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In vitro Screening of *Psidium guajava* Leaf Extracts against *Fusarium oxysporum* and *Aspergillus niger*

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ABSTRACT
This experiment was conducted to find out the antifungal activities of leaves of Psidium guajava screened against two fungal species, viz. Fusarium oxysporum and Aspergillus niger by using spread plate and well diffusion assay method. It was observed that aqueous and ethanol extracts of leaf of P. guajava had no effect on Fusarium oxysporum, rather the fungus showed luxuriant growth on aqueous extracts might be due to presence of some phyto-constituents that favours the fungal growth in comparison to the control plates without plant extracts. Very less zone of inhibition of 3.37± 0.05mm was observed in alcohol extracts against F.oxysporum. At the same time aqueous and ethanol leaf extracts inhibits the growth of Aspergillus niger showed 06.34 ± 0.02 and 8.24 ± 0.02mm zone of inhibition respectively, The aqueous extract showed lower antifungal activity as compared to ethanol extract might be because of presence of dissolved phytochemicals in ethanol has inhibitory antifungal components against these fungal species.
Keywords: *Antifungal activities, Fusarium oxysporum, phytochemicals, Psidium guajava, zone of inhibition*

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INTRODUCTION

Anti-microbial activity is a process of killing or inhibiting the disease causing microbes. Antimicrobial may be anti-bacterial, anti-fungal or antiviral. They have different modes of action by which they are able to suppress the infection. A number of laboratory methods are used to evaluate the in vitro antimicrobial activity of an extract or a pure compound. The most known and basic methods are the disk-diffusion and broth or agar dilution methods. Plants synthesize secondary metabolites like tannins, terpenoids, alkaloids etc which have antimicrobial activities. Now a day there is a development in the research and promotion of plants based drugs. The interest of peoples has become increasingly towards herbal medicines rather the laboratory synthesized drugs. In this study leaves of *Pisidium guajava* were selected to study the antifungal activity against *Fusarium oxysporum* and *Aspergillus niger.*

Psidium guajava belongs to family Myrtaceae, common name guava and it is believed that these are originated from the tropical South America and the subtropical areas like Egypt ,Asia, Hawaii, Florida .Among the genus *Psidium* constitutes about 150 species of small trees and shrubs, 20 species produce edible fruits and others are with inferior quality of fruits [1]. The guava tree is an evergreen small tree having leaves 2 to 6 inches long and 1 to 2 inches wide, opposite, simple, elliptic to ovate, green and aromatic when crushed and have pronounced veins [2]. Guava is about 4-12 cm long, round, oval , green in colour and turns yellow when it ripens [3]. The bark is smooth, mottled green and peels in thin flakes to reveal the attractive bony aspect of its trunk. Fruits are many seeded berries. The leaves of guava are very effective in cough and cold because guava is rich in ascorbic acid and iron due to which it reduces lungs congestion and mucous formation. It also keeps the respiratory tract free of any unfriendly pathogen. These components also found to cure influenza [4]. By the treatment with leaf extracts of guava the dental plaques which is the main cause of periodontitis can be prevented [5]. Guava extract acts against oral plagues without disturbing oral cavity homeostatis. It also prevents bacterial infection of the oral cavity and also discourages plague formation [6]. The ethyl acetate extract of guava inhibits Ig E mediated allergic reactions by blocking Fc E Ri signaling [7]. The leaf extract of guava has anti-inflammatory, antimicrobial, hepato-protective and anti-oxidant activities because of presence of phenolic compounds [8]. The guava leaves and fruits have the capacity to lower blood sugar levels of human. Therefore, the leaves are peeled and the fruits are taken in empty stomach which works against diabetes [9]. Leaves can be chewed directly to get instant relief from toothache [10]. There is a most

important antioxidant named lycopene present in guava that plays a great role in preventing cancer [11]. The leaves of guava have alkaline nature, so it prevents against the hyperacidity of stomach [12]. The guava leaves and fruits contain plenty of flavonoids and saponins, which are more effective against the ulceration of stomach [13]. Guava leaves have capacity to lower the blood sugar level and also its fruit shows antidiabetic activity when eaten without skin. The high fibres of guava slow down the absorption of glucose from the gut as a result it prevents the brisk rise in blood sugar level after a meal [14]. Quercetin is present in guava and it has the capacity to show antibacterial activity against the pathogens which are responsible for periodontitis [15]. The seeds of guava have powerful laxatives which helps a lot in chronic constipation and cleansing. It was also reported that guava fruits are rich in dietary fiber and vitamin c which helps in digestive disorders [16]. Guava leaves have wound healing capacity, as they contain tannins and flavonoids which are known for fast healing of wound [17]. Phytochemicals like phenol, tannin, saponin, flavonoids, triterpenes, steroids, amino acids, proteins are present in rich amount in guava [18]. This study was undertaken to know the anti-fungal properties of *P. guajava* against fungal pathogens *Fusarium oxysporum and Aspergillus niger.*

MATERIAL AND METHODS

Selection of Plants and Plant Parts

Psidium guajava plant was selected to explore the presence of bioactive compounds showing anti-fungal activity. The leaves of *P. guajava* were collected from Centurion University of Technology and Management, BBSR, Khordha.

Preparation of Leaf Extract

The collected leaf samples were shade dried at room temperature and powdered after drying using mechanical devices. The powdered material of the experimental plant was kept in a plastic zip lock bags and stored in a cool place until they are taken to laboratory for extraction.

Aqueous Extract

The aqueous extract of selected dried plant leaves was prepared by taking 50 ml of distilled water and 5grams leaf powder in a 250 ml sterile conical flask sealed with sterile cotton plug and was kept on shaking incubator for 24 hours. Then these solutions were filtered by using what man filter paper. This process was continued for 3 times to get a clear aqueous extract. Then these extracts were collected in screw capped bottles

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Fig. 1 Fresh leaves of *Psidium guajava*

Fig. 2 Shade dried leaves of *P. guajava*

Fig. 3 Powdered leaves of *P. guajava*

Ethanol Extract

The ethanol extract of selected dried plant leaves was prepared by taking 5grams of powdered leaf samples of P. *guajava* with 50 ml of ethanol. This mixture was kept in a 250 ml sterile conical flask tightly plugged by cork to avoid evaporation. After 24 hrs, extract was collected by using what man filter paper and stored in collected in screw capped bottles. This process was repeated for 2 times to get a clear ethanol extract.

Collection of Fungal Pathogen

The extracts of experimental plant parts were screened for antifungal activity against two species viz., *Fusarium oxysporum* and *Aspergillus niger*. These two species were obtained from Rama Devi Women's University, BBSR, Khordha. The fungal species were sub cultured using potato dextrose agar

(PDA) medium. Antifungal activity was done using slight modification of standard methods of agar well diffusion assay [19] and spread plate method.

Preparation of Culture Media

Potato dextrose agar medium was used for antifungal susceptibility test. Potato dextrose agar medium was prepared by taking 10 gm of potato dextrose agar and 500 liter distilled water , stirred well and autoclaved at 121^0C for 20 minutes. After autoclaving, it is allowed to cool. Then the medium was poured into sterilized petridishes with a uniform depth of 4mm.

Agar Well Diffusion Assay and Spread Plate Assay

Agar well diffusion method [19] was followed to test the antifungal activity of extracts of experimental plant parts against the two fungal species. Potato dextrose agar medium plates were prepared as per manufacturer's instructions. For well diffusion assay wells of about 6 mm were made using sterile borer. Ethanol and aqueous extracts were added by sterile syringes into the wells separately and allowed to diffuse at room temperature for 2 h. Plates were incubated at 35 ± 2°C for 18-24 h. For spread plate method, 2ml of extracts were poured separately for each extracts over the plates uniformly using a sterile spreader in a laminar hood. Then both are allowed to solidify. After media became solid, fungal strain (*F. oxysporum* and *A. niger*) was inoculated on the middle of two petridishes separately. After 24 hrs growths were observed. At the same time petridishes having PDA media were inoculated with the selected two fungal strains as control. Comparison of the growth of fungal strains between control, ethanol and aqueous extracts were observed. Triplicates were maintained and the experiment was repeated thrice. For each replicates the zone of inhibition readings were taken (in mm) and the mean values were recorded.

RESULTS AND DISCUSSION

Literature data revealed that both the fungal species are pathogenic to both plants and human beings and can grow within temperature ranges from 25-35^{0}C (Table 1).

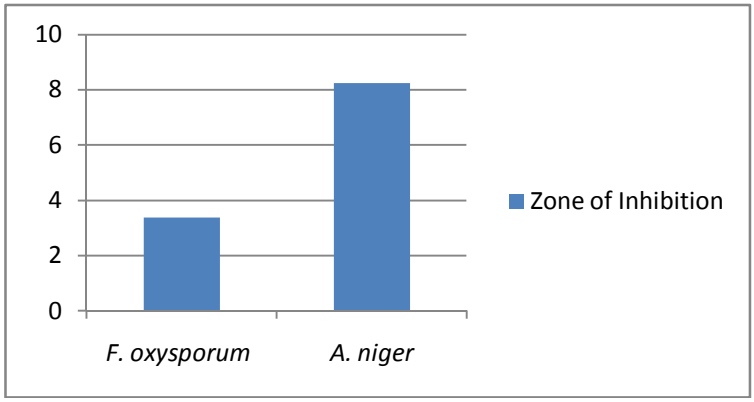
TABLE 1: Growth characteristics of *Fusarium oxysporum* and *Aspergillus niger*

Properties of Fungal Species			
Fungal species	**Properties**	**Media**	**Optimum Temperature (^{0}C) for growth**
Fusarium oxysporum	Pathogenic	PDA media	25-35
Aspergillus niger	Pathogenic	PDA media	25-35

TABLE 2: Effect of leaf extracts of *P. guajava* on growth of *Fusarium oxysporum* and *Aspergillus niger*

Zone of Inhibition (mm) of *P. guajava* Leaf			
Fungal species	**Aqueous extract**	**Ethanol extract**	**Growth in Control**
Fusarium oxysporum	No zone of inhibition and luxuriant growth seen covering the entire petriplates	3.37± 0.05	Growth occurs
Aspergillus niger	06.34 ± 0.02	8.24 ± 0.02	Growth occurs

Data recorded in Table 2 showed that aqueous and ethanol extracts of leaf of *P. guajava* had no effect on *Fusarium oxysporum*, rather the fungus showed luxuriant growth on aqueous extracts might be due to presence of some phyto-constituents that favours the fungal growth in comparison to the control plates with no plant extracts. Very less zone of inhibition of 3.37± 0.05mm was observed in alcohol extracts against *F.oxysporum.* At the same time aqueous and ethanol leaf extracts inhibits the growth of *Aspergillus niger* showed 06.34 ± 0.02 and 8.24 ± 0.02mm zone of inhibition respectively, which was found to be correlated with the findings of [20]. According to them tannin extracted from leaf of *P. guajava* has been functioning as antifungal at low concentration of 0.1%. *Fusarium* species can cause mycotoxicocity in human beings following ingestion of food and can cause local infections like septic arthritis, endophthalmitis, osteomyletis, cystitis and brain abscess. The fungus can be the cause for invasive or disseminated diseases and affects immuno-compromised individuals. This species also is the causal organism for fusarium wilt of tomato, sweet potatoes, banana and legumes. These results revealed that *P. guajava* leaf extract cannot be taken as a source to treat these diseases as the plant extracts has no impact on growth of *F. Oxysporum*. In contrast to this, *P. guajava* leaf extract in both showed antifungal activity against *A. Niger*. Comparing in between the aqeous and ethanol extracs, more inhibiting capacity was found for ethanolic extract of leaves than aqueous extract.



[Fig. 1. Zone of inhibition of *F. oxysporum* and *A.niger*]

CONCLUSION

This study revealed that the ethanolic extracts contains more inhibitory substances than aqueous extract. It was observed that in both well diffusion and spread plate assay both ethanol extracts showed good response against these two species in comparison to aqueous extracts by inhibiting the growth of *A. niger*. This study makes the advantages of this plant as antifungal agent and can be used as a bio fungicidal foliar spray for the crops affected by *Aspergillus* species. Further research is being carried out for isolation of bioactive chemical constituents from the active fractions and their mode of action on microbial cells.

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CONFLICT OF INTEREST

There was no conflict of interest.

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