flavonoids, tannins, and triterpenoids (Table 1).

Microbial Activity

The antimicrobial activity of the different extracts of *M. elengi* were studied against four pathogenic bacterial strains, two gram-positive (*S. aureus* MTCC 96, *Bacillus* MTCC 441) and two gram-negative (*E. coli* MTCC 443, *Xanthomonas* MTCC 2286), and one fungal strain (*C. albicans* MTCC 227). These strains have been selected for the basis of its application purpose of further formulation study

Antibacterial and antifungal potential of extracts were assessed in terms of zone of inhibition of bacterial growth. The results of the antibacterial and antifungal activities are presented in Tables 2 and 3, Graph 1 and 2, and Figures 1 to 5.

Antibacterial Activity



Graph 1: Result of antibacterial activity of different extract

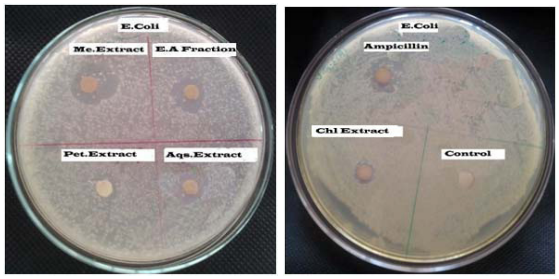


Figure 1: Antibacterial activity against *E. coli*

Table 1: Result of preliminary phytochemical screening of different extract of *M. elengi* Linn.

Test	*Pet. ether extract*	*Chloroform extract*	*Methanol extract*	*Aqueous extract*
Carbohydrates	-	+	+	+
Proteins	-	-	-	-
Amino acids	-	-	+	+
Steroids	+	+	+	+
Glycosides	+	-	+	+
Alkaloids	-	-	+	+
Flavonoids	-	+	+	+
Tannins	-	+	+	+
Triterpenoids	-	-	-	+
Saponins	-	-	+	+

Table 2: Result of antibacterial activity of different extract

	Zone of inhibition (mm)					
Pathogen	*Ampicillin*	*Methanol extract*	*Aqueous extract*	*Pet. ether extract*	*Chloroform extract*	*E. acetate fraction*
E. coli	17	18	15	11	08	15
Staph. a	21	16	13	7	11	14
Bacillus	23	15	17	08	10	13
Xanthomo	17	14	15	04	06	13

Table 3: Result of antifungal activity of different extract and standard

	Zone of inhibition (mm)					
Pathogen	*Std.*	*Methanol ext*	*Aqueous ext*	*E. acetate fraction*	*Chloroform ext*	*Pet. ether ext*
Candida albicans	16	12	09	10	05	08

Antifungal Activity

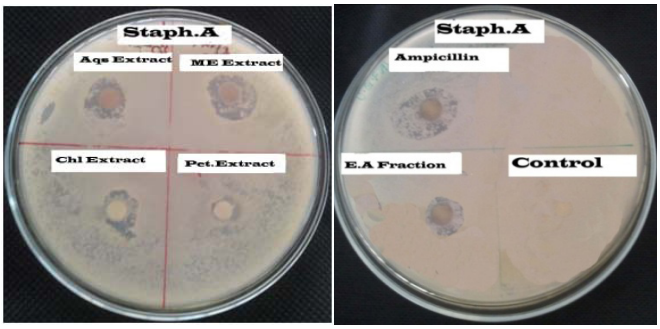


Figure 2: Antibacterial activity against *S. aureus*

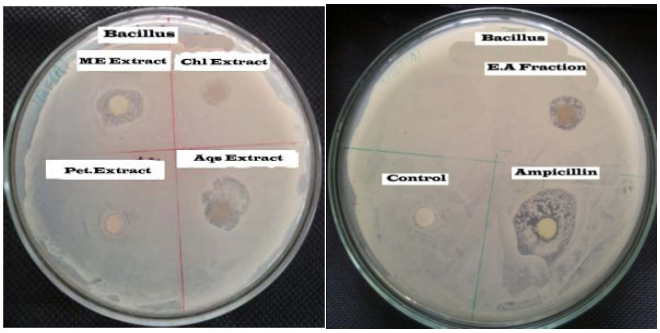


Figure 3: Antibacterial activity against *Bacillus*

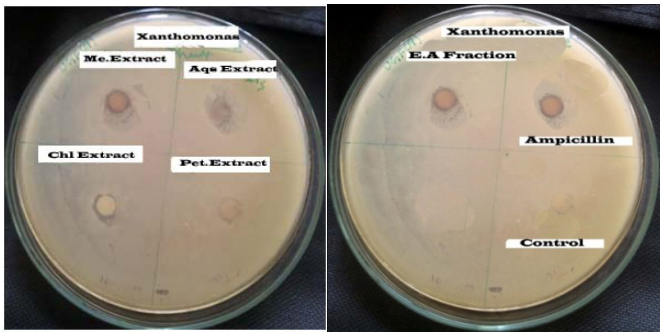


Figure 4: Antibacterial activity against *Xanthomonas* (plant pathogen)

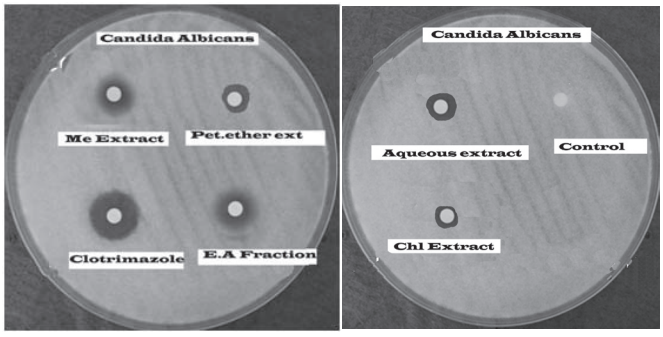
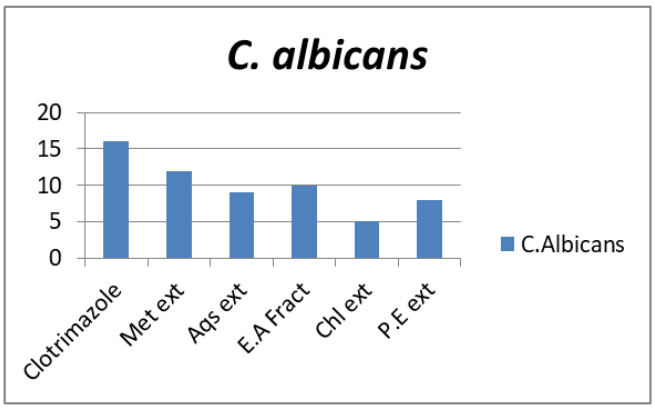


Figure 5: Antifungal activity against *C. albicans*

DISCUSSION

Preliminary phytochemical investigation of petroleum ether, chloroform, methanol, and aqueous extract were revealed that presence of tannins, flavonoids, alkaloids, steroids, saponins, triterpenoids, and glycosides, wherein, the steroids, glycosides



Graph 2: Result of antifungal activity of different extract and standard

may present in petroleum ether extract, the methanolic extract may contains steroids, flavonoids, alkaloids, tannins, and phenolic compounds, the aqueous extract may contains alkaloids, flavonoids, tannins, saponins, and phenolic compounds, and the chloroform extract contains steroids, tannins, and flavonoids.

The antibacterial activity of extract was evaluated by disc diffusion methods the methanolic, aqueous, petroleum ether, chloroform extracts, and ethyl acetate fraction of leaves of *M. elengi* was found to be effective as antibacterial. The results indicate that the extracts of *M. elengi* have antibacterial potential and can be used in the treatment of infectious diseases caused by resistant microorganisms. Methanol, and chloroform extracts obtained from *M. elengi* leaf have been shown to be mild to moderately effective against most of the tested bacteria, but no inhibitory effect of methanol and chloroform extract was observed against only the bacteria *B. subtilis*. But only aqueous extract showed higher activity against *B. subtilis.* The ampicillin as a standard antibiotic showed higher activity against all microorganisms, when compared with all extracts.

The antifungal activity of extract was evaluated by disc diffusion methods the methanolic and ethyl acetate fraction of leaves of *M. elengi* was found to be most effective as antifungal against *C. Albicans,* when compared with petroleum ether, chloroform, and aqueous extract. However, the activity of clotrimazole as a standard antifungal was significantly higher than that of all extracts.

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