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EVALUATION OF ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF *CYMBOPOGON CITRATUS* & *PSIDIUM GUAJAVA* FROM SIALKOT ORIGIN

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Abstract

Since few decades, Natural medications were used for the ailment of diseases in humans well as in animals. Natural medicines are preffered over allopathic medicines due to less side effects and toxicities in humans and animals and these were mainly derived from plants and animal source. In the recent era, new chemical compounds were extracted world widely from plants source that have potential therapeutic benefits and provide a base for the synthesis of different dosage forms. New chemical compounds were extracted from leaves of *Cymbopogon citratus* and *Psidium guajava* through process of shade drying and extraction through maceration. Different methods such as 96 well method and Agar Tube Dilution method were used to evaluate the antibacterial and antifungal activities of *Cymbopogon citratus* and *Psidium guajava*. In the present research, antibacterial and antifungal activities of specific concentration of methanolic extracts of *Cymbopogon citratus* and *Psidium guajava* against different strains of micro-organisms such as *Escheritia Coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeuroginosa*, *Salmonella typhi*, *Candida albicans*, *Trichophyton longifusis*, *Aspergillus flavus*, *Microsporrum canis* and *Fusarium solani* on the basis of percentage inhibition has been evaluated. We found that P. guajava have shown antibacterial activity against Bacillus subtilis and Staphylococcus aureus and shown percentage inhibition of 66.01% and 64.29% respectively, while C. citratus have shown characteristic percentage inhibition of 66.89% and 61.69% respectively. We also found that only P. guajava have shown antifungal activities against *Candida Albican* and *Microsporum conis* and shown percentage inhibition of 40% and 25%. While *C. citrates* has not any antifungal activity against strains of micro-organisms.

Keywords: *maceration, well method and Agar Tube Dilution method, strains.*

Introduction

Natural medications obtained from plants and animal source were used for the treatment of diseases in human beings and animals with minimum side effects and toxicities as compared to allopathic remedies. Ayurvedic medicines and new chemical compounds isolated from plants source have been used worldwide since 6000 BC and have a potential therapeutic benefits that provide a base for the synthesis of medicaments. In addition, people are relying more on natural products globally due to more safe and secure use of herbal drugs. As concerned with the treatment of several chronic and acute ailments, bacterial and fungal diseases are one of the major serious issue world widely, regarding to its therapy and adverse effects with different medications.2

Cymbopogon citratus is originated from lemongrass, which is also called lemon grass stalk and ropogon citrates, belongs to the family Poaceae and geographically found in Pakistan, Sri Lanka, South India. *Cymbopogon citratus* broadly cultivated in tropical areas of Asia and America.7 that grows up-to 6 feet height with short rhizomes. Size of leaves of lemongrass ranges from 0.5 to 1 inch wide, about 3 feet long, nice drooping tips, bluish-green color and feeling of excellent aroma flavor after crushing.9 This study primarily focused on the evaluation of antibacterial and antifungal activities of methanollic, chloroform and n-hexane extracts of C. citrates and its broad spectrum applications. Anti-bacterial and antifungal activities were evaluated by using different methods such as well plate method, diffusion method, dilution method and bioautographic method. In these methods, zone of inhibition of plant extracts were compared against different organisms such as Candida albicans, Trichphyton longifusus,Aspergilus flavus, Microsporum canis, Fusarium lini, Escheritia Coli, Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeuroginosa, Salmonella typhi.

Materials and Methods

Plant collection and extraction protocol of Cymbopogon citrates

Materials:

The aerial parts of *Cymbopogon citratus* were collected from Airport Nursery Sambrial, District Sialkot, West part of the Punjab, Pakistan. Herbarium specimen of the plant bearing voucher specimen # GC-Herb-Bot-2407 was deposited in Dr. Sultan Ahmed Herbarium, Department of Botany, Government College University, Lahore, Pakistan. 1

Method of Drying and extraction of drug

C. citratus was dried under shade and ground to obtain powder form. Dried powder (1000 g) was macerated with 5 L each of n-hexane, chloroform, and methanol for one week at room temperature. The bottles were gently shaken after every 12hr during the maceration process. The whole material was initially filtered through muslin cloth and subsequently with Whattmann's filter paper No. 1. The obtained filtrate was concentrated at 35 °C using rotary evaporator (IKA HB10 Basic, Made in Germany), which produced dark brown extract having semi-solid consistency. The percentage yield of each extract was calculated as; 1.52% for n-hexane extract, 3.2% for chloroform extract, and 8.4% for methanol extract. The extracts were stored at 4 °C for further use and doses were prepared freshly when required.9, 11

Psidium guajava:

Psidium guajava L. is a tiny 10 meter high tree belongs to the family of "Myrtaceae" with smooth, thin, and cracking bark. Leaves had oval blade with prominent pinnate veins (5 to 15cm long) and short-petiole. P. guajava is widely cultivated in Pakistan, native to Mexico and spread throughout the European, Asian, African and American cities. It preferably grows in dry climate but frequently cultivates in tropical and subtropical areas of the world. The common names of P. guajava in various countries are; amrood or amrut in Pakistan, English guava, in France called goyave or goyavier, guayave in German, banjiro in Japan, and goiabeiro in Portugal.4Leaves of P. guajava has been used for different therapeutic indications including; anti-diarrheal, antibacterial, hepatoprotective, antioxidant, and gastritis. Additionally, leaves extract can also be used in various pharmaceutical dosage form to treat sedative cough.6Due to numerous activities of P. guajava, we have focused on new activities such as anti-bacterial and anti-fungal on n-hexane, chloroform, and methanol extracts of the leaves.[6, 2]

Collection of plant and extraction procedure of P. guajava

The collection of P. guajava leaves was accomplished from a well-known garden (Pak garden) nearby vicinity in Sialkot city to Wazirabad Road, Pakistan. The voucher specimen GC-Herb-Bot-2408 issued by the Dr.

Sultan Ahmed Herbarium, Department of Botany, Government College University, Lahore, Pakistan on the deposit of herbarium specimen. Leaves were dried under shade for fortnight and made powder with electric grinder. Maceration of 1000g powder of plant material was done firstly with 5 liter n-hexane for one weak and stirred the flask mechanically twice a day for the whole week. The mixture was filtered through the muslin cloth first and then used whatman filter paper grade 1 for the further purification. The residue obtained after filtration was shade dried and followed the same procedure of maceration and filtration, firstly with 5 liter chloroform and subsequently with 5 liter methanol. All three filtrates were concentrated separately using rotary evaporator (IKA HB10 Basic, Made in Germany) at 35 °C which converted to dark brown gummy masses. The resulting percentage yield of each extract was determined as; n-hexane (0.83%), chloroform (2.3%), and methanol (18.2%). Extracts were labeled and stored at 4 °C for the further study and always freshly prepared solutions of each extracts were used when required.4, 11

Determination of Antibacterial activity by 96 Well Plate Method[8, 10]

In this method, Mueller hinton medium was used that facilitates the growth of Escheritia Coli, Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeuroginosa, Salmonella typhi. Mueller hinton medium was prepared by using the directions mentioned on the label. Initially inoculums of organisms were placed in 0.5 Mcfarland turbidity medium. On the other hand, standard stock solutions of different test compounds (plant extracts) were prepared in Dimethyl sulfoxide (DMSO) in ratio of 1:1. Transfer up-to 200µl prepared media in certain wells in triplicate manner. Now add compound in wells, control wells do not contain any test compound. Then add 5x106 cells in all wells including both control and test. All the plates were seal with liquid parafilm and incubate them for 18-20 hrs. After the incubation, Alamar Blue Dye was dispensed in each well and shaken at 80 RPM in a shaking incubator for 2- 3hrs. Finally cover the plates with aluminum foil and place them in shaking incubator. Change in color of Alamar Blue dye from blue to pink indicated the growth in bacterial strains. Then record the absorbance at 570 and 600nm by the ELISA reader.

Determination of Antifungal activity by Agar Tube Dilution

Agar tube dilution method was used to determine antifungal bioassay for screening. Following stages of screening were used for the test compound/ extract:

First screening (preliminary screening)

In the preliminary stage of the antifungal bioassay, the extract / test compound was screened against the following fungi:

- Trichophyton longifusis
- Candida albicans
- Candida glabarata
- Fusarium solani
- Microsporum canis
- Aspergillus flavus

If the extract/ test compound shows significant activity against the fungi mentioned above then it is further fractionated and screened.

Protocol of Agar Tube Dilution Protocol [3]

- Material
- Sabouraud dextrose agar (SDA) pH- 5.5-5.6
- Screw capped test tubes
- Micropipette (100-200 ul)
- Tips and tip box (Sterile)
- DMSO (Dimethyl sulfoxide)
- Glass vials

Methodology[5]

Preparation of test sample:

Dissolve 24mg of crude extract and 12 mg of pure compound in 1 ml sterile DMSO serving as stock solution.

Preparation of media:

Sabouraud dextrose agar (SDA) was used for the growth of fungus. Media with acidic (pH 5.5-5.6) containing relatively high concentration of glucose or maltose 2% is prepared by mixing 32.5 gm/500 ml D. water. Then it was heated to dissolve the contents and dispensed as volumes

4ml into screw caps tubes. Then steam sterilization was carried out by using autoclaved at 121° C for 15 min at 15psi.

Loading of sample:

Tubes were allowed to cool to 50° C and non-solidified SDA is loaded with 66.6 µl of compound pipette from the stock solution. This will give the final concentration of 400 µg/ml (Crude extract) and 200µg/ml of the media for pure compound respectively. Then tubes were allowed to solidify in slanting position at room temperature.

Inoculation of fungus:

Each tube was inoculated with 4mm diameter piece of fungus removed from a seven-day-old culture of fungus. For non-mycelial growth, an agar surface streak is employed.

Other media supplemented with DMSO and reference antifungal drugs used as negative and positive control respectively. Incubate the at 27-29° C for 3-7 days. During incubation, cltures were examined twice weekly. Growth in the compound amended media was determined by measuring linear growth (mm) and growth inhibition calculated with reference to the negative control.

Calculating % Inhibition of fungal growth:

$$\% \text{ Inhibition} = 100 - \frac{\text{linear growth in test (mm)}}{\text{linear growth in control (mm)}} \times 100$$

Results

Results of antibacterial activity of methanollic extracts of C. citratus and P. guajava on the basis percentage inhibition against different organism such as E.Coli, B. Subtilis, Staphylococcus, P.Aeriginosa and S. Typhi have been shown in the Table 3,4 and Figure 1,2. Upon inoculating 3000µg/ml concentration of compound on above mentioned stains, C. citratus have shown characteristic zone of inhibition against Bacillus subtilis and Staphylococcus aureus and shown percentage inhibition of 66.89% and 61.69% respectively, while 3000µg/ml concentration of standard drug (Ofloxacin) had shown percentage inhibition of 94.79% and 92.30% respectively Table 3 and Figure 1.

Upon inoculating 3000µg/ml concentration of compound (Methanollic extract of P. guajava) on above mentioned stains, P. guajava have shown characteristic zone of inhibition against Bacillus subtilis and Staphylococcus aureus and shown percentage inhibition of 66.01% and 64.29% respectively, while 3000µg/ml concentration of standard drug (Ofloxacin) had shown percentage inhibition of 94.79% and 92.30% respectively as shown in Table 4 and Figure 2.

It can be concluded that C. citratus have more antibacterial activity against B.Subtilis on the basis percentage inhibition as compared to P.guajava. SImilarly P.guajava have more antibacterial activity against Staphylococcus aureus on the basis of percentage inhibition as compared to C. citrates.

Results of antifungal activity of methanollic extracts of C. citratus and P. guajava on the basis percentage inhibition against different organism such as Candida albicans, Trichophyton longifusis, Aspergillus flavus, Microsporrum canis and Fusarium solani have been shown in the Table 5,6 and Figure 3. Upon inoculating 400µg/ml concentration of compound in DMSO on above mentioned stains, C. citratus have not shown any characteristic zone of inhibition against any above mentioned stains of micro-organism as shown in Table 5.

Upon inoculating 400µg/ml concentration of compound in DMSO on above mentioned stains at 27°C incubation time of 7days, methanolic extract of P. guajava have shown characteristic zone of inhibition against Candida Albican and Microsporum conis. Compound have shown percentage inhibition of 40% and 25% against test stains of Candida Albican and Microsporum conis respectively. Moreover standard drugs (Miconazole) have shown 98.8% and 98.1% minimum inhibition of zone upon inoculation of 400µg/ml concentration of compound in DMSO as shown in Table 6.

It can be concluded that C. citratus have no more antifungal activity against Candida albicans, Trichophyton longifusis, Aspergillus flavus, Microsporrum canis and Fusarium solani on the basis of percentage inhibition as compared to P.guajava. However P.guajava have more antifungal activity against Candida albicans and Microsporrum canis on the basis of percentage inhibition as compared to C. citrates.

Conclusion

C. citratus have more antibacterial activity against B.Subtilis on the basis percentage inhibition as compared to P.guajava. SImilarly P.guajava have more antibacterial activity against Staphylococcus aureus on the basis of percentage inhibition as compared to C. citrates.

C. citratus have no more antifungal activity against Candida albicans, Trichophyton longifusis, Aspergillus flavus, Microsporrum canis and Fusarium solani on the basis of percentage inhibition as compared to P.guajava. However P.guajava have more antifungal activity against Candida albicans and Microsporrum canis on the basis of percentage inhibition as compared to C. citrates.

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Name of the Fungus	Std. Drugs
Trichophyton longifusis	Miconazole
Candida albicans	Miconazole
Aspergillus flavus	Amphotericin
Microsporum canis	Miconazole
Fusarium solani	Miconazole
Candida glaberata	Miconazole

Table 1: Different standard drugs used against micro-organisms

CRITERIA	Percentage Inhibition Activity
0-39	Low
40-59	Moderate
60-69	Good
Above 70	Significant activity

(Janaki., et al 1998)

Table 2: Percentage inhibition activity

Name of Bacteria	Percentage inhibition of compound	Percentage inhibition of drug
Escheritia coli	No inhibition	91.88%
Bacillus subtilis	66.89%	94.79%
Staphylococcus aureus	61.69%	92.30%
Pseudomonas aeroginosa	No inhibition	96.38%
Salmonella typhi	No inhibition	96.87%

Table 3: Evaluation of antibacterial activity of Methanollic extract of *C. citratus against standard drugs*

Name of Bacteria	Percentage inhibition of compound	Percentage inhibition of drug
Escheritia coli	No inhibition	91.88%
Bacillus subtilis	66.01%	94.79%
Staphylococcus aureus	64.29%	92.30%
Pseudomonas aeroginosa	No inhibition	96.38%
Salmonella typhi	No inhibition	96.87%

Table 4: Evaluation of antibacterial activity of Methanollic extract of *P. guajava against standard drugs*

Name of Organisms	Linear Growth (mm)		%age Inhibition	Standard Drug	MIC (µg/ml)
	Sample	Control			
Candida albicans	100	100	0%	Miconazole	97.8
Trichophyton longifusis	100	100	0%	Miconazole	113.5
Aspergillus flavus	100	100	0%	Amphotericin B	20.70
Microsporum canis	100	100	0%	Miconazole	98.1
Fusarium solani	100	100	0%	Miconazole	73.50

Table 5: Evaluation of antifungal activity of Methanollic extract of *C. citratus against standard drugs*

Name of Organisms	Linear Growth (mm)		%age Inhibition	Standard Drug	MIC (µg/ml)
	Sample	Control			
Candida albicans	60	100	40%	Miconazole	98.8
Trichophyton longifusis	100	100	0%	Miconazole	113.5
Aspergillus flavus	100	100	0%	Amphotericin B	20.70
Microsporum canis	75	100	25%	Miconazole	98.1
Fusarium solani	100	100	0%	Miconazole	73.50

Table 6: Evaluation of antifungal activity of Methanollic extract of *P. guajava against standard drugs*

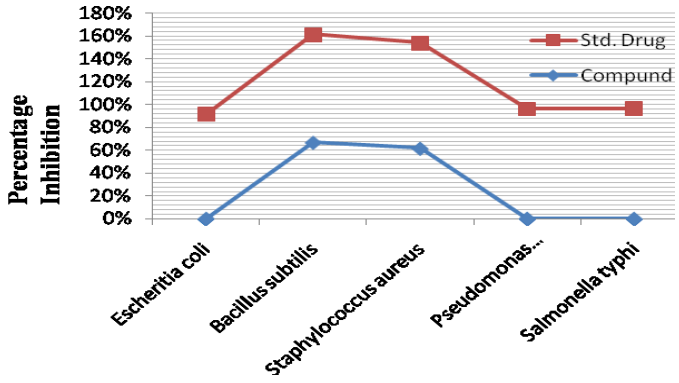

Figure 1: Evaluation of antibacterial activity of Methanollic extract of *C. citratus against standard drugs*

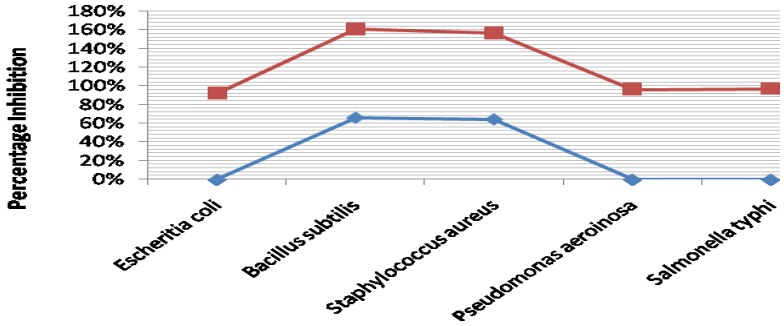

Figure 2: Evaluation of antibacterial activity of Methanollic extract of *P. guajava against standard drugs*

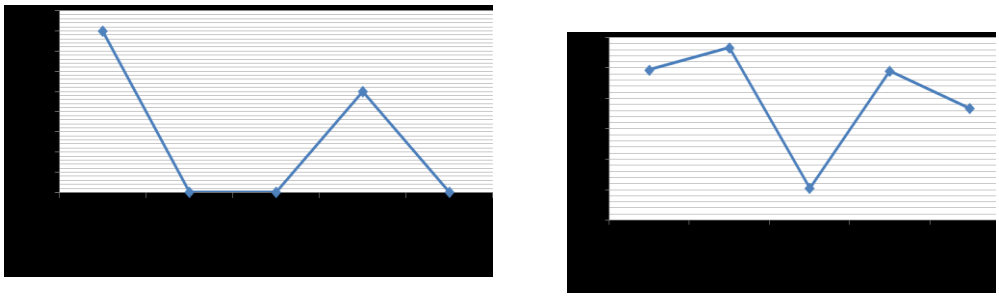

Figure 3: Comparative Evaluation of antifungal activity of Methanollic extract of *P. guajava against standard (MIC) drugs*