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Antifungal properties of *Psidium guajava* leaves and fruits against various pathogens

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Abstract: The present study was designated to evaluate the antifungal activities of ethanolic, methanolic, ethyl acetate and hot water extract from leaves and fruits of *Psidium guajava.* Compare to all parts, the fruits were showing best result and the zone of inhibition was obtained 29 mm in methanolic extract of fruits against *T. rubrum*. The antifungal activities of the extracts against fungus were tested by using agar well diffusion assay and the MIC values were determined by broth dilution assay. The hot water extracts showed lower antifungal activity as compared to methanolic, ethanolic and ethyl acetate extracts. The least concentrations were obtained 1.98 mg/ml in methanolic extract of fruits and ethanolic extract of leaves against *T. rubrum* and also 0.33 mg/ml for ethyl acetate extract of fruits against *T. rubrum*. The antifungal compound mainly found in *Psidium guajava* were tannins, phlobatannins, saponins, terpenoids, alkaloids and poly phenols.

Keywords: Antifungal properties, ethanolic, ethyl acetate and methanolic plant extract, MIC, zone of inhibition.

Introduction:

Present time the microorganisms have become resistance to many antibiotics due to increased use of drugs, which is decreasing efficiency of conventional medicines. So, it has become necessary to find out new antimicrobial agents. Prevention of pathogenic and spoilage microorganisms in food is usually achieved by using chemical preservatives but they are responsible for many carcinogenic and teratogenic attributes as well as residual toxicity and with growing concern of microbial resistance towards conventional preservatives, consumers tend to be suspicious of chemical additives and thus the exploration of naturally occurring antimicrobial for food preservations receives increasing attention. Present time medicinal plants being the effective source of medicines, either it can be modern or traditional medicines, the advantage of medicines are they are useful for health. WHO had given the remark that traditional medicines are safe treatment for the infections originated from microbial and non microbial origin [1] Some antibiotics do not have capability to treat diseases because of drug resistance capacity of pathogens [2]. The uses of herbal treatment are one of the possible ways to treat diseases caused by multi drug resistant bacteria. Though many pharmaceuticals industries have produced a number of antibiotics from several years but in many cases it was observed that the cultures were showing resistance against the medicines [3].

Psidium guajava is evergreen shrub native to tropical America that has neutralized in South East Asia. The part of guava has been reported the wide range of activity against the human ailments [4,5]. There are over 20 compounds have been reported present in leaves, stems, bark and roots of *P. guajava*[6-9]. Guava leaves were used to treat diarrhoea and stomach. The leaves were used in USA as an antibiotic in the form of poultice or decoction for wounds, ulcers and toothache. Guava fruits also contain vitamin C vitamin, iron calcium and phosphorus.

Guava plants contain some secondary metbolites. The roots were also rich in tannins. Guava plants contained phytochemicals. The leaves of guava were rich in flavonoids in particular quercetin, saponins, tannins, alkaloids anthraquinones, phlobatannins and cardiac glycosides. Much of guava therapeutic activity was attributed to these flavonoids. The flavonoids had demonstrated antibcterial activity. Guava also had antioxidant properties which were attributed to the poly phenols found in the leaves. Guava leaves were often boiled into a tea to treat diarrhoea on many pacific islands. In many of the developing countries the used of the plant drugs was increasing because modern life saving drugs and people spend 40-50% income in drugs for health care. Among ancient civilization, India had been known to be rich repository of medicinal plants. A wide spectrum of activities against a variety of human ailments found in guava leaf extract.

The present study is carried out by evaluation of antifungal properties of *Psidium guajava* against fungal pathogens. The used microorganisms were 4 fungus cultures- *A. niger, M. canis, T. rubrum* and *C. albicans* and also to check the phytochemicals present in sample which are responsible for antifungal activity.

Materials and methods:

Collection of plant:

The *Psidium guajava* leaves and fruits were collected from local area in Gomti Nagar, Lucknow.

Preparation of plant extract:

An extract is a mixture of phytochemicals from any plant which is obtained by extraction of specific parts of the plant. *Psidium guajava* leaves and fruits were washed with distilled water and kept in incubator at 37°C for 3-4 days and grinded into fine powder. Now plant material was dissolved in 70% ethanol and 80% methanol, ethyl acetate

and hot water (1:10); 1 g sample should be dissolved in 10 ml of solvent. Mixtures were kept in the dark for 3 days at room temperature in sterilized beakers wrapped with aluminum foil to avoid evaporation and exposure to sunlight was avoided. After 3 days, mixtures were filtered through whatman no.1 filter paper and kept it in incubator at 37°C till all solvents had completely evaporated from mixtures. Now all mixtures were dissolved in DMSO (Dimethyl sulfoxide).

Tested microorganisms:

Fungus cultures were obtained from IMTECH, Chandigarh. Subcultures were maintained by MRD LifeSciences, Lucknow. Fungus cultures were used; *Trychophyton rubrum, Microsporum canis, Aspergillus niger* and *Candida albicans.*

Antibiogram analysis:

The antifungal activity of *Psidium guajava* was evaluated against fungal strains in ethanolic, methanolic, ethyl acetate and hot water extracts by using agar well diffusion method [10]. Nutrient agar plates were prepared for all extracts, 20µl inoculum of each selected fungus was uniformly spreaded on agar plates with the help of glass spreader, after five minutes three wells approximately 5mm diameter was bored with the help of borer. The equal volume (50µl) of antibiotic (tetracycline), distilled water and plant extract were poured into the wells. The plates were incubated at 28°C for 24 hrs.-48 hrs.

Determination of minimum inhibitory concentration (MIC) of ethanolic, methanolic ethyl acetate and hot water extract:

The minimum inhibitory concentration (MIC) is defined as the lowest concentration of the antimicrobial agent that will inhibit the visible growth of a microorganism after overnight incubation at 37^0C in shaker Incubator [11,12]. MIC of all samples were determined by broth dilution method. A two fold serial dilution of the methanolic, ethanolic, ethyl acetate and hot water extracts were prepared and optical density was measured at 600 nm [13].

Phytochemical tests:

The leaves and fruits extracts were screened for some secondary metabolites like-saponins, tannins, alkaloids, anthraquinones, phlobatannins, flavonoids, terpenoids, reducing sugar and poly phenols.

Test for reducing sugar:

Take 1ml or 1gm of plant sample in a test tube and add 10ml deionized water then add few drops of Fehling solution (1ml Fehling solution A and B) and heat at 100^0C in a water bath. Brick red precipitate show positive result.

Test for tannins:

Take 2gm of aqueous extract in a test tube and add 2 drops of 5% ferric chloride, brown colour gives positive result.

Test for phlobatannins:

Take 2ml plant sample in a test tube and add 10 ml deionized water and boil at 100^0C with few drops of 1% HCl. Deposition of red precipitation gives positive result.

Test for saponins:

Saponins content is determined by boiling 1ml plant sample in 10 ml deionized water for 15 min. and after cooling the extract was shaken vigorously to record froth formation.

Test for terpenoids:

Take 5ml of aqueous extract and then add 2ml chloroform followed by addition of 3ml conc. sulfuric acid, observe the reddish brown interface for presence of terpenoids.

Test for alkaloids:

Take 1ml of aqueous extract in test tubes and add 2-3 drops of wagners reagent it gives orange red precipitation.

Test for flavonoids:

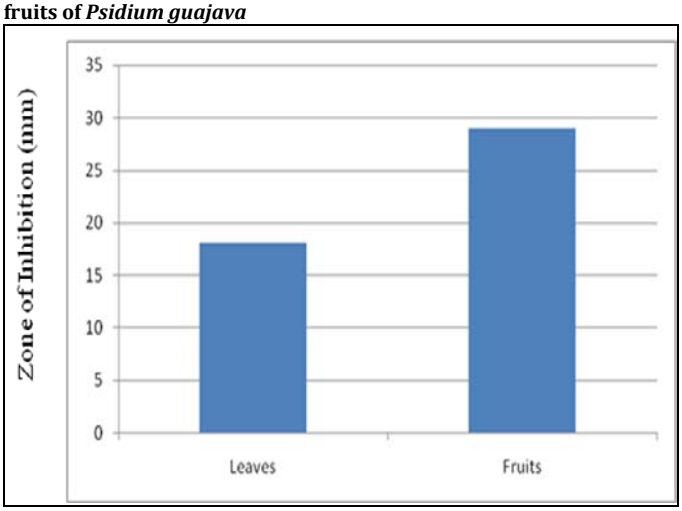
Take 1 ml of sample and add 1% NH_3 solution if yellow colour observed, showed presence of flavonoids then after this take ethanolic or aqueous extract and add 10 ml DMSO then heat it followed by adding Mg (magnesium chloride), add conc. HCl gives red color to confirmed flavonoids.

Test for poly phenols:

Take 2ml ethanolic extract of plant sample and add 1ml folin-ciocalteu reagent and 9ml of d/w. again add sodium carbonate solution (8ml), vortex to mix then kept test tube in dark and take O.D at 760nm.

Results:

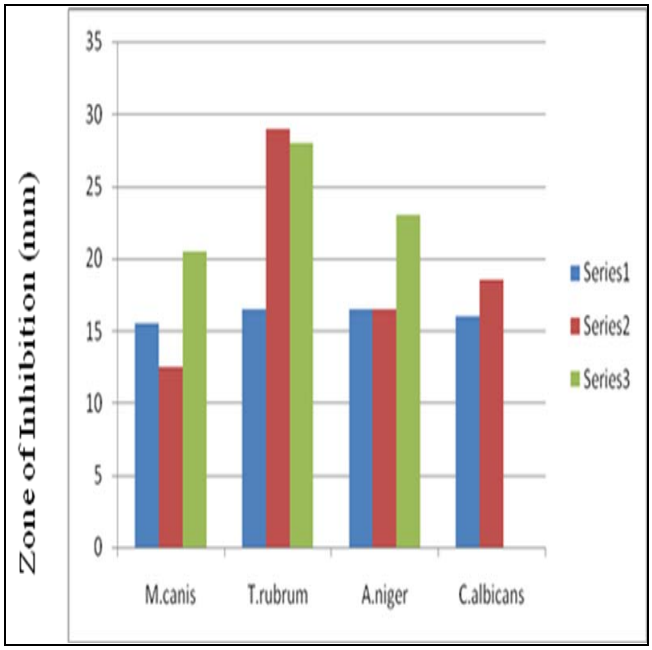
Graph 1: **Comparative analysis of antifungal activity of leaves and fruits of *Psidium guajava***



Graph 1 showed that the antifungal activity of *Psidium guajava* was higher in fruits compare to leaves.

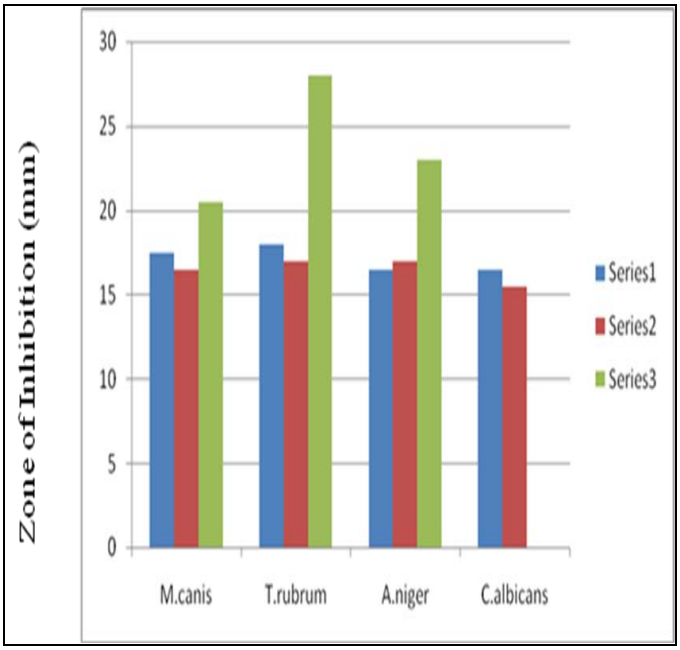
Table 1: **Antifungal activity in leaves and fruits (methanolic extract)**

Pathogens	ZOI of leaves sample (mm)	ZOI of fruits sample (mm)	ZOI of tetracycline (mm)
M. canis	15.5	12.5	20.5
T. rubrum	16.5	29	28
A. niger	16.5	16.5	23
C. albicans	16	18.5	0



Series 1= Leaves, Series 2= Fruits, Series 3= Tetracycline

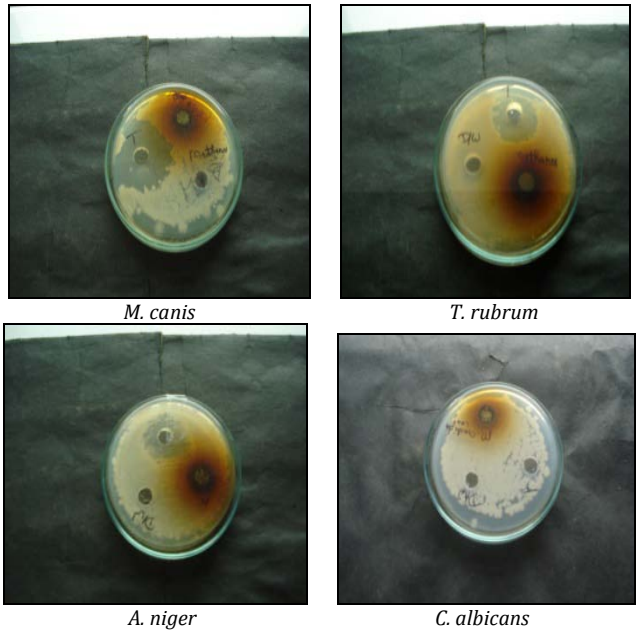
Graph 2: **Antibiogram analysis of methanolic extract of leaves and fruits against fungal pathogens**



Series 1= Leaves, Series 2= Fruits, Series 3= Tetracycline

Graph 3**: Antibiogram analysis of ethanolic extract of leaves and fruits against fungal pathogens**

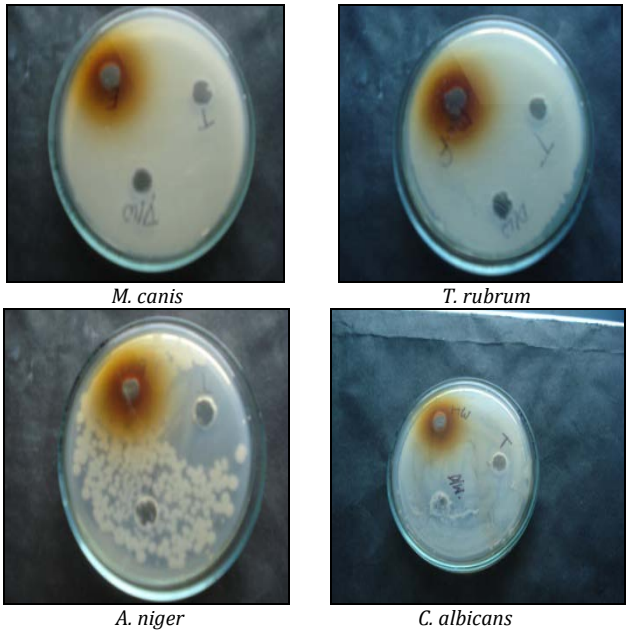
Figure 1: **Antifungal activity of methanolic extract of *Psidium guajava* fruits**



M. canis *T. rubrum*

A. niger *C. albicans*

Figure 2: **Antifungal activity of ethanolic extract of *Psidium guajava* leaves**



M. canis *T. rubrum*

A. niger *C. albicans*

Table 1, Graph 2 and Figure 1 showed that the maximum antifungal activity was found to be highest against *T. rubrum* in methanolic extract of fruits.

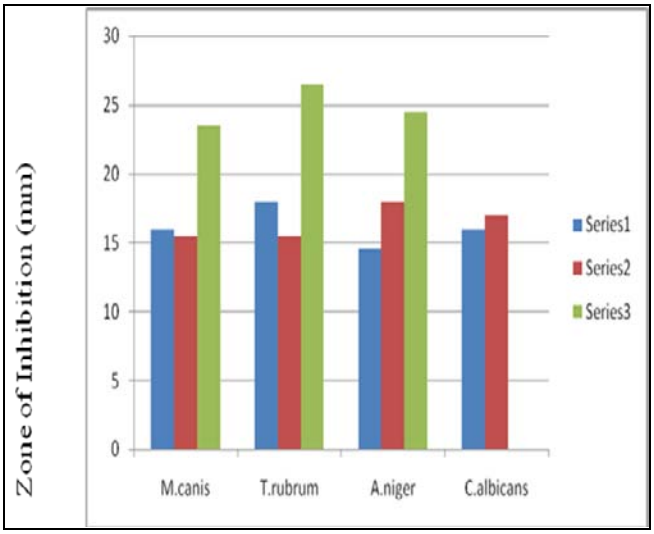
Table 2: **Antifungal activity in leaves and fruits (ethanolic extract)**

Pathogens	ZOI of leaves sample (mm)	ZOI of fruits sample (mm)	ZOI of tetracycline (mm)
M. canis	17.5	16.5	20.5
T. rubrum	18	17	28
A. niger	16.5	17	23
C. albicans	16.5	15.5	0

Table 2, Graph 3 and Figure 2 showed that the maximum antifungal activity was found to be highest against *T. rubrum* in ethanolic extract of fruits.

Table 3: **Antifungal activity in leaves and fruits (ethyl acetate extract)**

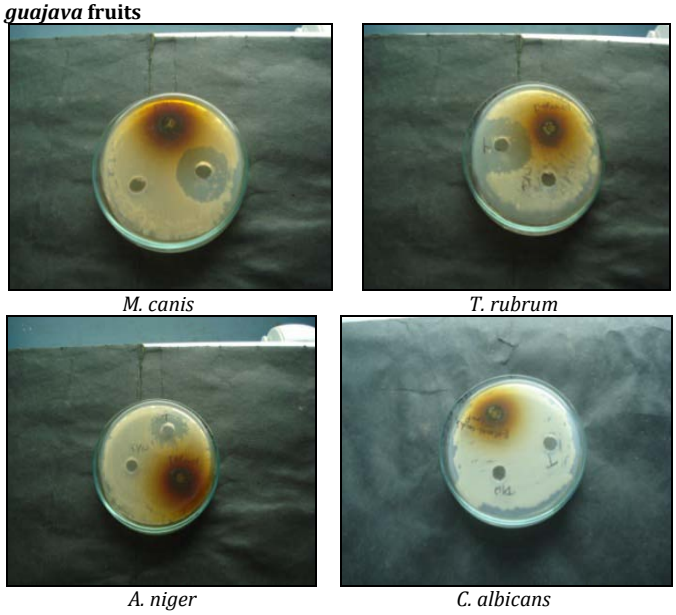
Pathogens	ZOI of leaves sample (mm)	ZOI of fruits sample (mm)	ZOI of tetracycline (mm)
M. canis	16	15.5	23.5
T. rubrum	18	15.5	26.5
A. niger	14.5	18	24.5
C. albicans	16	17	0



Series 1= Leaves, Series 2= Fruits, Series 3= Tetracycline

Graph 4: **Antibiogram analysis of ethyl acetate extract of leaves and fruits against fungal pathogens**

Figure 3: **Antifungal activity of ethyl acetate extract of *Psidium guajava* fruits**



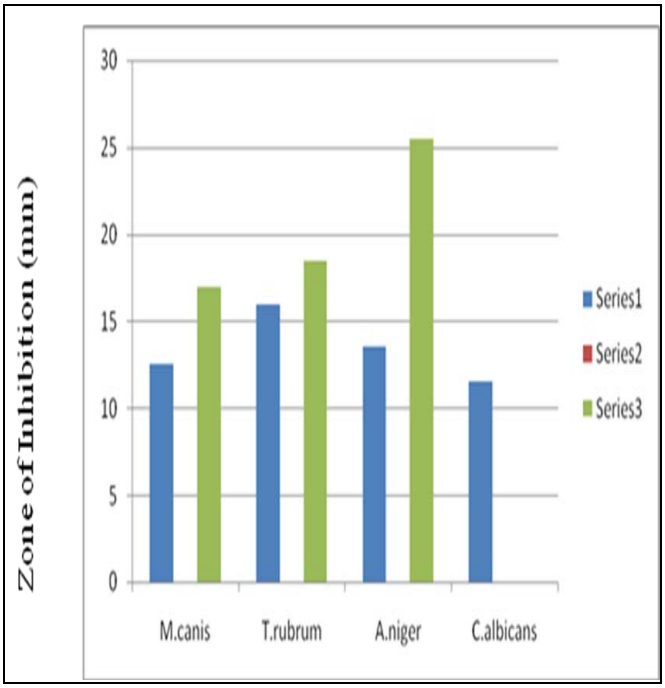
M. canis *T. rubrum*

A. niger *C. albicans*

Table 3, Graph 4 and Figure 3 showed that the maximum antifungal activity was found to be highest against *T. rubrum* in ethyl acetate extract of leaves and against *A. niger* in fruits.

Table 4: **Antifungal activity in leaves and fruits (hot water extract)**

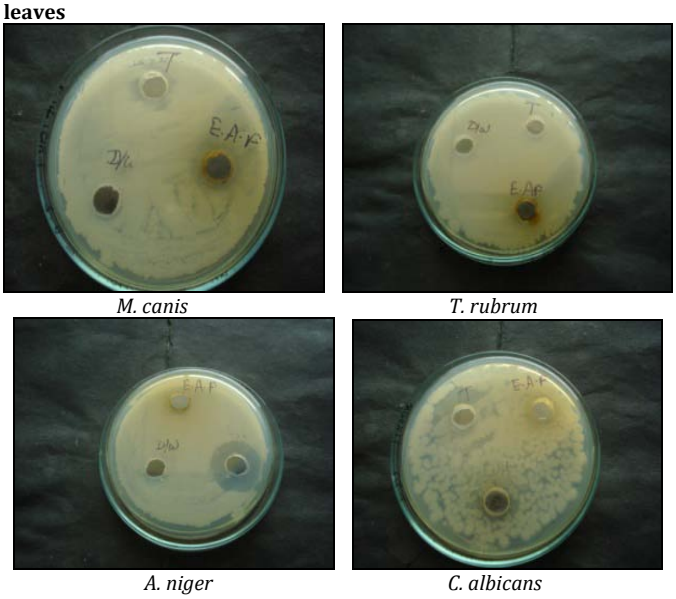
Pathogens	ZOI of leaves sample (mm)	ZOI of fruits sample (mm)	ZOI of tetracycline (mm)
M. canis	12.5	0	17
T. rubrum	16	0	18.5
A. niger	13.5	0	25.5
C. albicans	11.5	0	0



Series 1= Leaves, Series 2= Fruits, Series 3= Tetracycline

Graph 5: **Antibiogram analysis of hot water extract of leaves and fruits against fungal pathogens**

Figure 4: **Antifungal activity of hot water extract of *Psidium guajava* leaves**



M. canis *T. rubrum*

A. niger *C. albicans*

Table 4, Graph 5 and Figure 4 showed that the maximum antifungal activity was found to be highest against *T. rubrum* in ethyl acetate extract of leaves.

Table 5: **MIC value of fruits and leaves against fungal pathogens for solvents**

Test tube	Conc. of extracts (mg/ml)	Methanolic extract of fruits O.D against *T. rubrum* (600nm)	Ethanolic extract of leaves O.D against *T. rubrum* (600nm)	Ethyl acetate extracts of fruits O.D. against *T. rubrum* (600nm)
1	71.92	0.73	0.52	0.38
2	11.90	0.58	0.62	0.46
3	**1.98**	**0.42**	**0.34**	0.53
4	**0.33**	0.70	0.82	**0.40**
5	0.05	0.73	0.81	0.51
6	0.009	0.84	0.89	0.62

Table 5 showed that the least concentrations were obtained 1.98 mg/ml in methanolic extract of fruits and ethanolic extract of leaves against *T. rubrum* and also 0.33 mg/ml for ethyl acetate extract of fruits against *T. rubrum*.

Table 6: **Phytochemical Analysis**

Tests	Leaves	Fruits
Reducing sugar	-	+
Tannins	+	+
Phlobatannins	+	+
Saponins	+	+
Terpenoids	+	+
Alkaloids	+	-
Flavonoids	-	-
Poly phenols	+	+

+= Presence of phytochemical, - = Absence of phytochemical

Table 6 showed that the phytochemicals were present in leaves and fruits of *P. guajava*.

Discussion:

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the invitro antibacterial activity assay **[15].** Many reports are available on the antiviral, antibacterial, antifungal, antihelmintic, antimolluscal and anti-inflammatory properties of plants **[16-18]**. Some of these observations have helped in identifying the active principle responsible for such activities and in the developing drugs for the therapeutic use in human beings. However, not many reports are available on the plants for developing commercial formulations for applications in crop protection. In this present study the antifungal properties were found to be best in fruits of the *Psidium guajava* and compare to all solvents; ethanolic, methanolic and ethyl acetate extract were showing best result while the hot water extracts was showing minimum inhibition. The antibiogram analysis showed that zone of inhibition was observed 29mm against *T. rubrum* for methanolic extract. The least concentrations were obtained 1.98 mg/ml in methanolic extract of fruits and ethanolic extract of leaves against *T. rubrum* and also 0.33 mg/ml for ethyl acetate extract of fruits against *T. rubrum*. The antifungal compound mainly found in *Psidium guajava* were tannins, phlobatannins, saponins, terpenoids, alkaloids and poly phenols.

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