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Plant-Derived Bio-Fungicides: A Promising Tool for the Control of Soft Rot of Ginger (*Zingiber officinale Rosc.*)

S. Nazir Basha[1], K. Venkatesan[2], P. Irene Vethamoni[3], R. Kannan[4] and B. Anita[5]*

[1]PG Scholar, Department of Spices and Plantation Crops, HC&RI, TNAU, Coimbatore (Tamil Nadu), India.
[2]Professor & Head, Department of Spices and Plantation Crops, HC&RI, TNAU, Coimbatore (Tamil Nadu), India.
[3]Dean of Horticulture, HC&RI, TNAU, Coimbatore (Tamil Nadu), India.
[4]Professor, Department of Plant Pathology, TNAU, Coimbatore (Tamil Nadu), India.
[5]Professor, Department of Nematology, TNAU, Coimbatore (Tamil Nadu), India.

(Corresponding author: K. Venkatesan)*
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ABSTRACT: Ginger is a major spice in India which also has many medicinal properties. It is affected by various biotic and abiotic stress among them soft rot is a major disease caused by fungi. It causes about 70-90 per cent of crop loss if unchecked, even though many synthetic fungicides are available for control of the disease keeping the degradation of the environment due to excess use of chemicals, The present study had been taken up to identify some eco-friendly alternatives to synthetic fungicides has been screened. This study was carried out with different plant extracts *viz., Pimento dioca, Murraya koenigii, Adathoda visica, Piper longum, and Zingiber officinalis.* The results showed that the highest anti-fungal (*Fusarium* sps & *Pythium* sps) activity was recorded in Allspice (87.84 %&84.31%) followed by Curry leaf, Long-pepper, Adathoda, and Ginger. Quantitative studies are carried out for the analysis of phenols and anti-oxidant activity which was recorded highest in allspice (143.88 % and 87.52 %) among the all-plant extracts. This study concluded that the allspice plant extract had the potential to control this fungal disease.

Keywords: Ginger (*Zingiber officinale* Rosc.), Quantitative Studies, Secondary metabolites, DPPH, Phenols, Anti-oxidant and Antifungal.

INTRODUCTION

Ginger (*Zingiber officinale Rosc.*) is a tropical herb of the Zingiberaceae family. India is one of the largest producers of ginger, with an average area of 116.90 thousand hectares in India. The average production and productivity were 529.30 thousand MT and 4.30 MT/ha respectively (Santosh *et al.,* 2018). The major ginger-producing states of India contribute 65% to the country's total ginger production (Jayashree *et al*., 2015). According to the Spices Board figures, the production in India was 86,270 tons in the 2017-18 period. However, in 2021–22, the production was 54,260 tons. Madhya Pradesh is the largest producer of ginger in India, among the ginger-growing regions of Meghalaya, Arunachal Pradesh, Karnataka, Orissa, Assam, Gujarat, and Kerala, during the year 2022.

The production of ginger is affected by various factors and one of the most serious diseases of ginger is soft rot (rhizome rot), which is caused by *Pythium* spp., *Fusarium* spp., and bacteria such as *Ralstonia* spp (Rai *et al.,* 2018). Pathogens infect the roots, collar, and succulent sections of the rhizome, resulting in disease. Soft rot is said to be the most severe and devastating disease of ginger worldwide. Crop losses due to ginger soft rot are difficult to quantify, however, losses are believed to range from 50 to 90% (Dohroo *et al.,* 2005). According to a study by Le *et al.* (2014) more than 15 *Pythium* species have been reported as pathogen-causing soft rot of ginger. Further, they have isolated *P. aphanidermatum* from ginger soft rot, which causes about 60% yield loss.Apart from *Pythium, Fusarium* is another important fungus reported to cause the soft rot in ginger. The disease reduced the potential yields of ginger to a greater extent in field, storage, and market and many losses of even more than fifty percent (Ramteke and Kamble 2011). Excess use of chemical fungicides causes alteration of the soil microbial composition and disrupts the natural cycle of the metabolic process of crops which drastically causes low yield (Wijntjes *et al.,* 2022).

Secondary metabolites with antifungal activity against phytopathogenic fungi,which isa viable control measure. They are biodegradable to non-toxic products and could be a sustainable tool as bio-pesticides in integrated pest management programs. Thus, the objectives of this study were to identify the plant metabolites having anti-fungal properties against soft rot-causing pathogens and to evaluate the *in vitro* antifungal activity of extracts from study *Pimento dioca, Murraya koenigii, Adathoda visica, Piper longum, and zingiber officinalis.*

MATERIAL AND METHODS

The experiment was conducted during 2022-2023 at the Department of Plantation and Spices in HC and RI,

Tamil Nadu Agricultural University, Coimbatore Completely Randomized Design (CRD) was used, *in vitro* experiment with 26 treatments that were replicated three times. Following inoculation, samples of mycelial radial growth were observed at 48, 72, 96, and 120 hours to determine the effectiveness of each treatment.

A. Treatment details

Treatments No.	Plant extracts at different concentrations
T_1	Allspice extract at 2500 ppm
T_2	Allspice extract at 5000 ppm
T_3	Allspice extract at 7500 ppm
T_4	Allspice extract at 10000 ppm
T_5	Allspice extract at 15000 ppm
T_6	Curry leaf extract at 2500 ppm
T_7	Curry leaf extract at 5000 ppm
T_8	Curry leaf extract at 7500 ppm
T_9	Curry leaf extract at 10000 ppm
T_{10}	Curry leaf extract at 15000 ppm
T_{11}	Adathoda extract at 2500 ppm
T_{12}	Adathoda extract at 5000 ppm
T_{13}	Adathoda extract at 7500 ppm
T_{14}	Adathoda extract at 10000 ppm
T_{15}	Adathoda extract at 15000 ppm
T_{16}	Long Pepper extract at 2,500 ppm
T_{17}	Long Pepper extract at 5000 ppm
T_{18}	Long Pepper extract at 7500 ppm
T_{19}	Long Pepper extract at 10000 ppm
T_{20}	Long Pepper extract at 15000 ppm
T_{21}	Ginger extract at 2500 ppm
T_{22}	Ginger extract at 5000 ppm
T_{23}	Ginger extract at 7500 ppm
T_{24}	Ginger extract at 10000 ppm
T_{25}	Ginger extract at 15000 ppm
T_{26}	Control

B. Collection and preparation of plant extracts

Methanolic extract of five different plants *viz., Pimento dioca, Murraya koenigii, Adathoda visica, Piper longum, and Zingiber officinalis.* extracted by using the Soxhlet apparatus. The plant parts are collected and shade dried have been taken for extraction. 10 g of powder from each plant is taken and extracted by using methanol (200ml) as solvent (Redfern *et al.,* 2014).

(i) Qualitative Analysis as Methods Suggested by Shaik *et al.* (2020)

Test	Procedure
Alkaloids	
Hager's test	Few mL filtrate + 1-2 mL Hager's reagents
Iodine Test	3mL extract solution + few drops of iodine solution
Mayer's/ Bertrand's/ Valser's test	Few mL filtrate + 1-2 drops of Mayer's reagent (Along the sides of the test tube)
Flavanoids	
Lead acetate test	1mL plant extract + a few drops of 10% lead acetate solution
Ferric chloride test	Extract aqueous solution + a few drops of 10% ferric chloride solution
Phenols	
Ferric chloride test	Extra ct aqueous s solution + few with dra ops 5% ferric chloride sol.
Potassium dichromate test	Plant extract + few dra ops of potassium dichromate solution
Ellagic Acid Test	Plant extract aqueous solution + 5% glacial acetic acid + 5% sodium nitrite solution
Gelatin test	Plant extract is dissolved in 5mL distilled water + 1% gelatin solution + 10% NaCl
Tannins	
Braymer's test	1mL filtrated + 3mL distilled water + 3 drops 10% Ferric chloride solution
Gelatin	Plant extract is dissolved in 5mL distilled water + 1% gelatin solution + 10% NaCl
10% NaOH test	0.4mL plant extract + 4mL 10% NaOH + shaken well
Saponins	
Foam test	20mL water in measuring cylinder + 50gm extract (vigorously shaken for 15 min.)
$NaHCO_3$ TEST	Plant extract + few mL sodium bicarbonate solution + distilled water (vigorously shaken)
Terpenoids	
	2ml chloroform + 5mL plant extract, (evaporated on water bath) + 3mL conc. H_2SO_4 (boiled in water bath)

C. Quantification of Phyto-chemicals

(i) Estimation of total phenols in various plant extracts. Total phenols estimated in various extracts, 0.2 mL of each extract was diluted with respective solvent to adjust the absorbance within calibration limits. 1.0 mL of Folin-Ciocalteu reagent and 2.0 mL of Na_2CO_3 (20%, w/v) were added to the diluted extract, mixed, and volume was made up to 10.0 mL with water. After 8 min. The mixture was centrifuged at 6000 rpm for 10 min. The absorbance of the supernatant solution was measured at 730 nm using UV-VIS double beam Spectrophotometer Model 2203 (Systronics Co.) against a blank prepared similarly but containing respective solvent instead of extracts. The amount of total phenols present in the extracts was calculated from the standard curve and the results are expressed as milligrams of catechol equivalent per gram (mg CE/g) (Singleton and Rossi 1965).

(ii) Evaluation of DPPH free radical scavenging activity. DPPH assay (2, 2-diphenyl-1-picrylhydrazyl) of different extracts was determined by using Chang *et al.* (2001). The decrease in the absorption of the DPPH solution after the addition of an antioxidant was measured at 517nm. Ascorbic acid (10mg/ml DMSO) was used as a reference. Reagent preparation 0.1mM DPPH solution was prepared by dissolving 4mg of DPPH in 100ml of ethanol. Serial dilution (2 - 20µl) of plant extracts was made up to 40µl with DMSO and 2.96ml DPPH (0.1mM) solution was added. The reaction mixture was incubated in dark conditions at room temperature for 20 min. After 20 min, the absorbance of the mixture was read at 517 nm. 3ml of DPPH was taken as control. The % radical scavenging activity of the plant extracts was calculated using the following formula.

$$\% \text{ RSA} = \frac{\text{Absorbance control} - \text{Absorbance sample}}{\text{Absorbance control}} \times 100$$

Where, RSA= Radical Scavenging Activity

Abs control is the absorbance of DPPH radical + ethanol;

Abs sample is the absorbance of DPPH radical + plant extract

D. Antifungal activity of extracts

Bioassay of methanol extracts bio-actives were employed in evaluation at concentrations of 2500 ppm, 5000 ppm, 7500 ppm, 10000 ppm, and 15000 ppm after being dissolved in distilled water. At around 50°C, the conical flask holding the PDA medium was well mixed to integrate the various concentrations, and 20 ml of the mixture was then put into each Petri dish with a 9 cm diameter. With a cork borer, the freshly developing mycelium from the chosen culture plate was chopped into 5 mm pieces and put into the center of the Petri dish in an isolation chamber under aseptic conditions. Additionally, controls were kept in place without any extraction (Grover *et al*., 1962; Mohana *et al*., 2007).

RESULTS AND DISCUSSION

Plant extracts give positive results for alkaloids except Adathoda, For Phenols, Tannins, Flavanoids, and Saponins all five extracts showed positive. Terpenoids are present in Allspice, Curry leaf, and Adathoda (Table 1). The present results are results confirmed by Dashade *et al.* (2023); Bhagat *et al.* (2022), Sharma *et al.* (2018); Prathiba *et al.* (2014); Thrivedi *et al.* (2011). The total phenol and DPPH activity is high in allspice 143.88 mg CE/g (Fig. 1, Table 2) and 87.52 % (Fig. 2, Table 3), followed by the Curry leaf and Long Pepper. The phenol and anti-oxidant activity of allspice is higher than in ginger extract (Turgay *et al.,* 2018). Phenols and Anti-oxidant activity are greater in methanolic extracts of immature leaves of *P. dioica*. In general, the spices belonging to the Myrtaceae family are rich in polyphenols and antioxidant activity (Dharmadasa *et al.,* 2015).

Anti-fungal properties against *Fusarium solani* by plant methanolic extract showed maximum inhibition by Allspice (87.84 %) followed by Curry leaf (70.27%), Long pepper (61.25 %), Adathoda (59.55) and Ginger (47.62%) (Table 4). For *Pythium aphanidermatum*, the maximum inhibition is 84.31% (allspice) followed by 73.33 % (curry leaf),69.08% (Long pepper), 58.82% (Adathoda), and 56.75%(Ginger) (Table 5). All five extracts have anti-fungal properties but they follow the trend of allspice, curry leaf, long pepper, adathoda, and ginger for the pathogens causing soft rot in ginger. These results are by Ramanathan *et al.* (2004) *S. persica* extract was found to be effective against *P. aphanidrematum* and *R. solani* inhibiting their mycelial growth to 77.83 % and 56.43% respectively and slightly inhibited mycelial growth of *F. oxysporum* to 14.69 %. The extracts of *P. amboinicus* and *P. dioica* inhibited mycelial growth, and spore germination in *Colletotrichum* spp. at concentrations of 5, 10, and 20 mg/mL, while the extracts of *P. guajava* and *P. auritum* did so at concentrations of 30 and 40 mg/mL. *P. amboinicus, P. dioica*, and *P. auritum* modified the size of the spores, causing degradation and formation of vacuoles in the spores of *C. acutatum* and *C. gloeosporioides* (Silva *et al.,* 2021). Antifungal activity of *Alstoniascholaris, Argemone maxicana,* and *Datura alba* studied by Malik (2015) at different alcoholic extracts concentrations with (10%, 25% and 50%) were observed on the growth performances of *Candida albicans* causing human skin diseases. Based on the research by Raut *et al*. (2015), it was found that clove extracts containing propanol and acetone can completely inhibit *S. rolfsii*, in comparison to the untreated control. Propanol alone demonstrated a mycelium growth of 14.67 mm and an inhibition rate of 83.70 percent

The results showed that propanol and acetone clove extracts showed cent per cent inhibition of *S. rolfsii* compared to the untreated control. Propanol alone showed 14.67 mm of mycelium growth and 83.70 per cent inhibition. The results showed that propanol and acetone clove extracts showed cent per cent inhibition of *S. rolfsii* compared to the untreated control. Propanol alone showed 14.67 mm of mycelium growth and 83.70 per cent inhibition.

Anti-fungal activity of these extracts due to the presence of secondary metabolites allspice (eugenol),

curry leaf α.-Caryophyllene), ginger (*gingerols*, shogaols), adathoda (Squalene), and long pepper (beta-caryophyllene) as per Ashok *et al.* (2022); Shankaracharya *et al.* (1997); Nandhini *et al.* (2020); Mao *et al.* (2019); Hema *et al.* (2011). The antifungal activity of allspice is due to the presence of major constituents in *Pimento dioca* were eugenol (67.67%), β-myrcene (10.40%), p-chavicol (8.03%), and D-limonene (4.55%) (Ashok *et al.,* 2022). The biological activity of eugenol inhibits biofilm formation, disrupts the cell-to-cell connections, detaches the existing biofilms, and kills the bacteria in the biofilms of both MRSA and MSSA with equal effectiveness (Yadav *et al.*, 2015). Other researchers attributed the inhibitory effect of these plant extracts to the hydrophobicity characteristics of these plant extracts and their components. This enables them to partition in the lipids of the fungal cell wall membrane and mitochondria disturbing their structure and rendering them more permeable. Leaking of ions and other cell contents can then occur causing cell death (Burt, 2004).

Table 1: Qualitative analysis of different extracts.

Metabolites	Allspice	Curry leaf	Adathoda	Long Pepper	Ginger
Alkaloids	+	+	-	+	+
Phenols	+	+	+	+	+
Tannins	+	+	+	+	+
Flavonoids	+	-	+	+	+
Saponins	+	+	+	+	+
Terpenoids	+	+	+	-	-

"+" Indicates presents of compounds, "- "Indicates presence of compounds

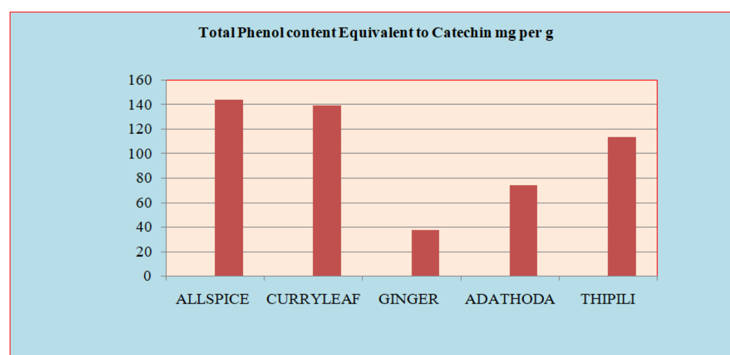

Fig. 1. Graphical representation of Total phenols content equivalent to Catechin mg per g.

Table 2: Total phenol content of plant extracts.

Sr. No.	Sample	Od at 730nm	Total phenols (mg CE/g)
1.	Allspice	2.14	143.88
2.	Curry leaf	2.07	139.02
3.	Ginger	0.47	37.22
4.	Adathoda	1.04	73.57
5.	Long pepper	1.66	112.92

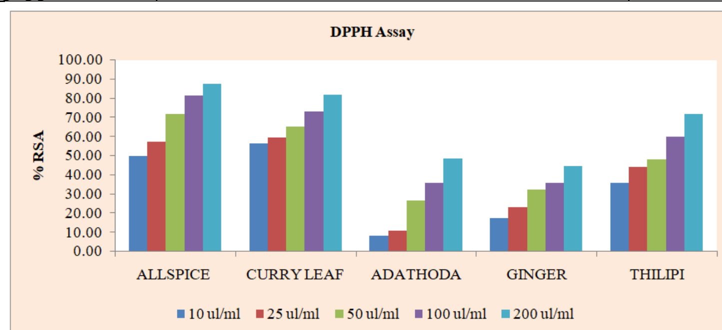

Fig. 2. Graphical representation of DPPH assay.

Table 3: DPPH activity of plant extracts.

Concentration	Allspice	Curry leaf	Ginger	Adathoda	Long-pepper
10 ul/ml	50.00	56.38	8.33	17.57	35.96
25 ul/ml	57.39	59.52	11.10	23.11	44.46
50 ul/ml	71.81	65.53	26.81	32.54	48.43
100 ul/ml	81.43	73.11	36.14	35.87	60.08
200 ul/ml	87.52	81.98	48.80	44.74	72.09

Table 4: Growth inhibition of *Pythium aphanidermatum* by plant extracts.

Treatments	Plant extracts at different concentrations	MIC
T_1	Allspice extract at 2500 ppm	10.98(3.41)
T_2	Allspice extract at 5000 ppm	28.63(5.44)
T_3	Allspice extract at 7500 ppm	60.00(7.795)
T_4	Allspice extract at 10000 ppm	72.94(8.59)
T_5	Allspice extract at 15000 ppm	84.31(9.23)
T_6	Curry leaf extract at 2500 ppm	13.33(3.73)
T_7	Curry leaf extract at 5000 ppm	28.63(5.43)
T_8	Curry leaf extract at 7500 ppm	41.57(6.52)
T_9	Curry leaf extract at 10000 ppm	60.78(7.85)
T_{10}	Curry leaf extract at 15000 ppm	73.33(8.62)
T_{11}	Adathoda extract at 2500 ppm	12.94(3.70)
T_{12}	Adathoda extract at 5000 ppm	21.18(4.69)
T_{13}	Adathoda extract at 7500 ppm	28.24(5.40)
T_{14}	Adathoda extract at 10000 ppm	31.76(5.71)
T_{15}	Adathoda extract at 15000 ppm	58.82(7.73)
T_{16}	Long Pepper extract at 2,500 ppm	23.69(4.96)
T_{17}	Long Pepper extract at 5000 ppm	30.12(5.57)
T_{18}	Long Pepper extract at 7500 ppm	50.20(7.14)
T_{19}	Long Pepper extract at 10000 ppm	51.41(7.23)
T_{20}	Long Pepper extract at 15000 ppm	69.08(8.36)
T_{21}	Ginger extract at 2500 ppm	9.92(3.24)
T_{22}	Ginger extract at 5000 ppm	19.44(4.51)
T_{23}	Ginger extract at 7500 ppm	31.35(5.68)
T_{24}	Ginger extract at 10000 ppm	44.84(6.76)
T_{25}	Ginger extract at 15000 ppm	56.75(7.59)
T_{26}	Control	
	SEm	0.25
	CD	0.71

Table 5: Growth inhibition of *Fusarium solani* by plant extracts.

Treatments	Plant extracts at different concentrations	MIC
T_1	Allspice extract at 2500 ppm	51.80(7.41)
T_2	Allspice extract at 5000 ppm	74.77(8.084)
T_3	Allspice extract at 7500 ppm	80.63(8.36)
T_4	Allspice extract at 10000 ppm	84.23(8.36)
T_5	Allspice extract at 15000 ppm	87.84(8.36)
T_6	Curry leaf extract at 2500 ppm	32.43(5.78)
T_7	Curry leaf extract at 5000 ppm	46.85(6.91)
T_8	Curry leaf extract at 7500 ppm	59.91(7.80)
T_9	Curry leaf extract at 10000 ppm	68.92(8.35)
T_{10}	Curry leaf extract at 15000 ppm	70.27(8.43)
T_{11}	Adathoda extract at 2500 ppm	15.00 (3.99)
T_{12}	Adathoda extract at 5000 ppm	22.08(4.79)
T_{13}	Adathoda extract at 7500 ppm	26.25(5.21)
T_{14}	Adathoda extract at 10000 ppm	36.25(6.10)
T_{15}	Adathoda extract at 15000 ppm	59.58(7.78)
T_{16}	Long Pepper extract at 2,500 ppm	20.83(4.67)
T_{17}	Long Pepper extract at 5000 ppm	25.42(5.13)
T_{18}	Long Pepper extract at 7500 ppm	29.58(5.52)
T_{19}	Long Pepper extract at 10000 ppm	37.50(6.20)
T_{20}	Long Pepper extract at 15000 ppm	61.25(7.88)
T_{21}	Ginger extract at 2500 ppm	15.58(4.05)
T_{22}	Ginger extract at 5000 ppm	22.51(4.84)
T_{23}	Ginger extract at 7500 ppm	28.14(5.39)
T_{24}	Ginger extract at 10000 ppm	38.53(6.28)
T_{25}	Ginger extract at 15000 ppm	47.62(6.97)
T_{26}	Control	0.00
	SEm	0.15
	CD	0.42

CONCLUSIONS

The extracts are screened for phytochemicals which showed the presence of phenols, flavonoids, terpenoids, saponins, tannins, and alkaloids. Quantitative studies are carried out for the analysis of phenols and anti-oxidant activity the plant extracts are rich in phenols and anti-oxidant activity showed significant antifungal activity against *Pythium aphanedermatum* and *Fusarium solani*. Due to the richness of bioactive compounds in the five botanical extracts, they can be used as a source of substances with the antifungal potential of low toxicity for the management of soft rot disease in Ginger. For future control of disease organically or through IPM these are the best alternatives.

FUTURE SCOPE

The plants under study showed potential anti-fungal properties which can be alternatives for synthetic fungicides making them into standard bio formulations.

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Conflict of Interest. None.

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