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Phytochemical Analysis of Different Plant Extracts and their Inhibitory Effects on *Aspergillus niger* and *Fusarium solani*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Plant extracts constitutes versatile phytochemicals which can be substituted with commercial synthetic chemicals to inhibit the growth of pathogens. In this study, the methanol and aqueous extracts of guava, jackfruit, tulsi, peppermint and eucalyptus leaves were evaluated in-vitro against Aspergillus niger and Fusarium solani isolated from Pomegranate and Guava respectively. Plant extracts were prepared using Soxhlet apparatus and condensed the extract by rotary evaporation. Highest yield was obtained in methanol plant extracts compared to aqueous extract, as it causes differences in the polarity of the extraction which leads to wide variation in the level of bioactive compounds in the extract. Poison food technique was used to test the antifungal activity of plant

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extracts. Jackfruit leaves methanol extract at 7000ppm showed maximum inhibition of 88.89% and guava leaves aqueous extract at 3000ppm showed minimum inhibition of 48.15% against Aspergillus niger. In case of Fusarium solani maximum inhibition was observed in jackfruit leaves methanol extract at 7000ppm of 87.89% and minimum inhibition was recorded in eucalyptus leaves aqueous extract of 57.41% at 3000ppm.The qualitative phytochemical screening of selected plant extracts showed various phytochemicals in plant extracts like tannins, phenols, flavonoids, terpenoid, saponin and alkaloid. GC-MS analysis of jackfruit methanol extract showed the presence of 9-Octadecenoic acid (Z)-, 2-hydroxy-1, Octadecanoic acid and Benzene, 1, 2-dimethoxy-4-(1-propenyl). All these findings implied the availability of various phytochemicals might be a source of antifungal agent for inhibition of pathogens and to improve the postharvest quality of fruits.

Keywords: Phenols; inhibition; methanol; plant extracts; bioactive.

1. INTRODUCTION

The use of plant extracts and essential oils as a natural alternative to commercial synthetic chemicals in the treatment of the major postharvest diseases of fruits and vegetables is getting prominence in recent days as they have already shown potential antibacterial, insecticidal, antioxidant and herbicidal activity for the agri-food sector [1]. Secondary metabolites such as flavonoids are implicated in plant resistance against diseases, flavones, flavonols and anthocyanins are crucial in attracting pollinating insects and fruit-eating mammals due to their attractive hues. They can easily adsorb and can express their effects at very low concentrations. The use of botanical pesticides works well with integrated disease management strategies to protect native biodiversity through healthy crops and natural enemies [2]. Additionally, the plant compounds change the structure of hypha and mycelia, preventing certain fungi, like *Aspergillus spp.* from producing substances like aflatoxin. Terpenes, phenols, alkaloids, tannins, and other phytochemicals present in botanical pesticides cause toxicity to fungal cell membranes, cell walls, and organelles [2]. Moreover, plant extracts are biodegradable, have minimal effects on non-target organisms and slow down the occurrence of resistance in pests [3].

Guava (Psidium guajava), where all parts of the plant have been utilised for various purposes, including hepatoprotection, antioxidant, antiinflammatory, anti-spasmodic, anti-cancer [4]. The "tree bread" jackfruit (*Artocarpus heterophyllus*) has been shown to contain a wide range of secondary metabolites, including flavonoids, carotenoids, prenylflavones, and sterols, among which are artocarpine, artocarpetine, norartocarpetine, morine, artonin, isocarpine, artocapesine, tannins, and sapogenins act as potential antifungal effects on postharvest pathogens (Hari et al., [5], Vazhacharickal et al*.,* [6]. Tusli (*Ocimum sanctum L*) also known as king of herbs is regarded as one of the most important sources of medicine and drugs. Furthermore, it exhibits anticancer, antifungal, antibacterial, antifertility, hepatoprotective, antispasmodic, antiemetic, analgesic, adaptogenic [7]. *Mentha piperita* (Lamiaceae), the peppermint plant is an aromatic perennial herb with antioxidants and antibacterial properties against a variety of gastrointestinal infections, also preferred in the food and cosmetic industries [8,9].

Thus, the current study focuses on gathering information and results from several studies on plant extract properties, with an emphasis on the screening the phytochemicals present in the plant extracts and to test their efficacy against major postharvest pathogens such as *Aspergillus niger* and *Fusarium solani.*

2. MATERIALS AND METHODS

Aspergillus niger and *Fusarium solani* were collected from Department of Plant Biotechnology, University of Agricultural Sciences, GKVK, Bangalore. Cultures were subcultures at the regular intervals using potato dextrose agar media at 25± 1℃ 50% relative humidity.

2.1 Collection of Plant Samples

The leaves samples of *Psidium guajava* guava and eucalyptus *(Eucalyptus globulus)* were collected from Department of Plant Biotechnology, GKVK, UAS (B). Jackfruit (*Artocarpus heterophyllus)* leaves were collected from Jackfruit DUS center, germplasm conservation block, UAS, GKVK, Bangalore, samples of Tulsi (*Ocimum tenuiflorum*) and mint

(*Mentha piperita*) samples were collected from Sanjeevini Vatika, Department of Horticulture, GKVK, UAS(B). The samples were then washed with running tap water to remove dirt, insects, and plankton before being dried in the shade for further work. The shade dried plants were blended using an electric mixer and kept in an airtight container.

2.2 Preparation of Plant Extracts Using Soxhlet Apparatus

Solvents used for extraction are methanol and water. For the preparation of extracts, same procedure followed by Mahira and Patil, [10].The extracts were then diluted with solvent to achieve the desired concentrations of 3000, 5000, and 7000 ppm. The resulting plant extracts were used for phytochemical analysis, pathogen growth inhibition assays, and biochemical analysis.

Percentage yield = Final weight of dry extract / Initial weight of dry powder *100

2.3 Testing the Antifungal Activity of Plant Extracts against *Aspergillus niger* and *Fusarium solani*

The efficacy of *Guava, Jackfruit, Tulsi, Mint and Eucalyptus* were evaluated against *Aspergillus niger* and *Fuasrium solani* under *in vitro* conditions on the PDA media using poison food technique. The required quantities of individual plant extracts 3000, 5000 and 7000 ppm of 500µl were added separately into molten and cooled PDA to get the desired concentration. The efficacy of a plant extract was expressed as per cent inhibition of mycelial growth over control that was calculated by using Adhikari et al., [11].

$$I = C - T \ / \ C * 100$$

Where,

I = Per cent inhibition of mycelium
C = Growth of mycelium in control
T = Growth of mycelium in treatment

2.4 Phytochemical Screening of Plant Extracts

Plant extracts from the soxhlet extractor and rotary evaporator were subjected to qualitative biochemical or phytochemical test of steroids, terpenoids, alkaloids, phenols, glycosides, saponins, flavonoids and tannins using standard procedures with slight modifications [7].

2.5 Quantitative Estimation of Total Phenol and Flavonoids in Plant Extracts

The crude extracts of *Guava, Jackfruit, Tulsi, Mint and Eucalyptus* were dissolved in distilled water (5mg/ml) were used for the estimation of total phenolics and flavonoids. Total phenols were estimated using protocol Jayan et al*.,* [12] and total flavonoids were estimated using the protocol mentioned by Chang et al., [13].

2.6 GC-MS Analysis

Phytochemical analysis using Gas Chromatography Mass Spectrometry**.** Basic principle of GC-MS is separation technique which will help in identify the phytochemicals present in jackfruit leaf extract. The same procedure followed (Kurian et al.,2018) with slight modifications.

2.7 Sample Preparation

Dried powdered (leaves) material of Jackfruit was subjected to the solvent extraction for 16 hours with the ethanol in soxhlet apparatus. 50 g of dried plant powder is extracted in 250 ml of solvent (methanol). Then the extract is evaporated to dryness. Mixed with ethyl acetate and hexane, charcoal filtration is done to remove the colour of the sample. It is condensed to 5ml.

2.8 GC-MS Analysis

Gas chromatography equipped with DB wax column which had thickness of (0.25mm), diameter (0.32mm) and length (30m) with inert mass spectrometer detector with triple –axis detection using helium as carrier gas, running in electron impact mode at 70 eV; Helium was used as carrier gas at a constant flow of 1ml / min and an injection volume of 2.0 µl was employed (split ratio of 10:1); injector temperature 28 ℃. The oven temperature was programmed from 50 °C (for 1 min.), with an increase of 6 ℃/ min to 280 ℃, then ending with a isothermal for 15 min at 280 °C.

2.9 Identification of Compounds

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST). The

spectrum of the unknown component was compared with the spectrum of the known components stored in the NIS library. The name, molecular weight and structure of the compounds of the test materials were ascertained. (Kurian et al., 2018).

2.10 Statistical Analysis

The collected data on various parameters were statistically analyzed using OP STAT statistical package program. Mean comparisons were made using factorial completely randomized design (FCRD) test at 1% probability level. All the data were statistically analysed by using analysis of variance (ANOVA) followed by Duncan's Multiple Range test (DMR) at Pd"0.01 [14].

3. RESULTS AND DISCUSSION

The yield residues of methanol and aqueous extracts after soxhlet and rotary evaporation. Methanol extracts showed the highest yield compared to aqueous extracts shown in the Table 1. Highest extraction was observed in Jackfruit 9% with methanol as the solvent while the lowest of 4% yield was observed when the solvent was water. The extraction method, temperature, extraction time, phytochemical makeup and solvent utilised all have a significant impact on extraction efficiency. According to the findings, one of the most important aspects was solvent. Results showed that different solvents resulted in various extraction yields because of differences in the polarity of the extraction solvents could cause a wide variation in the level of bioactive compounds in the extract [15]. A higher extraction yield was observed in methanolic extract compared to distilled water extract indicating that the extraction efficiency favors the highly polar solvents [15].

3.1 Evaluating the Efficiency of Plant Extracts *In-vitro* against *Aspergillus niger* and *Fusarium solani*

The methanol extract of jackfruit leaves inhibited fungal growth at the maximum possible speed of 88.89% at 7000ppm. Among the three treatments, 7000 ppm of methanol plant extract caused the greatest amount of colony development inhibition. The aqueous extracts of jackfruit leaf at 7000 ppm had maximum inhibitory effects of 77.78%. Hiyadati et al. (2016) used the jackfruit leaf extracts against *Aspergillus niger, Aspergillus terreus*, and *Aspergillus glaucus*. At 75% concentration it has shown 74.77 percentage of mycelia

Table 1. Plant extracts yield from different solvents

Plant Extracts	Methanol Extract	Aqueous Extract
Guava	8%	4%
Jackfruit	9%	5%
Tulsi	8%	5%
Pepper mint	7%	4%
Eucalyptus	6%	4%

Table 2. Effect of methanol and aqueous plants against colony growth of *Aspergillus niger* and *Fusarium solani*

Plant extracts	Mycelia inhibition % *(Aspergillus niger)*		Mycelia inhibition% *(Fusarium solani)*	
	Methanol Extract	Aqueous Extract	Methanol Extract	Aqueous extract
	7000ppm	7000ppm	7000ppm	7000ppm
Guava	75.93^{c} (60.59)	72.22^{b} (58.17)	83.33^{b} (65.88)	75.93^{b} (60.58)
Jackfruit	88.89^{a} (70.50)	77.78^{a} (61.85)	87.04^{a} (68.87)	77.78^{a} (61.85)
Tulsi	85.19^{b} (67.34)	70.37^{b} (57.00)	83.33^{b} (65.88)	75.93^{b} (60.59)
Pepper mint	72.22^{d} (58.17)	66.67^{c} (54.71)	81.48^{b} (64.49)	75.78^{b} (61.34)
Eucalyptus	75.93^{c} (60.59)	64.81^{d} (53.60)	77.78^{c} (61.85)	68.52^{c} (52.49)
Control 1	00.00	00.00	00.00	00.00
Control 2		93.45	94.34	94.34

Control 1- Distilled water, Control 2- Bavistin

(Figures in the parentheses are arc sine transformed values)

Values in same column with different superscript letters (a, b, c, d, e & g) differs significantly at p<0.01(DMRT)

inhibition. Antifungal activity of plant extracts indicates the rich source of phytochemicals mainly flavonoids, saponins and tannins which acts as antifungal compounds to prevent mycelia growth.

The methanol extract of jackfruit leaves inhibited fungal growth at the maximum possible speed of 88.89% at 7000ppm. Among the three treatments, 7000 ppm of methanol plant extract caused the greatest amount of colony development inhibition. The aqueous extracts of jackfruit leaf at 7000 ppm had maximum inhibitory effects of 77.78%, Ramaiah and Garamapalli, (2015) investigated the effects of different extracts of selected plants were observed for the percentage of mycelial growth inhibition of *Fusarium oxysporum f. sp*. *Lycopersici,* highest inhibition was showed in at the four tested concentrations viz., *Solanum indicum* (78.33%), *Oxalis latifolia* (70.33%), *Azadirachta indica* (75.00%) which helps to control the pathogen in eco-friendly and cost effective manner.

3.2 Qualitative Phytochemical Screening of Plant Extracts

Qualitative phytochemical screening of plants confirms the presence of various biologically active secondary metabolites and the present investigation was carried out to screen the presence of secondary metabolites. The aqueous and methanol extracts of guava, jackfruit, tulsi, mint and eucalyptus leaves showed the significant antimicrobial activity against the isolated pathogens *Aspergillus niger* and *Fusarium solani*. So, the phytochemical analyses were done for those extracts and were presented in the Table 3.

The phytochemical screening of aqueous and methanol extracts of guava, jackfruit, tulsi, peppermint and eucalyptus confirm the presence of different bioactive compounds. In case of jackfruit leaf methanol extract which showed the presence of tannins, phenols, flavonoids, terpenoids, saponins, glycosides and alkaloids. Aqueous extracts of jackfruit showed the presence of phenols, flavonoids, terpenoids, glycosides. This result was complementary to the results obtained by the methanol extracts of jackfruit which showed the presence of tannins, alkaloids, saponins, phenols which helps in exhibiting the maximum antimicrobial activity Vijaya poojitha and Devarakonda Ramadevi., [16]. In case of tulsi methanol extract phenols, flavonoids, terpenoids, saponins, glycosides and alkaloids were present, while in tulsi aqueous extract phenols, flavonoids, glycosides and alkaloids were present. Similar results were shown by Borah and Biswas, (2018).

3.3 Estimation of Total Phenol and Flavonoid Contents in Different Plant Extracts

The phenolic content and flavonoid content were estimated in methanol extract and aqueous extracts of guava, jackfruit, tulsi, peppermint and eucalyptus leaves using Folin Ciocalteu reagent as described in materials and methods [17,18].

From the Table 4 it was observed that the total phenolic content in methanolic extracts of plants was highest in methanol extract of tulsi leaf with 76.58 GAE mg/g of dry extract followed by jackfruit with 58.01GAE mg/g of dry extract and least content was found in eucalyptus leaf extract with 55.34 GAE mg/g of dry extract [19].

Table 3. Phytochemical screening of plant extracts

Phytochemicals	Guava		Jackfruit		Tulsi		Peppermint		Eucalyptus	
Solvents	**A**	**M**	**A**	**M**	**A**	**M**	**A**	**M**	**A**	**M**
Tannins	+	+	-	+	-	+	+	+	+	+
Phenols	+	+	+	+	+	+	+	+	+	+
Flavanoids	+	+	+	+	+	+	+	+	-	+
Terpenoids	+	+	+	+	-	+	-	+	-	+
Saponins	-	+	-	+	-	+	-	+	+	+
Glycosides	+	-	+	+	+	+	-	-	-	+
Alkaloids	-	+	-	-	+	+	-	-	+	-

A- Aqueous Extract. M- Methanol Extract; + sign indicates presence, - sign indicates absence

Table 4. Estimation of phenols and flavonoids in methanol plant extracts

Plant extracts (Methanol)	Phenols (GAE mg/g of dry extracts)	Flavonoids (mg/g of dry extracts)
Guava	69.78[c]	35.34[c]
Jackfruit	72.01[b]	51.98[b]
Tusli	76.58[a]	57.69[a]
Peppermint	65.34[d]	21.34[e]
Eucalyptus	55.34[e]	27.98[d]

Table 5. Estimation of phenols and flavonoids in aqueous plant extracts

Plant extracts (Aqueous)	Phenols (GAE mg/g of dry extracts)	Flavonoids (mg/g of dry extracts)
Guava	36.61[c]	15.78[e]
Jackfruit	41.48[a]	26.98[a]
Tusli	31.34[d]	16.91[d]
Peppermint	26.89[e]	18.67[c]
Eucalyptus	39.67[b]	19.58[b]

Table 6. GC-MS analysis of phytochemicals present in the jackfruit leaf methanol extract

Sl.No	Retention time	Name of the compound	Activity
1	15.77	1,3,Oxazolidinecarboxylic acid	Acidifier
2	15.66	Methyl N-acetylalanine, 3,3'-dithiobis-	Anticancer, NO-Scavenger, Neuroprotector
3	19.36	Hexadecanoic acid, methyl ester	Antioxidant, hypocholesterolemia, nematicide, pesticide, antiandrogenic, hemolytic, Acidifier
4	17.86	9-Aminofluorene	Insecticide, Psychotic effect, Neurotoxic effect
5	20.98	Phytol	Antinociceptive and antioxident
6	21.301	Octadecanoic acid	Helps in the synthesis of Antibiotics
7	21.94	9-Octadecenoic acid (Z)-, 2-hydroxy-1-	Antimicrobial, Anticancer, Diuretic, Antiinflammatory,
8	13.34	Quinoline, 7-propyl-	Antibacterial agent
9	19.39	1-Methyl-3,5-diisopropoxybenzene	Antibacterial, Antiviral, Antiseptic
10	21.84	Benzene, 1, 2-dimethoxy-4-(1-propenyl) -	Antibacterial,Nematicide Insect-attractant Perfumery, Flavour

Table 4 shows that the total flavonoid content was highest in jackfruit leaves in methanol extract with 41.98 mg/g of dry extract also in aqueous extract the total flavonoid content was highest in case of jackfruit leaves 26.98 mg/g. Similar content of total phenol about 368.61 µg/100g GAE and 162.92 µg/100g of flavonoids from the aqueous extracts of guava leaves (Redfern et al*.,* 2014). Pathak and Niraula ,2019 estimated the total phenol content in (*Ocimum sanctum linn*) tulsi leaves methanol extract was 180.21mg GAE/g and cholorofom extract

showed about 67.11 mg GAE/g which was more as compared *Ocimum tenuiflorum* [20]. The total flavonoids content was 67.11 mg/g of dry weight in methanol extract which showed highest phenol and flavonoids content compared to hexane, aqueous and hexane tulsi leaf extracts.
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In Table 5, total phenolic content was highest in aqueous extract of jackfruit leaf with 41.38 GAE mg/g of dry extract followed by eucalyptus leaves aqueous extract with 39.67 GAE mg/g of dry extract and least was observed in guava leaves with 15.78 GAE mg/g [21].

3.4 GC-MS Analysis

The volatile matter, long chain, branched chain hydrocarbons, alcohols, acids, esters, and other substances found in the extract can all be identified using the GC-MS technique. More than two hundred phytochemical components that might contribute to the plant's therapeutic properties were found in *A. heterophyllus* after a GC-MS study of the methanol extract. By comparing the retention indices with the mass spectral fragmentation patterns of the known compounds stored in the research library of the National Institute of Standards and Technology (NIST), it was possible to identify the chemical composition of the components in the extract. Based on peak area, molecular weight, and molecular formula, the phytochemical substances are identified. By comparing each component's average peak area to the sum of all areas, the relative percentage quantity of each component was computed. The components of the extracts were identified by name, molecular weight, and structure [22].

The presence of various bioactive compounds justifies the uses of the jackfruit leaves for various ailments. It is evident from the GC-MS analysis that jackfruit leaves possesses bioactive compounds such as Methyl N-acetylalanine, 3,3'-dithiobisHexadecanoic acid, methyl ester, Quinoline, 7-propyl-, Benzene, 1, 2-dimethoxy- 4-(1-propenyl) – may serve as a new potential source of medicines due to the presence of these phytochemicals and bioactive compounds having the antimicrobial property helped in inhibiting the growth of isolated postharvest pathogen that is *Aspergillus niger* and *Fusarium solani* [23,24]. Similar phytochemicals were obtained from the jackfruit leaves were screened for bioactive compounds which inhibited the growth of *Aspergillus nige* (43.56%) inhibition was observed (Kurian et al*.,* 2018).

4. CONCLUSION

The methanol and aqueous extracts of guava, jackfruit, tulsi, peppermint and eucalyptus leaves were evaluated *in-vitro* against *Aspergillus niger* and *Fusarium solani* isolated from Pomegranate and guava respectively. Plant extracts were prepared using Soxhlet apparatus and condensed the extract by rotary evaporation. Highest yield was obtained in methanol plant extracts compared to aqueous extract, as it causes differences in the polarity of the extraction which leads to wide variation in the level of bioactive compounds in the extract. Poison food technique was used to test the antifungal activity of plant extracts. Jackfruit leaves methanol extract at 7000ppm showed maximum inhibition of 88.89% and guava leaves aqueous extract at 3000ppm showed minimum inhibition of 48.15% against *Aspergillus niger.* In case of *Fusarium solani* maximum inhibition was observed in jackfruit leaves methanol extract at 7000ppm of 87.89% and minimum inhibition was recorded in eucalyptus leaves aqueous extract of 57.41% at 3000 ppm.The qualitative phytochemical screening of selected plant extracts showed various phytochemicals in plant extracts like tannins, phenols, flavonoids, terpenoid, saponin and alkaloid. GC-MS analysis of jackfruit methanol extract showed the presence of 9-Octadecenoic acid (Z)-, 2-hydroxy-1, Octadecanoic acid and Benzene, 1, 2-dimethoxy-4-(1-propenyl). All these findings implied the availability of various phytochemicals might be a source of antifungal agent for inhibition of pathogens and to improve the postharvest quality of fruits.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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