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Antimicrobial activity of Neem, Clove, Curry leaves, Cardamom, Tulsi stem and Tulsi leaves

Sonali Sewani* **and Mahajbeen Qureshi**
Department of Biotechnology, Govt. Holkar Science College, Indore, MP, INDIA
sonalisewani@gmail.com

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Abstract

Antimicrobial activity refers to the ability of a substance to reduce or inhibit the growth of microorganisms. Different substances have different extent of antimicrobial activity. The present study deals with the antimicrobial activity of different samples like neem, clove, curry leaves, cardamom, tulsi stem and tulsi leaves. The antimicrobial activity of these samples has been determined against Escherichia coli and Aspergillus and Rhizopus species of fungi. In this work, the Escherichia coli was cultured on nutrient agar plates and fungi was cultured on Potato Dextrose Agar plates. The aqueous and ethanolic extracts of samples were prepared and introduced in these culture plates by ditch method and disc method. The zone of inhibition was observed and measured in each case after incubation of 24 and 48 hours.

Keywords: Neem (Azadirachta indica), Tulsi (Ocimum tenuiflorum), Clove (Syzygium aromaticum), Cardamom (Elletaria *cardamomum*), Curry (*Murraya koenigii*).

Introduction

Microorganisms are the organisms which can be seen only through a microscope and not naked eyes. Microorganisms include a wide range of bacteria, fungus, virus, protozoa etc.

These microbes may be useful as well as harmful for plants, animals, humans. These microorganisms also prove to be source of contamination in food and hence can cause various food and water borne diseases like typhoid, cholera, jaundice etc. This increases the importance of antimicrobial agents that kill or inhibit the growth of microorganisms. Some spices like clove, cardamom, turmeric etc and some medicinal plants like neem, tulsi, amla, aloevera etc also have some bioactive molecules and antioxidants that show both antibacterial and antifungal activity. These agents protect the host from cellular oxidation reactions.

In the present sudy, antimicrobial activity of the aqueous and ethanolic extracts of clove, cardamom, curry leaves, neem, tulsi stem and tulsi leaves have been determined against *E.coli*, *Aspergillus* species, *Rhizopus* species by Agar well diffusion method and Disc diffusion method. The diameter of zone of inhibition is measured and the antimicrobial activity of the sample was reported accordingly.

Cloves are the aromatic flower buds of a tree in the family Myrtaceae harvested as spices. These are about 2 cm long and consist of along calyx that terminates in four spreading sepals It has eugenol, caryophyllene, eugenylacetate, caryophyllene oxide, grerillin A, tri azolopyrimidine phthalazine derivative as its chemical components[1].

Cardamom is a spice widely used. The chemical constituents of cardamom are 1, 8-cineole (43.7%), α-terpineol (9.5%), terpinen-4-ol(3.2%), spathulenol (2.7%) and α-pinene (1.6%)[2].

Curry tree is a tropical tree with 2-4 cm long aromatic leaves. These leaves are often used in making dishes. The chemical components of curry leaves include linalool (32.83%), elemol (7.44%), geranylacetate (6.18%), mycrene (6.12%), alllo-ocimene (5.02%), α-terpiene (4.9%), β-ocimene (3.68%) and nerylacetate (3.45%) (3).

Neem is a fast growing evergreen tree having 20-40 cm long leaves. It has acetic acid, hydroxy piralic, phytol, 4-cyclooctenol, 1, 3 diphenyl-2-azafluorene, acetate, germnicol[4].

Tulsi (*Osimum tenuiflorum*) is an aromatic plant native to Indian subcontinent. It is a shrub having oleanolic acid, ursolic acid, rosamarinic acid, eugenol, carvacrol, linalool, β- elemene, germacrene as chemical constituents that have antimicrobial activity[5].

Materials and Methods

Requirements: This research work was carried out in the laboratory of Department of Biotechnology, Govt. Holkar Science College, Indore, M.P. in the period of June 2015.

Composition of media: Nutrient agar media – 28 gm in 1000 ml, *Constituents*[6]. *gm/lt.* Peptic digest of animal tissue: 5.0, Sodium chloride: 5.0, Beef extract: 1.50, Yeast: 1.50, Agar: 15.00, pH at 25°C = 7.2 – 7.6, Potato Dextrose agar – 54 gm in 1000 ml, *Constituents*: *gm/lt,* Potatoes in fusion form: 200.00,

Dextrose: 20.00, Agar: 30.00, pH at 25 ° C = 5.4 – 5.8.

Procedure: ***Preparation of sample*****:** *Drying and grinding:* The leaves of neem, tulsi, curry and stem of tulsi plant were washed and dried in sunlight and then grinded to form the powder. The clove and cardamom were also grinded to fine powder. These powdered samples were then stored in airtight bottles.

Preparation of aqueous extract: Aqueous extract was prepared by heating 10 gm of dried powder in 100 ml of distilled water (0.1 gm per ml) for six hours at slow heat. Two hours after removing from heat, it was filtered through eight layers of muslin cloth and centrifuged at 15000 rpm for 15 minutes. The supernatant was stored aseptically at 4°C[7].

Preparation of crude ethanolic extracts: The samples were extracted by ethanolic extraction by soaking 10gm of sample powder in 100 ml of 90% ethanol for four days at ambient temperature. The mixture was then filtered. The filterate was then concentrated on a rotary evaporator at 45°C for ethanol elimination and the extracts were kept in sterile bottles under refrigerated conditions until use[8].

Preparation of media: Nutrient agar media was prepared by dissolving definite amount of media in distilled water as per 28 gm in 1000 ml. Similarly, Potato dextrose agar was prepared by dissolving definite amount of media in distilled water as per 54 gm per 1000 ml. It was heated to boiling to dissolve the media completely[9]. It was then sterilized by autoclaving at pressure of 15 lbs (121°C) for 15 minutes. Mixed well and poured into sterile petriplates in a Laminar Air Flow.

Test Organisms: The *Escherichia coli* and fungus isolated from air was cultured on Nutrient Agar media and Potato Dextrose Agar plates respectively and characterized, identified. *E.coli* cells observed were circular and rod shaped, small sized, smooth, shiny non pigmented and tansluscent[10]. *Aspergillus* was black colored, cottony and fluffy in nature. The *rhizopus* was elevated, fluffy and smooth in nature with fine thread like structures, single sporangia.

Antimicrobial assay: The screening of ethanolic and aqueous extracts of the samples for antimicrobial activity was performed using two methods, the agar well diffusion and the disc diffusion method.

Agar well diffusion method: A volume of 20 ml of agar medium was inoculated with culture of indicator strain. In each agar plate, two wells of 4mm in diameter were punched. One of the well was filled with 10 µL of aqueous extract of sample, while the other well was filled with 10 µL of ethanolic extract of the sample. After holding the plates at room temperature for two hours to allow diffusion of extract into agar, the plates were incubated at 37°C for 48 hours. These plates were then examined for the inhibition of microbial lawn and diameter of the inhibition zones were measured after 24hours in case of *E.coli* and after 48 hours in case of fungus[8].

Disc diffusion method: The inoculum suspension of each microbial strain was swabbed on the entire surface of agar plates. Two sterile 5 mm filter paper discs (Whatmann filter no.1) were asceptically placed on agar surfaces and crude ethanol extracts was immediately added to one disc in volume of 10µL. The other disc was added by 10 µL of aqueous extract in the same manner. The plates were left at ambient temperature for 15 minutes to allow excess prediffusion of extracts prior to incubation at 37°C for 48 hours. Diameter of the zone of inhibition was measured after 24 hours in case of *E.coli* and after 48 hours in case of fungus[8].

Results and Discussion

The zone of inhibition for the ethanolic and aqueous extracts of clove, cardamom, neem leaves, curry leaves, tulsi stem and tulsi leaves was determined by agar well diffusion and disc diffusion method. The diameter of each zone was measured which are as follows:

Table-1
Diameter of zone of inhibition (millimeters.) after 24 hours

Sample	Agar Well Diffusion		Disc Diffusion	
	Ethanolic	Aqueous	Ethanolic	Aqueous
Clove	12.0	7.0	8.0	6.0
Cardamom	7.0	6.0	5.5	6.0
Curry leaves	18.0	5.0	9.0	5.0
Neem leaves	12.0	7.5	9.5	8.0
Tulsi stem	6.5	4.8	5.1	5.1
Tulsi leaves	7.0	4.0	6.0	5.0

Table-2
Diameter of zone of inhibition (in millimeters.) after 48 hours, E = Ethanolic, A = Aqueous

Sample	*Aspergillus*		*Rhizopus*	
	E	A	E	A
Clove	10.0	6.0	12.0	4.0
Cardamom	11.0	6.0	9.0	5.0
Curry leaf	8.0	5.5	10.0	4.5
Neem leaf	4.0	4.1	6.0	4.0
Tulsi stem	10.0	5.0	4.2	4.0
Tulsi leaf	4.8	4.3	6.0	4.0

Discussion: Escherichia coli: The zone of inhibition was measured after the duration of 24 hours in case of *E.coli*. Observations show that in case of ethanolic extracts inoculatd by well diffusion method and disc diffusion method, the zone of inhibition was maximum in case of curry leaves (18 mm and 9 mm) respectively. The ethanolic extract of neem (12mm and 9 mm) and clove (12mm and 8 mm) have similar activity. Likewise, cardamom (7mm and 5.5 mm) and tulsi leaves (7mm and 6 mm) exhibit similar antibacterial activity as depicted by their respective zone of inhibition. While, tulsi steams have least zone of inhibition (6.5 mm and 5.5 mm) amongst the ethanolic extracts of all the samples.

In case of aqueous extracts, neem leaves prove to exhibit maximum zone of inhibition i;e 7.5 mm and 8 mm in case of well and disc respectively. The zone of inhibition of clove (7 mm and 6mm) has a still lower value. Cardamom (6mm and 6mm) proves to be less effective than clove, while curry leaves (5mm and 5mm) have low zone of inhibition as compared to cardamom. Tulsi stem (4.8mm and 5.1mm) shows a greater antibacterial activity than tulsi leaves (4 mm and 5 mm) against *E.coli*.

Table-3
Diameter of zone of inhibition (in millimeters.) after 48 hours, E = Ethanolic, A = Aqueous

Sample	*Aspergillus*		*Rhizopus*	
	E	A	E	A
Clove	7.0	5.0	6.0	5.0
Cardamom	5.0	5.0	5.0	5.0
Curry leaf	6.5	5.1	5.0	5.0
Neem leaf	5.0	5.0	5.0	5.0
Tulsi stem	5.0	5.0	5.0	5.0
Tulsi leaf	5.0	5.0	5.0	5.0

Here are the images for zones observed in neem, clove, curry leaves, cardamom, tulsi stem and tulsi leaves respectively.

Aspergillus: The ethanolic extract of clove has maximum antifungal activity giving 11 mm and 5mm as zone of inhibition by well diffusion and disc diffusion method respectively. Clove and tulsi stem have an equal zone diameter (10 mm and 5mm), hence they have similar effectiveness. Curry leaves have a still lower value; 8mm and 6.5 mm. While, tulsi leaves (4.8 mm and 5. mm) have more antifungal activity than neem (4mm and 5 mm).

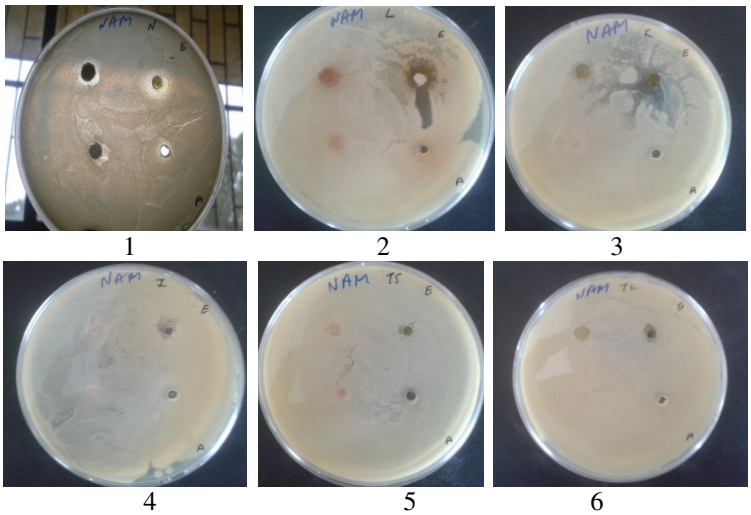
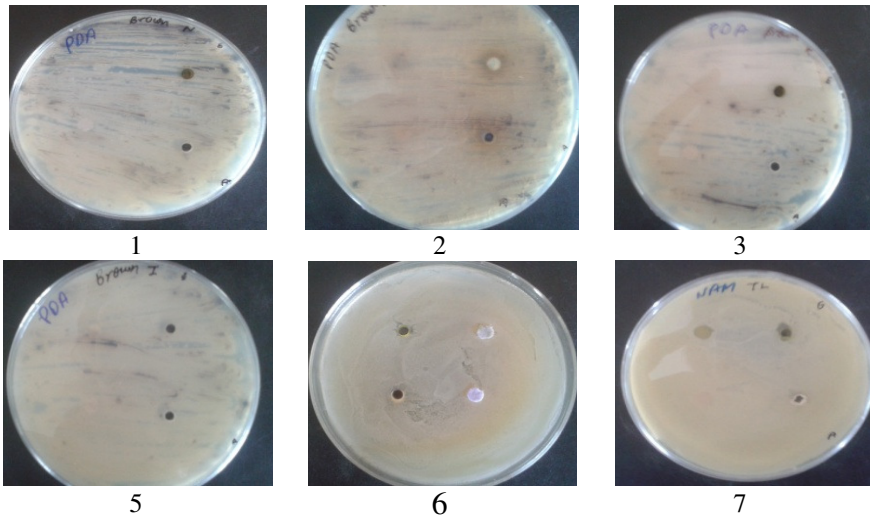


Figure-1
Zones of neem, clove, curry leaves, cardamom, tulsi stem, tulsi leaf in *Aspergillus*



1 2 3

5 6 7

For aqueous extracts, clove and cardamom have similar activity with 6mm and 5mm as the diameter of zones by well diffusion and disc diffusion method respectively. The decreasing order of the antifungal activity of the samples is curry leaves (5.5 mm 5.1 mm) ; tulsi stem (5 mm and 5mm) tulsi leaf (4.3 mm and 5mm); neem (4.1mm and 5mm).

Rhizopus: The observations show that among the ethanolic extracts of all the samples, clove has maximum antifungal activity against *rhizopus* with 12 mm as zone diameter for well and 6 mm for disc.

Next is the effectiveness of curry leaves (10 m and 5 mm) followed by cardamom (9 m and5 mm).

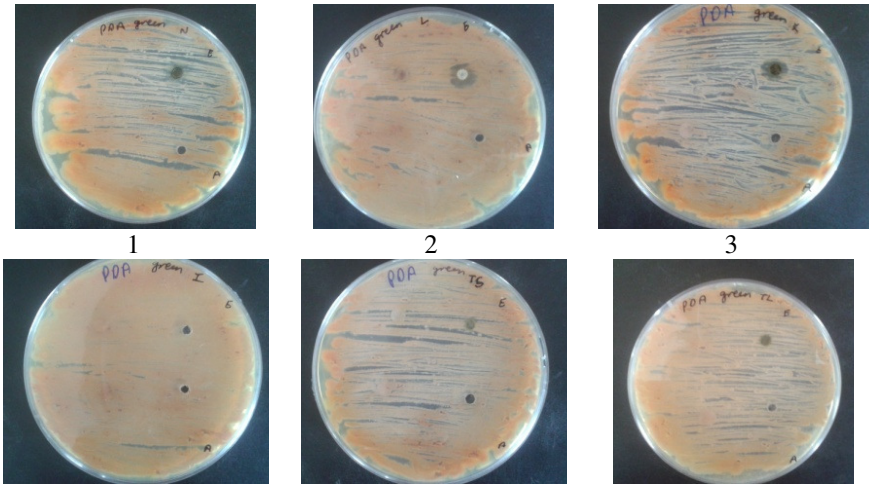
Neem and tulsi leaves exhibited similar activity with 6mm and 5 mm zone diameter by the two respective methods. Tulsi stem (4.2 mm and 5 mm) has the lowest antifungal activity against *rhizopus.*

In case of aqueous extracts, cardamom (5m and 5mm) gave best results followed by curry leaves (4.5 mm and 5 mm) and neem, tulsi stem, tulsi leaves and clove have similar efficiency with 4 mm (well diffusion) and 5 mm (disc diffusion) as zone diameter.

Observed zones in case of neem, clove, curry leaves, cardamom, tulsi stem and tulsi leaves are a follows:

Conclusion

Thus, after the entire study it can be conclude that, curry leaves have maximum antibacterial activity against *E.coli* whereas, clove proves to be most effective against the *rhizopus*. However, in case of *aspergillus*, cardamom exhibits maximum antifungal activity. Hence, the ethanolic extracts of these substances have the potential to be used as sanitizing agents and therapeutic agents.



1 2 3

4 5 6

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