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(RESEARCH ARTICLE)



Synergistic antibacterial activity of *Murraya koenigii* and *Cynodon dactylon* against pathogenic strains

Patil Rupali Bhushan ^{1,*}, Chavan Pranita Sandip ¹ and Desai Deepa Mahendra ²

¹ Department of Pharmacognosy, N.S.S. College of Pharmacy, Maharashtra State Board of Technical Education, Mumbai, India.

² Department of Pharmaceutical Chemistry, N.S.S. College of Pharmacy, Maharashtra State Board of Technical Education, Mumbai, India.

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Abstract

The present study deals with the synergistic antibacterial activity of *Cynodon dactylon* and *Murraya koenigii*. Combined concentration of the drugs extracts was tested against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis* by agar well diffusion technique. The results of antibacterial activity revealed that the aq. extracts of *Cynodon dactylon* exhibited maximum to moderate inhibitory activity against all test pathogens. Maximum antibacterial activity by aqueous extract of *Cynodon dactylon* were exhibited against *Escherichia coli*, *Staphylococcus aureus* followed by *Bacillus subtilis* and *Pseudomonas aeruginosa* whereas aqueous extract of *Murraya koenigii* showed moderate sensitivity on all pathogens. Combined concentration of both drugs showed good antibacterial activity against all four pathogens.

Keywords: Antibacterial activity; Cynodon dactylon; Murraya koenigii; Agar well diffusion technique

1. Introduction

Now a days many infectious diseases have been known to be treated with herbal remedies as it has fewer side effects. Therefore, there has been increasing demand for the drugs from natural sources. *Murraya koenigii* and *Cynodon dactylon* are used in traditional medicine as a source of many therapeutic agents in the Indian culture and grows well in the tropical countries [11]. *Murraya koenigii* known a Curry leaves, locally also known as Karipatha, belonging to Rutaceae family are widely used as a medicinal herb and has characteristic aroma [1].

Murraya koenigii is commonly used as spice due to aromatic nature of leaves and traditionally used as antiemetic, antidiarrheal, blood purifier, antifungal, anti-inflammatory, for kidney pain and to prevent vomiting [2,3]. The Leaves are also a source of an essential oil which finds use in soap and cosmetic aromatherapy industry [4]. It is rich source of Carbazole alkaloids, Carbohydrates, Steroids and Flavonoids[5,6]. *Cynodon dactylon* is known as Durva grass and is a member of the Family Rutaceae. Some of the medicinal uses/actions of *Cynodon dactylon* are antiemetic, antidiabetic, diuretic, anti-inflammatory, hepatoprotective activity etc [15]. Its chemical constituents are beta Sitosterol, Beta Carotene, Vitamin C, Palmitic acid, triterpenoids, P-coumaric acid, Cyanogenic glucoside etc.

In the present investigation, attempt has been made to investigate combined antibacterial activity of aq. extract of *Cynodon dactylon* and *Murraya koenigii* against some pathogens.

^{*} Corresponding author: Patil Rupali Bhushan

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2. Material and methods

2.1. Collection of plant material

Disease free Leaves of *Murraya koenigii* were collected from the local market of Thane district, Maharashtra. The leaves of *Cynodon dactylon* were collected from the medicinal garden, Thane, Maharashtra. Leaves were washed thoroughly with tap water and then with distilled water in order to remove any dust, dirty particles present on the surface [7,8]. Then leaves were air dried in shade for preparation of extracts.

2.2. Aqueous extract preparation

The plant material of *Murraya koenigii* and *Cynodon dactylon* were shade dried and powdered by a mechanical grinder. The plant materials of *Murraya koenigii* and *Cynodon dactylon* about 20 gms each were sequentially extracted with 200 ml cold water by maceration process for 5 days at room temperature. The extracts obtained were filtered by using Whatman No.1 filter paper. The extracts were boiled to obtain a syrupy solution [13,16].

2.3. Selection of bacterial strains

Medicinally important bacterial pathogens used in this study were Staphylococcus aureus, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Escherichia coli* and were procured from the Microbiology department, Bharti Vidyapeeth Institute of Pharmacy, Navi Mumbai, India.

2.4. Test Microorganisms

Bacterial cultures *viz.*, Staphylococcus aureus, *Pseudomonas aeruginosa, Bacillus subtilis, Escherichia coli*, were maintained on Nutrient Agar (NA) slants at "4 °C". For further study, cultures have been grown in Nutrient agar slants for 24 hours as overnight cultures [14].

2.5. Preparation of inoculum

The pure cultures of bacteria were grown on nutrient agar slants and incubated at "37 °C" for 24 hrs. Nutrient broth and the slants were stored at "4 °C" and maintained in active state by regular sub-culturing for further use [12].

2.6. Standard reference antibiotic

The reference antibiotic used was Ciprofloxacin.

2.7. Drugs and chemicals

Ciprofloxacin, Agar, DMSO (Dimethyl sulfoxide) were used in the experiment.

2.8. Agar well diffusion method

The modified agar well diffusion method was employed [9]. The petri plates were washed and placed in a hot air oven for sterilization [10]. The Nutrient Agar was prepared and poured into different petri plates. The plates were made to solidify in a laminar air flow. Initially, the stock cultures of bacteria were revived by inoculating in broth media and grown at "37 °C" for 18 hrs. The agar plates of the above media were prepared, and wells were made in the plate. The wells were filled with the different concentrations of sample. The control wells were filled with Ciprofloxacin (0.5mg/ml). All the plates were incubated at "37 °C" for 24 hrs. and the diameter of inhibition zones were noted.

3. Results

Antibacterial activity results obtained in the present study revealed that aqueous leaf extract of *Murraya koenigii* and *Cynodon dactylon* tested individually possess potential antibacterial activity against bacterial species *viz., Escherichia coli*, Staphylococcus aureus, *Pseudomonas aeruginosa, Bacillus subtilis*.

The antibacterial efficacy of the extracts of *Murraya koenigii* and *Cynodon dactylon* leaves was quantitatively assessed based on Zone of inhibition in Cm by agar well diffusion method.

The antibacterial activity of both the leaf extract was compared with that of Ciprofloxacin standard against the tested microorganism.

Pathogen	0.5 mg/ml	40	mg/ml	0.5 mg/ml	Sample 1	0.5 mg/ml	Sample 2
	STD	С	Μ	STD	(1:2)	STD	(2:1)
Escherichia coli	4	2.8	1.8	3.8	2.4	3	2
Bacillus subtilis	3	1.1	1	3.4	2.7	3.8	1.8
Staphylococci	3	1.8	2.5	3.3	2.8	3.4	2.4
Pseudomonas aeruginosa	3.8	1.4	-	3.6	2	3.6	1.8

Table 1 Zone of inhibition in centimeter against the pathogens

Note: C- Cynodon dactylon M- Murraya koenigii Sample 1 - 1:2 (M:C) = 40mg/ml:80mg/ml Sample 2 - 2:1 (M:C) = 80mg/ml:40mg/ml

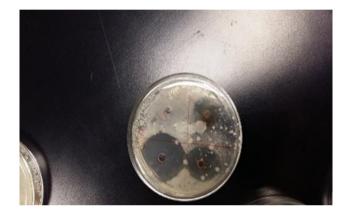


Figure 1 Zone of inhibition of both the drugs against Staphylococci



Figure 2 Zone of Inhibition of both drugs against Escherichia coli



Figure 3 Zone of Inhibition for Sample 1 and sample 2 against Staphylococci and Escherichia coli respectively

4. Discussion

In the present investigation, aq. extract of both the drugs tested individually showed moderate zones of inhibition at the conc. of 40 mg/ml against pathogens. So, combination of both the extracts was used for investigation and it was found to give better results than the individual effect.

Aqueous extract of *Cynodon dactylon* showed higher sensitivity to all pathogens, except *Bacillus subtilis*. For aqueous extract of sample 1 (at the ratio specified in the table) maximum zone of inhibition was found to be 2.7 Cm, 2.8 Cm, 2.4 Cm and 2 Cm at the concentration of 80 mg/ml of *Cynodon dactylon* and 40 mg/ml of *Murraya koenigii* of plant extracts against *Escherichia coli*, Staphylococcus aureus, *Pseudomonas aeruginosa* and *Bacillus subtilis*. Whereas aq. extract of sample 2 (at the ratio specified in the table) showed moderate sensitivity on all pathogens and zone of inhibition was found to be 2.4 Cm, 1.8 Cm, 1.8 Cm and 2 Cm at the concentration of 80 mg/ml of *Cynodon dactylon* of plant extract against *Escherichia coli*, Staphylococcus aureus, *Pseudomonas aeruginosa* and *Bacillus subtilis*. Whereas aq. extract of *Sumple 2* (at the ratio specified in the table) showed moderate sensitivity on all pathogens and zone of inhibition was found to be 2.4 Cm, 1.8 Cm, 1.8 Cm and 2 Cm at the concentration of 80 mg/ml of *Murraya koenigii* and 40 mg/ml of *Cynodon dactylon* of plant extract against *Escherichia coli*, Staphylococcus aureus, *Pseudomonas aeruginosa* and *Bacillus subtilis*.

The present study indicated that when the two drugs were tested at a dose of 40 mg/ml and 80 mg/ml in the form of combined extract, the synergistic antibacterial effect is observed. Further, when these two combinations were tried against different bacteria, sample 1 shows reasonably good activity compared to sample 2.

Compliance with ethical standards

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Disclosure of conflict of interest

There are no conflicts of interest.

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