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World Journal of Science and Research

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Research Article

Chemistry

ANTIMICROBIAL ACTIVITY OF *Tagetes erecta* FLOWER AND *Mango ginger* RHIZOME EXTRACTS

P. Jegajeevanram¹, N.M.I. Alhaji^{2*}¹Research scholar, Department of Chemistry, Khadir Mohideen College, Adirampattinam - 614 701 (India)²Associate Professor, Department of Chemistry, Khadir Mohideen College, Adirampattinam - 614 701 (India)*Corresponding author: alhajinmi@hotmail.com

ABSTRACT

Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country. Emergence of pathogenic microorganisms that are resistant/multi-resistant to major class of antibiotics has increased in recent years due to indiscriminate use of synthetic antimicrobial drugs. The *in vitro* antimicrobial activity of the *Tagetes erecta* flower and *Mango ginger* rhizome extracts against *Escherichia coli* and *Staphylococcus aureus* species of bacteria and *Candida albicans* species of fungi were evaluated using the standard agar disc diffusion method and were qualitatively assessed by the presence of inhibition zones. Among the two plants, *Mango ginger* rhizome extract possess potential activity than *Tagetes erecta* flower extract. These new findings may helpful to be applied in integrated control strategies to gain maximum impact on vector control.

Citation: P. Jegajeevanram and N.M.I. Alhaji (2016) Antimicrobial activity of *Tagetes erecta* flower and *Mango ginger* rhizome extracts. *World Journal of Science and Research*. 1 (3): 65-70.

Article Info:

Received on 15th Sep. 2016Accepted on 22nd Oct.

2016

Online October 2016

Keywords:

Antimicrobial activity, *Tagetes erecta* flower, *Mango ginger* rhizome

*Corresponding author

Dr. N.M.I. Alhaji,
Department of
Chemistry, Khadir
Mohideen College,
Adirampattinam Tamil
Nadu, India.

1. INTRODUCTION

Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen-substituted derivatives (Klink, 1997). Most are secondary metabolites, of which at least 12,000 have been isolated, a number estimated to be less than 10% of the total (Schultes, 1978). In many cases, these substances serve as plant defense mechanisms against predation by microorganisms, insects and herbivores. Some, such as terpenoids, give plants their odors; others (quinones and tannins) are responsible for plant

pigment. Many compounds are responsible for plant flavor (e.g., the terpenoid capsaicin from chili peppers), and some of the same herbs and spices used by humans to season food yield useful medicinal compounds

Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country. Emergence of pathogenic microorganisms that are resistant/multi-resistant to major class of antibiotics has increased in recent years due to

indiscriminate use of synthetic antimicrobial drugs. In addition, high cost and adverse side effects are commonly associated with popular synthetic antibiotics, such as hypersensitivity, allergic reactions, and immunosuppressant and are major burning global issues in treating infectious diseases (Karaman *et al.*, 2003). This situation forced scientists to search for new antimicrobial substances with plant origin. To study the antimicrobial activity of *Tagetes erecta* flower and *Mango ginger* rhizome extracts.

2. MATERIALS AND METHODS

Collection of Plant materials

During the month of February, *Curcuma amada* rhizome and *Tagetes erecta* flowers were collected from various gardens in Keelavandanviduthy Village, Pudukkottai district, Tamil Nadu, India.

Authentication of plants

The plant was identified and carefully examined with the help of region floras. Specimens were further confirmed with reference to herbarium sheets available in the Rapinat Herbarium, St. Joseph's College, Tiruchirappalli, Tamil Nadu, India.

Determination of Antibacterial activity

The antibacterial activity was performed by disc diffusion method.

Preparation of Media

Preparation of medium:

Suspend 28.0 grams of nutrient agar in 1000 ml distilled water. Heat to boiling and dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well and pour into sterile Petri plates.

Preparation of medium for Fungi

Suspend 39.0 grams of Potato dextrose agar in 1000 ml distilled water. Heat to boiling and dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well before dispensing in specific work, when pH 3.5 is required; acidify the medium with sterile 10 % tartaric acid. The amount of acid required for 100 ml of sterile cooled medium is approximately 1 ml. do not heat the medium after addition of acid.

Microorganisms

The microbial strains employed in the biological assays were Gram – **Positive** bacteria: *Staphylococcus aureus* (MTCC 96) Gram – **negative** bacteria: *Escherichia coli* (MTCC 119) and *Candida albicans* (MTCC 227) Obtained from Microbial type culture collection (MTCC) at the institute of Microbial Technology (IMTECH), Chandigarh, India.

Preparation of 24 hours pure culture

A loop full of each of the microorganisms was suspended in about 10ml of physiological saline in a Roux bottle. Each of these was streaked on to the appropriate culture slants and was incubated at 37°C for 24 hours. After completion of incubation period, when growth was observed the tubes were kept into 2-8°C until use.

Preparation of plant extracts solutions for the experiment

The sample were weighed (10mg/10ml) and dissolved in sterile distilled to prepare appropriate dilution to get required concentrations of about 50µl (50µg) 100µl (100µg) and 150µl (150µg). They were kept under refrigerated condition unless they were used for the experiment. Standard solution as Chloromphenical for bacteria and fluconazole (25mg/ml distilled water- 30µl) for fungi used to compare the test solution. They were kept under refrigerated condition unless they were used for the experiment.

Preparation of dried filter paper discs

Whatman filter paper (No:1) was used to prepare discs approximately 6 mm in diameter, which are placed in hot air for sterilization. After sterilization, the discs were loaded with different concentrations of prepared plant extract solutions and again kept under refrigeration for 24 hrs.

Application of discs to inoculated agar plates

Previously prepared paper discs were dispensed onto the surface of the inoculated agar plate. Each disc was pressed down firmly to ensure complete contact with the agar surface. The discs were placed on the medium suitably apart and the plates were incubated at 5°C for 1 hr to permit good diffusion and then transferred to incubator at 37°C for 24 hrs. After completion of 24hrs, the plates were inverted and placed in an incubator set to respective temperature for 24 hrs.

Antimicrobial assay

Antibiogram was done by disc diffusion method (NCCLS, 1993; Awoyinka *et al.*, 2007) using plant extracts. Petri plates were prepared by pouring 30 ml of NA /PDA medium for bacteria/fungi. The test organism was inoculated on solidified agar plate with the help of micropipette and spread and allowed to dry for 10 mins. The surfaces of media were inoculated with bacteria/fungi from a broth culture. A sterile cotton swab is dipped into a standardized bacterial/ fungi test suspension and used to evenly inoculate the entire surface of the Nutrient agar/PDA plate. Briefly, inoculums containing *Escherichia coli*, *Staphylococcus aureus* specie of bacteria were spread on Nutrient agar plates for bacteria and *Candida albicans* was spread on potato dextrose agar

for fungus strains. Using sterile forceps, the sterile filter papers (6 mm diameter) containing the crude extracts (50µl, 100 µl and 150 µl) were laid down on the surface of inoculated agar plate. The plates were incubated at 37°C for 24 h for the bacteria and at room temperature (30±1) for 24-48 hr. for yeasts strains. Each sample was tested in triplicate.

Measurement of zone of inhibition

The antimicrobial potential of test compounds was determined on the basis of mean diameter of zone of inhibition around the disc in millimeters. The zones of inhibition of the tested microorganisms by the extracts were measured using a millimeter scale.

3. RESULTS

Now-a-days there is a renewed interest in drugs of natural origin simply because they are considered as green medicine and green medicine is always supposed to be safe. Another factor which emphasizes this attention is the incidences of harmful nature of synthetic drugs which are regarded as harmful to human beings and environment. The advantage of natural drugs is their easy availability, economic and less or no side effects but the disadvantage is that they are the victims of adulteration (Dineshkumar, 2007).

Antimicrobial activity of *Tagetes erecta* flower and *Mango ginger* rhizome extracts

Antimicrobial activity of *Mango ginger* rhizome extract

The *in vitro* antimicrobial activity of the *Mango ginger* rhizome extract against these bacteria and fungi were qualitatively assessed by the presence of inhibition zones represented in the photographic Fig 1. The inhibitory activities in culture media of the

Mango ginger rhizome reported in Table 1 were comparable with standard antimicrobial viz. chloromphenical and Fluconazole.

E. coli and *St. auerus*, which already known to be multi-resistant to antibiotics, were resistant to tested plant extract. The mean inhibition zone of *Mango ginger* rhizome extract was 3.70±0.26mm for 50 µl, 6.60±0.46mm for 100 µl, 8.20±0.56mm for 150 µl for *E. coli*. The mean inhibition zone of *Mango ginger* rhizome extract was 3.10±0.22mm for 50 µl, 5.50±0.37mm for 100 µl, 7.10±0.50 mm for 150 µl for *St. auerus*. The mean inhibition zone for standard is 11.10±0.78 and 10.80±0.76for *E. coli* and *St. auerus*

In addition, *C. albicans* was strongly influenced with a mean inhibition zone of 2.80±0.18mm for 50 µl, 4.70±0.34mm for 100 µl, 6.60±0.46mm for 150 µl by *Mango ginger* rhizome extract and 10.60±0.75 for standard. This result is very interesting because *C. albicans* has been the most extensively studied pathogen in antifungal resistance because of their morbidity and mortality associated with infections in immunocompromised patients (Casalinuovo *et al.*, 2004; Redding *et al.*, 1993).

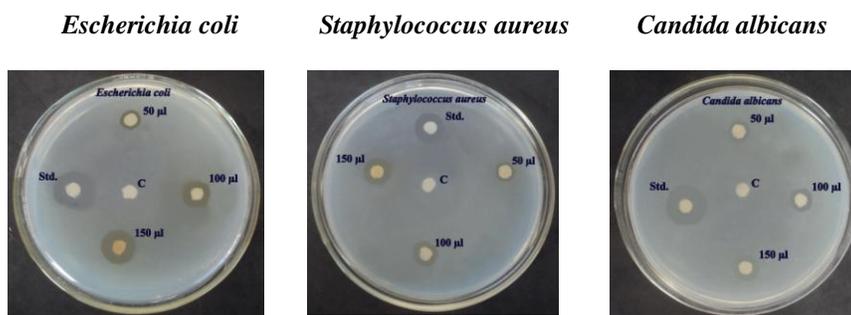
The results showed that the antimicrobial activity was directly proportional to the concentration of *Mango ginger* rhizome extract. The *Mango ginger* rhizome extracts hows highest antimicrobial activity was observed against *S. aureus*, when compared with *E. coli* and *Candida albicans*. The high doses (150µl) of *Mango ginger* rhizome extract possess similar activity to standard drug as chloromphenical for bacteria and Fluconazole for fungi.

Table 1: Antimicrobial activity of *Mango ginger* rhizome extract

Microorganisms	50 µl	100 µl	150 µl	Standard (30 µl)	Control (30 µl)
<i>Escherichia coli</i> (mm)	3.70±0.26	6.60±0.46	8.20±0.56	11.10±0.78	0
<i>Staphylococcus aureus</i> (mm)	3.10±0.22	5.50±0.37	7.10±0.50	10.80±0.76	0
<i>Candida albicans</i> (mm)	2.80±0.18	4.70±0.34	6.60±0.46	10.60±0.75	0

Values were expressed as Mean ± SD; Bacterial standard - Chloromphenical
Fungal standard - Fluconazole

Fig 1: Antimicrobial activity of *Mango ginger* rhizome extract



Antimicrobial activity of *Tagetes erecta* flower extract

The *in vitro* antimicrobial activity of the *Tagetes erecta* flower extract against these bacteria and fungi were qualitatively assessed by the presence of inhibition zones represented in the photographic Fig 2. The inhibitory activities in culture media of the *Tagetes erecta* flower reported in Table 2 were comparable with standard antimicrobial viz. chloromphenical and Fluconazole.

E. coli and *St. auerus*, which already known to be multi-resistant to antibiotics, were resistant to tested plant extract. The mean inhibition zone of *Tagetes erecta* flower extract was 2.30±0.16mm for 50 µl, 4.90±0.32mm for 100 µl, and 7.70±0.54mm for 150 µl for *E. coli*. The mean inhibition zone of *Tagetes erecta* flower extract was 1.60±0.11 mm for 50 µl, 3.70±0.26mm for 100 µl, and 6.80±0.48 mm for 150 µl for *St. auerus*. The mean inhibition zone for standard is 11.30±0.77 and 10.60±0.75 for *E. coli* and *St. auerus*

In addition, *C. albicans* was strongly influenced with a mean inhibition zone of 2.20±0.13 mm for 50 µl, 4.60±0.31 mm for 100 µl, 7.50±0.51 mm for 150 µl by *Tagetes erecta* flower extract and 10.70±0.76 mm for standard. This result is very interesting because *C. albicans* has been the most extensively studied pathogen in antifungal resistance because of their morbidity and mortality associated with infections in immunocompromised patients (Casalnuovo *et al.*, 2004; Redding *et al.*, 1993).

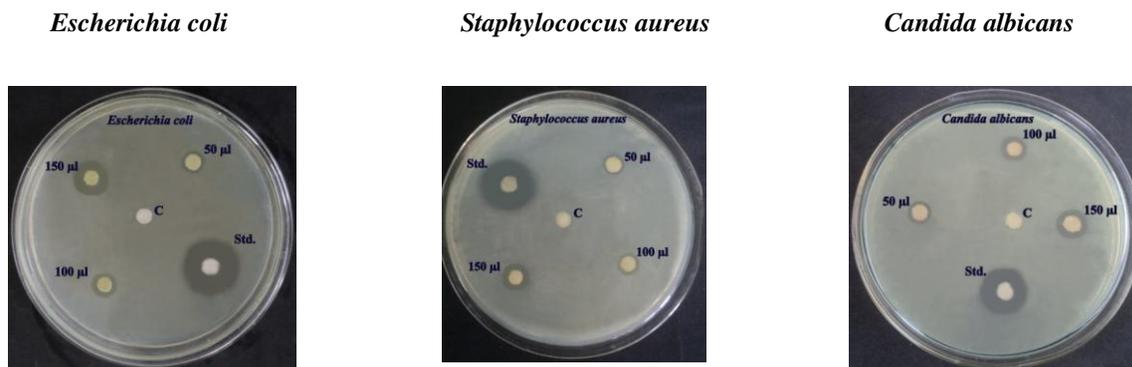
The results showed that the antimicrobial activity was directly proportional to the concentration of *Tagetes erecta* flower extract. The *Tagetes erecta* flower extracts hows highest antimicrobial activity was observed against *S. aureus*, when compared with *E. coli* and *Candida albicans*. The high doses (150µl) of *Tagetes erecta* flower extract possess similar activity to standard drug as chloromphenical for bacteria and Fluconazole for fungi.

Table 2: Antimicrobial activity of *Tagetes erecta* flower extract

Microorganisms	50 µl	100 µl	150 µl	Standard (30 µl)	Control (30 µl)
<i>Escherichia coli</i> (mm)	2.30±0.16	4.90±0.32	7.70±0.54	11.30±0.77	0
<i>Streptococcus pyogenes</i> (mm)	1.60±0.11	3.70±0.26	6.80±0.48	10.60±0.75	0
<i>Candida albicans</i> (mm)	2.20±0.13	4.60±0.31	7.50±0.51	10.70±0.76	0

Values were expressed as Mean ± SD
 Bacterial standard - Chloromphenical
 Fungal standard - Fluconazole

Table 2: Antimicrobial activity of *Tagetes erecta* flowerextract



4. DISCUSSION

Plants have basic nutritional importance by their content of protein, carbohydrate, fats and oils minerals, vitamins and water responsible for growth and development in man and animals. Phytochemical simply means plant chemicals. “Phyto” is the Greek word for plant. Phytochemicals are classified as primary or secondary constituents, depending on their role in plant metabolism. Primary metabolism is important for growth and development of plants include the common sugars, aminoacids, proteins, purines and pyrimidines of nucleic acids, chlorophyll’s etc. Secondary metabolism in a plant plays a major role in the survival of the plant in its environment. Attractions of pollinators, natural defense system against predators and diseases, etc., are examples of the roles of secondary metabolites (Sofowara, 1993).

The secondary metabolites formed also are an important trait for our food plants (taste, colour, scent, etc.) and ornamental plants. Moreover, numerous plant secondary metabolites such as flavonoids, alkaloids, tannins, saponins, steroids, anthocyanins, terpenoids, rotenoids etc. have found commercial application as drug, dye, flavour, fragrance, insecticide, etc. Such fine chemicals are extracted and purified from plant materials (Das *et al.*, 2010).

Antimicrobial activity of *Curcuma amada* rhizome (Mango ginger) and *Tagetes erecta* flower (Marigold)

Antimicrobial activity

Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country. Emergence of pathogenic microorganisms that are resistant/multi-resistant to major class of antibiotics has increased in recent years due to indiscriminate

use of synthetic antimicrobial drugs. In addition, high cost and adverse side effects are commonly associated with popular synthetic antibiotics, such as hypersensitivity, allergic reactions, and immunosuppressant and are major burning global issues in treating infectious diseases (Karaman *et al.*, 2003). This situation forced scientists to search for new antimicrobial substances with plant origin.

Methanolic extract of *Tagetes erecta* flower and *Mango ginger* rhizome extracts were screened against *Escherichia coli* and *Staphylococcus aureus* species of bacteria and *Candida albicans* species of fungi were evaluated using the standard agar disc diffusion method. The disc diffusion method is used to detect the antimicrobial activity of plant extract. The solidified Nutrient agar plates were swapped with the test organism and the samples were impregnated. After the incubation the zone was measured. The antimicrobial activity of plant extracts was detected by the indication of zone around the disc. The *in vitro* antimicrobial activity of the *Tagetes erecta* flower and *Mango ginger* rhizome extracts against these bacteria and fungi were qualitatively assessed by the presence of inhibition zones represented in the photographic Fig 4.19 and 20. The inhibitory activities in culture media of the *Tagetes erecta* flower and *Mango ginger* rhizome reported in Table 4.25 and 4.26 were comparable with standard antimicrobiotic viz. chloromphenical and Fluconazole. Among the two plants, *Mango ginger* rhizome extract possess potential activity than *Tagetes erecta* flower extract.

Pavithra *et al.* (2010) reported on antibacterial activity of *Delonix elata*, *Enicostemma axillare*, *Merremia tridentata*, *Mollugo cerviana* and *Solanum incanum* which are used in traditional Indian medicine for the treatment of various ailments like rheumatism, piles fever, skin diseases and snake bite. The antibacterial activity of organic solvent

extracts of these plants were determined by disc diffusion and broth dilution techniques against gram-positive bacterial strains (*Bacillus subtilis*, *Staphylococcus aureus*) and gram-negative bacterial strains (*Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*). Results revealed that the chloroform and methanol extracts of *D. elata* and methanol extracts of *M. cerviana* exhibited significant antibacterial activity against gram-positive and gram-negative strains with minimum bactericidal concentration (MBC) ranging from 1.5 to 100 mg/ml. Methanol extracts of *M. tridentata* exhibited activity only against gram-positive bacterial strains with MBC ranging from 12.5 to 100 mg/ml. Extracts of *E. axillare* and *S. incanum* showed activity only against *B. subtilis* and were not bactericidal at 100 mg/ml. The most susceptible organism to the organic extracts from all the studied plants was *B. subtilis* and the most resistant organism was *P. aeruginosa*. The presence of phytochemicals such as alkaloids, tannins, triterpenoids, steroids and glycosides in the extracts of these plants supports their traditional uses as medicinal plants for the treatment of various ailments. The present study reveals potential use of these plants for developing new antibacterial compounds against pathogenic microorganisms.

The *in vitro* antimicrobial activity of the *Tagetes erecta* flower and *Mango ginger* rhizome extracts against *Escherichia coli* and *Staphylococcus aureus* species of bacteria and *Candida albicans* species of fungi were evaluated using the standard agar disc diffusion method and were qualitatively assessed by the presence of inhibition zones. Among the two plants, *Mango ginger* rhizome extract possess potential activity than *Tagetes erecta* flower extract. These new findings may helpful to be applied in integrated control strategies to gain maximum impact on vector control.

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Source of support: Nil;

Conflict of interest: None declared