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Characterization of leaf extract of *Lawsonia inermis* L. by GC–MS analysis, and its efficacy on post harvest decaying fungi of *Psidium guajava* L.

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ABSTRACT

Different fungi affect *Psidium guajava* L. in the post-harvest period, causing considerable economic losses and risks to consumer health due to the mycotoxins that some of these fungi produce. Studies on the efficacy *of Lawsonia inermis* L. methanolic leaf extract were determined on causative agents of guava post-harvest fruit. Different concentration of *Lawsonia inermis* L. methanolic leaf extract (25, 50 and 75%) was used. The treatments were laid out in a completely randomized design (CRD) with three replications. *F. oxysporum, A. alternata, R. stolanifer, Gloesporium psidii* and *Phomopsis psiddi* were isolated and identified to be responsible for the Guava fruit rots. All concentrations of the tested leaf extract of *Lawsonia inermis* L. significantly suppressed the mycelial growth of the post-harvest decaying fungi. The effect was proportional to the concentration of the leaf extract and suppression was highest at 75% and lowest at 25% concentration. The study revealed that leaf extract of Lawsonia *inermis* L. proved to be effective in the control of postharvest rots of Guava and serves as a good option to synthetic agrochemicals which are not eco-friendly and biologically safe. The GCMS analysis study revealed the presence of 11 major bioactive compounds in which the presence of Phenol, Benzoic acid, Phytol, and Squalene are known to have antifungal activities.

Keywords: Anti-fungal, Post-harvest, *Lawsonia inermis* L., Leaf extracts, GCMS analysis.

INTRODUCTION

Nature has endowed guava or *Psidium guajava* L. with many essential nutrients fruit crop believed to have originated in Mexico or Central America (Arevalo-Marin *et al*. 2021). Guava has about 133 genera and more than 3,800 species in word but out of these five are edibles and remaining are wild and nonedible in test and quality (Nwinyi *et al.*,

2008; Shruthi *et al.,* 2013; Paull and Duarte, 2012). Guava fruits are typically 4 to 12 cm (1.6 to 4.7 in) long, round or oval, depending on the species. Guava cultivation is of great economic significance in many countries around the world, owing to its high yield and the variety of products derived from its fruit. (Morton JF, 1987) Guava is grown in over 60 countries and its worldwide production is estimated to be around 40 million tons (2020). (Irshad, *et al*., 2020), Maharashtra ranking at the top, the cultivated states are Satara, Pune, Beed, Aurangabad, Amravati, Nagpur, Buldhana under cultivation area and production of the crop with 3.6 thousand hectares yielding approximately 3.11 lakh MT guava fruit (Anonymous, 2014).

Fungal infection on the fruit may occur during the growing season, the harvesting, handling, transport and storage and marketing post-harvest conditions or after purchase by the consumer. Fruits contain high levels of sugars and nutrients and their low pH makes them vulnerable to fungal decay (Singh and Sharma 2007). The most prominent pathogenic fungal agents responsible for post harvest diseases of guava which attack fruits and cause considerable damage during transit, storage and final transportation to the market. Around 90-100% fruits have been found to be infected with fungi, namely, *Alternaria, Aspergillus, Colletotrichum, Fusarium, Penicillium, Pestalotia, Phytopthora* and *Rhizopus* etc. during transportation and storage periods (Chaube and Pundhir, 2005).

Though biological and other methods of controlling plant disease are effective. Control of plant diseases typically depends upon the application of chemical fungicides, despite their potentiality toxic effects on non-target organisms and the environment (Santos *et al*., 2008; Ferrer Alcon *et al*., 2009). Plant extracts are used effectively in controlling diseases caused by fungi Though the fungicides are the most common method to control of plant diseases, however their use is costly as well as environmentally undesirable (Song and Goodman, 2001).

Lawsonia inermis Linn. (Family: Lythraceae) which is commonly known as henna, mainly distributed in subtropical and tropical areas and is used in all over the world. The preliminary phytochemical analysis of the aqueous extract of *Lawsonia inermis* revealed the presence of carbohydrates, phenolic compounds, flavonoids, saponins, proteins, alkaloids, terpenoids, quinones, coumarins, xanthones, 6% fat, 2-3% resin and 7-8% tannins (Sharma *et al*., 2016).

The pharmacological studies showed that *Lawsonia inermis* showed antibacterial, antifungal, antiparasitic, and many other pharmacological effects. (Borade *et al*., 2011). Considering economical importance of *Psidium guajava* fungal pathogen associated with post harvest diseases that must be controlled to obtain desired quality and good yields. Therefore, studies on the effective control of fungal infection are highly requisite.

MATERIAL AND METHODS

Collection of Plant Material

Mature plant leaf of *Lawsonia inermis* L. were collected Millind College of Science, Campus Aurangabad, Maharashtra. The Plant identification and authentication was confirmed by Department of Botany, R.G. Bagdia Arts, S.B. Lakhotia Commerce and R. Bezonji Science College, *Jalna*.

Extract Preparation and Phytochemical Analysis

Plant leaf extract of *Lawsonia inermis* L. are used for estimation of GC-MS analysis. To obtain sample, the powdered plant material of leaves part was taken separately and subjected to Soxhlet extraction procedure (Redfern *et al.,* 2014). Preliminary phytochemical analysis was carried out for each solvent extract according to the standard procedure. (Evans *et al*., 2009; Yadav *et al*., 2011).

Gas Chromatography-Mass Spectrometry (GCMS) analysis

Gas Chromatography-Mass Spectrometry (GCMS) analysis GC-MS analysis of plant leaf extract (methanolic) was done at the Sophisticated Analytical Instrument Facility (SAIF) labs, MIT CARS, Department of agriculture Engineering College, Aurangabad, using standard GCMS model as explained below. The procedure followed was of Dandekar *et al*., 2015.

Instrument details,

The Shimadzu GC-MS analyzer (GC 2010 Plus, GCMS-OP2020) used was equipped with an automated gas valve with helium (He) as the carrier gas, quadruple detector, capillary column, flame ionization detector (FID), and a thermal conductivity detector (TCD).

The analyses were performed using a GC-MS system (GC-2010 plus) Shimadzu, Agilent Technologies Inc.,) equipped with an HP-5 MS capillary column (30 m × 0.25 mm, 0.25 mm, Agilent Technologies Inc.,). The injection volume of each sample was 1 µL. Helium (99.999%) was used as the carrier gas at a flow rate of 1 mL/min. The temperature of the injection port was 250 ^{0}C, and the column temperature program was as follows: 50 ^{0}C for 2 min, followed by an increase to 180 ^{0}C at a rate of 5 ^{0}C/min, an increase to 270 ^{0}C at a rate of 20 ^{0}C/min, and maintenance at 270 ^{0}C for 5 min. The MS (QP-2020) conditions included an EI ion source temperature of 230 ^{0}C, ionization energy of 70 eV, and a mass scan range of 40–500 amu.

Determination of antifungal activity

Five-millimeter diameter wells were cut from the agar using a sterile cork-borer, and 50 µl of the samples solutions of different concentrations (25%, 50%, and 75%) was subjected into the wells. Each Petri dish was inoculated at 28±2 o C for 7 days. The radial growth of the colony was recorded on 3rd, 5th and 7th day of intervals and % inhibition of mycelial growth was calculated over control. The tests were carried out in triplicate. The antifungal activity was evaluated by measuring the inhibition-zone diameter observed. (Huda *et al*., 2015; Ameera *et al*., 2015). The percent inhibition in growth due to different treatments at different concentrations was computed as follows:

Mycelial growth inhibition (%) = [(dc – dt) / (dc)] x 100

Where dc = average diameter of fungal colony in control and dt = average diameter of fungal colony.

RESULTS,

Preliminary Phytochemical Analysis

The outcomes of extracted contents of plant leaf extract (methanolic) tested for presence or absences of various phytochemicals (in qualitative form) are noted in Table 1. The results show that *Lawsonia inermis* L. plant contains a maximum ten types of phytochemical groups, such as Carbohydrates, Alkaloids, Flavonoids, Proteins, Phenols, Steroids, Glycosides, Saponins and Terpenoids.

Table 1. Phytochemical screening of methanolic extracts of *Lawsonia inermis* Linn results, where (+) sign indicates presence of corresponding phytocomponents.

Sr. No.	Test	Reagent	Result
1	Flavonoids	Shibita's reaction test	+
2	Phenols	Lead test	+
3	Steroids	Salkowski's Test	+
4	Glycosides	Borntrager's	+
5	Saponins	Froth test	+
6	Terpenoids	Salkowski test	+
7	Tennins	Ferric chloride test	+

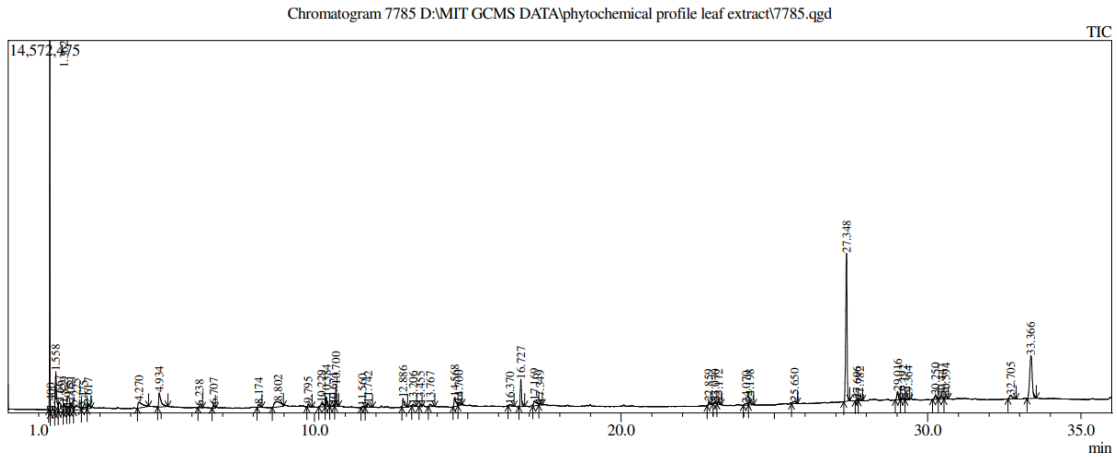

Fig. 1: Chromatogram of GCMS analysis of leaf extract of *Lawsonia inermis* L.

GC-MS Analysis

Gas chromatography and mass spectroscopy analysis of compounds was carried out in methanolic leaves extract of *Lawsonia inermis.* Chromatogram GC-MS analysis showed the presence of 11 major peaks and the components corresponding to the peaks were determined is shown in Figures 1. In this observation, the active principle with their Retention time (RT), Molecular formula, Molecular weight (MW), Concentration (%) and biological activities of these identified bio-compounds are presented in Table 2.

Table. 2: Components Identified and their biological activity in *Lawsonia inermis L.*

Sr. No.	Name of the compound	RT	MF	M W	Peak area %	Biological activity
1	Dimethyl ether	1.362	C2H6O	46	15.9549	Anti-microbial
2	Propanedioic acid	1.558	C3H4O6	136	5.4693	Anti-microbial
3	2-Propenoic acid, oxiranylmethyl ester	1.667	C6H8O3	128	3.0323	Anti-bacterial
4	2(3H)-Furanone, 5-methyl-	2.225	C5H6O2	98	2.0478	Anti-malarial
5	Benzoic acid	4.270	C7H6O2	122	2.6239	Anti-fungal
6	Benzofuran, 2,3-dihydro-	4.379	C8H8O	120	3.2519	Anti-bacterial
7	Beta.-D-Glucopyranose, 1,6-anhydro-	8.802	C6H10O5	162	3.4859	Anti-bacterial and Antioxidant
8	4,4,5,8-Tetramethylchroman-2-ol	10.700	C13H18O2	206	2.57	unknown
9	Phytol	16.727	C20H40O	338	3.2155	Anti-bacterial and Anti-fungal
10	Squalene	27.348	C30H50	410	20.6777	Anti-fungal
11	Alpha.-Tocopherol-.beta.-D-mannoside	33.366	C35H60O7	592	10.8314	Antioxidant, *Antibacterial*, Antiviral, Anti-inflammatory

RT*- Retention time, MF* - Molecular Formula, MW* - Molecular Weight

Molecular weight (MW) and Concentration (%) are presented in (Table 1 and Fig 1) with their Retention time(RT), Molecular formula, Molecular weight (MW) and Concentration (%) are presented in (Table 1 and Fig 1)

Table 3: Efficacy of leaf extracts against some post harvest disease causing fungi on 3rd day after inoculation

Sr. No.	Treatment	Concentration (%)	*Fusarium oxysporum*	*Alternaria alternata*	*Rhizopus stolanifer*	*Gloesporium psoddi*	*Phomopsis psidii*
1	T0	Control	56.73	62.32	59.34	58.95	52.32
2	T1	25	47.43	28.22	24.11	43.22	33.22
3	T2	50	22.33	12.22	10.11	22.21	18.23
4	T3	75	08.22	04.21	06.22	06.06	10.07

Table 4: Efficacy of leaf extracts against some post harvest disease causing fungi on 5th day after inoculation

Sr. No.	Treatment	Concentration (%)	*Fusarium oxysporum*	*Alternaria alternata*	*Rhizopus stolanifer*	*Gloesporium psiddi*	*Phomopsis psidii*
1	T0	Control	66.63	73.32	65.34	68.65	72.32
2	T1	25	49.23	38.09	28.09	23.01	27.06
3	T2	50	34.33	18.22	17.08	26.02	17.12
4	T3	75	12.22	07.32	07.80	08.02	10.22

Table 5: Efficacy of leaf extracts against some post harvest disease causing fungi on 7th day after inoculation

Sr. No.	Treatment	Concentration (%)	*Fusarium oxysporum*	*Alternaria alternata*	*Rhizopus stolanifer*	*Gloesporium psiddi*	*Phomopsis psidii*
1	T0	Control	72.82	79.42	69.42	73.32	74.43
2	T1	25	51.43	28.22	29.31	26.92	29.22
3	T2	50	37.01	20.11	21.11	29.01	19.09
4	T3	75	14.74	08.42	07.98	09.06	11.07

Antifungal Activity

The results of the present study indicated (Table 3, 4 and 5) that leaf extract of *Lawsonia inermis* is active against growth of fungi as per the % of the zone of inhibitions. It was observed from the results that the inhibitory activity of plant leaf extracts showed significant variation against the mycelial growth of all the tested fungi, viz. *F. oxysporum, A. alternata, R. stolonifer, Gloeosporium psidii* and *Phomopsis psiddi.* It was revealed (Table 3, 4 and 5) that different concentration (25%, 50% and 75%) plant extract caused significant inhibition in the mycelial growth among the *A. alternata, R. stolonifer, Gloeosporium psidii* at highest concentration, as compared *to F. oxysporum* and *P. psiddi*. It was found to be the most effective against *R. stolonifer* and caused highest inhibition in the mycelial growth (06.22%) followed by 5th and 7th day of incubation (07.80% and 07.98%).

DISCUSSION

Thus, it is clear from the above study that the plant leaf extracts of *Lawsonia inermis* L. in their different concentrations proved to be effective against some tested post-harvest diseases causing fungi in guava. In the present study, the highest antifungal activity of leaf extract against some post-harvest decaying fungi could be associated with the presence of 11 bioactive compounds identified using GCMS. The presence of Phenol, Benzoic acid, Phytol and Squalene are known to have antifungal activities (Sofu *et al*. 1998; Song, *et al*., 2019). 2-Propenoic acid, oxiranylmethyl ester Benzofuran, 2, 3-dihydro-, Beta.-D-Glucopyranose, 1, 6-anhydro-, Alpha.-Tocopherol beta.-D-mannoside has antioxidant, antibacterial, antimicrobial, antitoxic effects. Dracheva, *et al*., 2009; Shakya *et al*., 2011. Gololo *et al*., 2016.

Squalene is the most abundant in this crude extract. It is a naturally occurring triterpenoid and a precursor for the synthesis of secondary metabolites such as sterols, hormones, or vitamins. Squalene has been shown to have excellent antioxidant, anticancer, antibacterial, and antifungal biological activities (Reddy *et al*., 2009). Phytol is the second-highest compound in this extract. It was classified in the diterpene group. Yoshihiro *et al.,* 2005 reported that diterpene of phytol could disrupt the cell membranes of the fungus, resulting in K+ ions leaking from the cells, and causing the fungus hyphae to wither. In this case, the composition of major antifungal compounds, including squalene, phytol and Benzoic acid as well as minor antifungal compounds, acted as synergistic effects in controlling the harvest decaying fungi of *Psidium guajava* L.

In similar studies, several reports stated that the extracts of medicinal plants play an important role in controlling many phytopathogenic fungi (Lin *et al*. 2001; Okemo *et al*. 2003; Choi *et al*. 2004; Mares *et. al*. 2004; Perez-Sanchez *et. al.* 2007; Raji & Raveendran, 2013). Many researchers have been applied different plant extracts to study the effect on the growth and reproduction of different pathogenic fungi. However, reports are available on the inhibitory effect of *L. inermis* plant extract on other plant pathogenic fungi. The present study indicated that the inhibitory effect of the plant extracts on these pathogenic fungi might be attributed to the presence of some partially effective antifungal ingredients in the plant extracts.

CONCLUSIONS

Biological formulations can be successfully used in agriculture to treat plant diseases and can limit the use of chemical control agents to safer levels. Thus, the use of plant extract against phytopathogenic fungi will become an important research area and will be the best alternative to existing chemical antifungal agents in the near future. The antifungal activity of the plant extract is mainly due to the presence of secondary metabolites. Further purification of phyto-components by GC-MS analysis is a very sensitive and complex fractionation method. In this study, benzoic acid, phytol, and squalene are known to have antifungal activity, and their derivatives were found in the active fraction of the column, which had already been reported for their antimicrobial activity on various plants. In summary, our results showed that the methanolic extract of *L. inermis* leaves has an antifungal effect on mycelium growth and spore germination of some post-harvest decaying fungi. The fungicidal effect of the tested plant extract is recommended as a promising candidate in the biological control of fungal pathogens, which limits over-reliance on chemical fungicides.

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Conflicts of Interest: The authors declare no conflict of interest.

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