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Antifungal activity of medicinal plants, *Adathoda vasica* and *Andrographis paniculata* against *Colletotrichum capsici*, the chilli fruit rot pathogen

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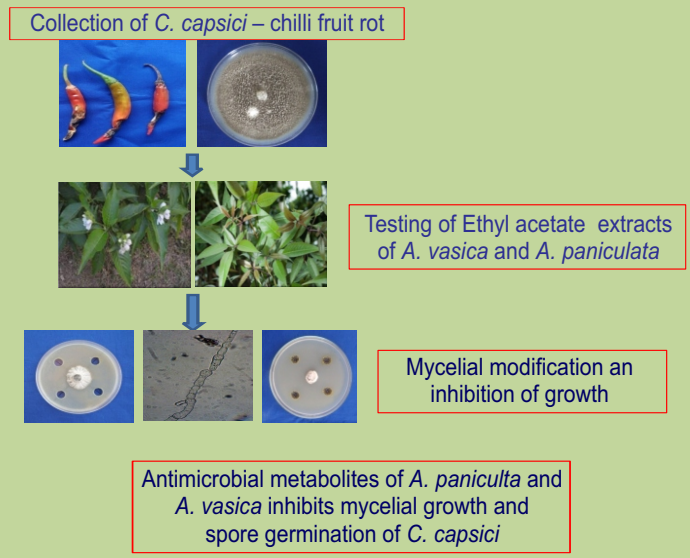
Abstract

Aim: The objective was formulated to screen the extracts of medicinal plants for tapping the antimicrobial activity against *Collectotrichum capsici.* Further, the work was planned to characterize and identify the nature of antimicrobial compounds and their functional groups.

Methodology: Extracts of eleven medicinal plants were tested against the mycelial growth and spore germination of *C. capsici* under *in-vitro* conditions. Based on these results, the potential plant extracts of *A. vasica* and *A. paniculata* found effective against *C. capsici* were assayed for the presence of antimicrobial metabolites through TLC, GC-MS and FTIR analysis.

Results: Among the medicinal plants screened, the crude extracts from *Adathoda vasica* and *Andrographis paniculata* inhibited mycelial growth and spore germination of *C. capsici* by 53.33% and 38.14%, respectively, under *in-vitro* conditions. GC-MS analysis of ethyl acetate extracts of *A. vasica* indicated antimicrobial compound, 1H-Pyrrolo[2,1-b]quinazolin-9-one,3-hydroxy-2,3-dihydro- and *A. paniculata* showed the presence of two compounds, docosahexaenoic acid and oleic acid. Similarly, FTIR analysis revealed esters, alcohols, and halide groups, which are known antimicrobials.

Interpretation: The medicinal plants, *A. paniculata* and *A. vasica* possessed antimicrobial metabolites, which was responsible for inhibiting the mycelial growth and spore germination of *C. capsici*.



Key words: *Adhatoda, Andrographis*, Anthracnose, Antimicrobial compounds, Chilli, *Colletotrichum*, Fruit rot

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Introduction

Chilli is widely prone to anthracnose/fruit rot caused by *Colletotrichum* spp., which is a significant problem in tropical and subtropical regions of India, resulting in severe yield losses up to 10–84% (Ramachandran and Rathnamma, 2006; both in the pre- and post-harvest conditions (Than *et al.,* 2008; Ramachandran and Rathnamma, 2006). Even a small lesion on the fruits under field conditions is carried to storage units where the pathogen inoculum builds up and lowers the dry fruit quality. Chilli anthracnose, dieback and fruit rot is caused by a complex of 24 species of the genus *Colletotrichum* (de Silva *et al.,* 2019), including *C. capsici*, the predominant fungi causing fruit rot in ripe fruits. The use of fungicides has led to environmental pollution and health hazards besides the high cost of production. Currently, modern research is focused on the search for antimicrobials from plants that serve as reservoirs of secondary metabolites of antimicrobial substances and finds application in the management of plant diseases (Sateesh *et al.,* 2004). Traditional medicinal plants like Azadiracta indica, Ocimum sanctum, Curcuma longa, Agegle marmelos, Vitex negundo and Catharanthus roseus have received significant attention in pharmaceutical industry in the recent years owing to its antimicrobial properties coupled with immunomodulatory properties which led to new drug discoveries (Amor *et al*., 2009).

Plants and their derivatives have been extensively studied for the control of phytopathogenic fungi. Several studies have been carried out on inhibitory potential of many botanical extracts against phytopathogenic fungi including species of Colletotrichum (Bajpai and Kang, 2012; Ajith *et al*., 2012). The synergistic activity of diverse bioactive metabolites is responsible for the antimicrobial activity of the medicinal plants (Manilal and Idhayadhulla, 2014). The presence of ester compounds, such as eugenol, vanillin, ferilic acid, 1, 2-diphenylethane, mono- and sesquiterpenes (oxidized or not), as well as triterpenes, has been available in the plant, which clearly indicates its potential as a fungicide against plant pathogens (Custódio and Veiga-Junior, 2012). Management of plant diseases with fungi and plant product derived molecules has a special significance in the context of mitigating the problems of environmental pollution, accumulation of toxic substances in the produce and development of resistance by plant pathogens. In this context, the present study was formulated to identify the efficacy of plant extracts of medicinal importance against *C. capsici*. In addition, the research work was planned to elucidate the nature of antimicrobial compounds by thin layer chromatography (TLC), gas chromatography-mass spectrometry (GC-MS) and Fourier transform-infrared spectroscopy (FTIR).

Materials and Methods

Pathogen: Infected chili fruits showing typical fruit rot symptoms were collected from severely infected chilli plants in fields of Coimbatore district. The pathogen was isolated from the specimen by using potato dextrose agar (PDA) medium by the tissue segment method and purified by single hyphal tip method. The microscopic characters of the mycelium, septation, shape of conidia, and acervuli were documented for the pathogen under a microscopic image analyser at 40x. Molecular characterization was performed by isolating DNA and PCR amplification as per the conventional method (White *et al.,* 1990). The amplified products were sequenced (Genei Pvt. Ltd., Bangalore, India) and partial 18S rRNA were compared with deposited entries available in the National Centre for Biotechnology Information (NCBI) database, using the basic local alignment search tool (BLAST) algorithm. Based on the comparative database statement, nucleotide sequences were deposited in GenBank, NCBI, and obtained with an accession number.

Preparation of plant extracts: The samples of medicinal plants *viz.,* leaves of *Adhatoda vasica, Tribulus terrestris, Andrographis paniculata, Coleus aromaticus, Vitex* spp., *Catharanthus roseus, Ocimum sanctum, Vitex negundo, Phyllanthus amarus*, root of *Andrographis paniculata* and neem seed kernel extract (*Azadirachta indica*) were collected from the Department of Medicinal and Aromatic Crops, Tamil Nadu Agricultural University, Coimbatore, washed in running tap water, surface sterilized with NaOH 1% and then with sterile distilled water and air-dried over a blotting paper. The plant parts were ground with a sterilized pestle and mortar with sterile water at the rate of one gram^{-1} of sterile distilled water. The extract was obtained by squeezing the macerate with cotton wool and strained through Whatman No. 1 filter paper and passed through Millipore filters (0.2 µm) using vacuum pump assembly under aseptic conditions to free it from bacterial contaminants. This formed a standard plant extract solution (100%). This extract was further diluted with sterilized distilled water to the required concentrations and used for further studies.

Preliminary screening of plant extracts against *C. capsici*: The extracts of eleven medicinal plants were prepared at 10% concentration and tested against *C. capsici* by poisoned food technique (Shekhawat and Prasada, 1972). Respective plant extracts were added separately to the molten PDA medium to get a required concentration of 10% under aseptic conditions. PDA medium without any plant extract served as control. After solidification, each plate was inoculated with mycelium disc (9 mm diameter) of *C. capsici* placed in the centre of plate. The plates were then incubated at 25°C for 7 days. Each treatment was replicated three times. Percent inhibition of pathogen growth was calculated by following the method described by Vincent (1947).

Testing of solvent extracts of medicinal plants, *A. vasica* and *A. paniculata* against *C. capsici*: The solvent extracts were added into agar wells separately @ 100 µl per well. The extracts of *A. paniculata* and *A. vasica* were selected for further studies based on the inhibition of mycelial growth along with morphological and cultural modifications of C.capsici. Further the leaf extracts of *A. paniculata* and *A. vasica* were extrcated using different solvents and tested against *C. capsici* by agar well diffusion test (Stokes and Ridgeway,1980). Actively growing ten

days old mycelium discs (9 mm in diameter) of *C. capsici* were inoculated at the centre of each Petri dish and incubated at 28±2ºC for seven days. The percent growth inhibition of *C. capsici* was recorded. The ethyl acetate solvent performed better in extracting the antifungal compounds and used for further studies. The ethyl acetate solvent extracted metabolites of *A. vasica* and *A. paniculata* were made up to different concentrations (1000, 2000, 3000, 4000, and 5000 ppm) and tested by agar well diffusion and the percent inhibition of mycelial growth of *C. capsici* was recorded. Similarly, the best performing ethyl acetate solvent extracts of *A. vasica* and *A. paniculata* were tested separately against spore germination of *C. capsici* using cavity slides. Spore germination was observed and recorded after 6, 12 and 18 hrs under a phase-contrast microscope and the percent inhibition of spore germination was calculated using the formula described by Akhter *et al.* (2006).

Characterisation of antimicrobial compounds from extracts of *A. vasica* and *A. paniculata* through GC-MS, FTIR and TLC: The antimicrobial compounds from the solvent extracts of *A. vasica* and *A. paniculata* were characterized through GC-MS analysis (Trace GC Ultra and DSQII model MS, Thermo Fisher Scientific Limited) instrument at the Department of Agricultural Microbiology, Tamil Nadu Agricultural University (TNAU), Coimbatore. The FTIR studies were performed to identify the chemical nature of antimicrobial compound from the ethyl acetate solvent extracts of *A. vasica* and *A. paniculata* by FT-IR (Jassco FTIR 6800) instrument of Agricultural Microbiology, TNAU, Coimbatore.

Specific bands detected from the TLC plate of *A. vasica* and *A. paniculata* were scrapped separately using sterile blade along with the silica gel and mixed with 1 ml of HPLC methanol, and vortexed for 10-15 min. The methanol and the compound and silica were separated by centrifugation at 10,000 rpm for 5 min. The supernatant was collected and filtered with a membrane filter

Table 1: Screening of medicinal plants against *C. capsici* by poisoned food technique

Plant extracts	*Average mycelial growth (mm)	% inhibition over control	Mycelial abnormalities
Adathoda vasica	42.00^{a} (40.40)	53.33	Whitish puff like mycelial growth
Tribulus terrestris	57.33^{b} (49.22)	36.30	Puffy whitish growth followed by slight mycelial growth
Neem seed kernel Extract (*Azadirachta indica*)	56.00^{b} (48.45)	37.77	Puffy whitish growth
Andrographis paniculata	55.67^{b} (48.25)	38.14	Puffy growth at centre followed by retarded growth
Coleus aromaticus	64.33^{c} (53.33)	28.52	Whitish radial mycelial growth with wavy margin
Vitex negundo	69.00^{d} (56.17)	23.33	Whitish radial mycelial growth with wavy margin
Catharanthus roseus	73.33^{f} (58.91)	18.52	Initially whitish growth followed by normal growth
Oscimum sanctum	71.00^{e} (57.42)	21.11	Normal mycelium with irregular margins
Vitex spp.	71.67^{e} (57.84)	20.36	Normal mycelium with irregular margins
Phyllanthus amarus	75.00^{g} (60.00)	16.66	Whitish puffy growth with irregular margins
Nilavembu root	78.00^{h} (62.03)	13.33	Normal mycelial growth
Control	90.00^{i} (71.57)	0.00	Normal mycelial growth
SEd	0.7698		
CD (p=0.05)	1.5888		

*Values are mean of three replications. Means followed by common letters are not significantly different at 5%level by DMRT

Table 2: Antimicrobial activity of solvent extracts from *A. vasica* and *A. paniculata* against *C. capsici*

Treatments	Solvent growth (mm)	*Average mycelial growth (mm)	% inhibition over control
Adathoda vasica	Ethyl acetate	18.00^{a} (25.10)	80.00
	Chloroform	41.00^{b} (39.82)	54.44
	Diethyl ether	42.25^{c} (40.54)	53.05
Andrographis paniculata	Ethyl acetate	17.00^{a} (24.35)	81.11
	Chloroform	50.00^{e} (45.00)	44.44
	Diethyl ether	46.00^{d} (42.71)	48.52
Control		90.00^{f} (71.57)	0.00
Sed		0.5510	
CD (p=0.05)		1.1458	

*Values are mean of five replications. Means followed by common letters are not significantly different at 5%level by DMRT

Table 3: Testing ethyl acetate solvent fractions from *A. vasica* and *A. paniculata* extracts against mycelial growth of *C. capsici*

Treatment	Concentration (ppm)	*Average mycelial growth (mm)	% inhibition over control
Adathoda vasica	1000	46.25^{f} (42.85)	48.16
	2000	44.08^{e} (41.60)	51.02
	3000	40.00^{d} (39.23)	55.55
	4000	35.5^{c} (36.57)	60.55
	5000	32.00^{a} (34.45)	64.44
Andrographis paniculata	1000	49.00^{g} (44.43)	45.55
	2000	46.00^{f} (42.71)	48.88
	3000	40.75^{d} (39.82)	54.72
	4000	36.00^{c} (36.87)	60.00
	5000	33.50^{b} (35.67)	62.77
Control		90.00^{h} (71.57)	0.00
SEd CD (p=0.05)		0.5727 1.1652	

*Values are mean of five replications. Means followed by common letters are not significantly different at 5% level by DMRT.

Table 4: Testing ethyl acetate solvent fractions from *A. vasica* and *A. paniculata* extracts against spore germination of *C. capsici*

Plant extracts	6 hr		12 hr		18 hr	
	SG	PI	SG	PI	SG	PI
A. vasica	-	100	36.60^{a} (37.23)	63.40	19.60a (26.28)	80.40
A. paniculata	-	100	42.20^{b} (40.51)	57.80	21.00a (27.27)	79.00
Control	-	100	51.00^{c} (45.57)	49.00	78.60c (62.44)	21.40
Control without metabolite	-	100	47.00^{d} (43.28)	53.00	68.20b (55.67)	31.80
			1.0488		1.5100	
			2.2234		3.2010	

SG: No. of spores germinated; PI: Percent inhibition of spore germination. *Values are mean of five replicates. Means followed by common letter are not significantly different at 5% level by DMRT.

(0.2 µm) was assayed for the antimicrobial activity against *C. capsici* by agar well diffusion method as mentioned earlier. The specific bands eluted from the TLC plate of *A. vasica* and *A. paniculata* were further analyzed by GC-MS (Trace GC Ultra and DSQII model MS from Thermo Fisher Scientific Limited) at the Department of Agricultural Microbiology, TNAU, Coimbatore to find out the nature of antimicrobial compound.

Statistical analyses: The data was subjected to appropriate transformations, and statistically analysed using the IRRISTAT software package (Biometrics Unit, International Rice Research Institute, Philippines).

Results and Discussion

The morphological characters of purified fungal colony in PDA medium resembled the characters of *Colletotrichum* sp. Microscopic observations showed the presence of hyaline, septate mycelium and acervuli with brown coloured septate setae that entrapped short cylindrical conidiophores bearing falcate-shaped conidia with acutely pointed tips. Amplification of the PCR product of rDNA fragment of *Colletotrichum* sp. using universal primers ITS 1 and ITS 4 showed the amplicon size at 550 bp. The purified PCR product subjected to partial sequencing and BLAST analysis carried out in NCBI database showed 100% similarity with *C. capsici*. The isolate was deposited in TNAU Microbial Culture Collection Bank. The sequence was submitted in the NCBI GenBank database (Accession number Mk758061). *In-vitro* assay was carried out to screen the antimicrobial effect of plant extracts against *C. capsici* revealed that the leaf extracts of *A. vasica, A. paniculata,* neem seed kernel extract, and leaf extracts of *T. terrestris* reduced the mycelial growth of *C. capsici* by 53.33, 38.14, 37.77 and 36.30 percent (Table 1) with abnormalities in the growth pattern of *C. capsici* .

Microscopic observations of the plant extract treated cultures showed swellings in the mycelium of *C. capsici* due to antimicrobial compounds from leaf extracts of *A. vasica, A. paniculata, T. terrestris* and neem seed kernel extract (Fig.1a-e). Qammer (2020) reported the antibacterial effect of *A. vasica* may be due to the presence of secondary secondary metabolites such as alkaloid, flavonoids, tannins, and phenol. The presence of andrographolide compound in *A. paniculata* may be the reason for the antifungal nature against *C. capsici* as reported by

Table 5: Determination of functional groups of compounds from ethyl acetate extracts of *A. vasica* and *A. paniculata* through FT-IR

Absorption (cm^{-1})	Group	Compound class	Intensity
A. vasica			
3309.25	N-H stretching	Aliphatic Primary amine	Medium
2940.91	N-H stretching	Amine salt	Strong, Broad
2830.03	N-H stretching	Amine salt	Strong, Broad
2522.43	S-H stretching	Thiol	Weak
1699.09	C=O stretching	Conjugated aldehyde	Strong
1449.24	O-H bending	Carboxylic acid	Weak
1416.46	O-H bending	Alcohol	Medium
1114.65	C-O stretching	Secondary alcohol	Strong
1020.16	S=O stretching	Sulfoxide	Strong
624.823	C-Br stretching	Halo compound	Strong
A. paniculata			
3309.25	N-H stretching	Aliphatic Primary amine	Medium
2941.88	N-H stretching	Amine salt	Strong, Broad
2830.03	N-H stretching	Amine salt	Strong, Broad
2523.4	S-H stretching	Thiol	Weak
1913.04	C=C=C stretching	Allene	Medium
1661.37	C=O stretching	Conjugated ketone	Strong
1449.24	O-H bending	Carboxylic acid	Medium
1417.42	O-H bending	Alcohol	Medium
1114.65	C-O stretching	Secondary alcohol	Strong
1020.16	S=O stretching	Alcohol	Strong

Table 6: Testing antimicrobial activity of compounds eluted from TLC band against *C. capsici*

Treatments	Bands	Rf value	Average mycelial growth (mm)	% inhibition over control
A. vasica	Band 1	0.57	36.00^{b} (36.87)	60.00
	Band 2	0.17	39.80^{c}	(39.11)55.77
A. paniculata	Band 1	0.41	33.00^{a} (35.06)	63.33
Control		90.00d (71.57)	0.00	
Sed			0.3283	
CD (P=0.05%)			0.6960	

*Values are mean of five replicaties. Means followed by a common letter are not significantly different at 5%level by DMRT

Sebastian *et al.* (2015). Similar to our study, the extracts of *Polygonum amplexicaule* had more than 90 percent inhibitory effect on mycelial growth of *C. capsici* (Sattar *et al.,* 2018). Leaf extracts of *Acacia nilotica, Calotropis procera*, *Datura stramonium* and *Dadonea viscosa* suppressed uredospore germination in wheat (Rahber-Bhatti, 1988) and neem-based formulations were effective against *C. capsici* (Rajput and Palakshappa, 2014) where they found that azadirachtin was effective in inhibiting mycelial growth and sporulation of pathogens. Among the different solvent extracted metabolites of *A. vasica* and *A. paniculata*, ethyl acetate extracts showed maximum inhibition of mycelial growth with 80% and 81.11%, respectively (Table 2).

Further, different concentrations of ethyl acetate fractions of *A. vasica* and *A. paniculata* tested showed that 5000 ppm exhibited 64.40% and 62.77% inhibition of mycelial growth of *C. capsici*. (Table 3). The ethyl acetate fractions of *A. vasica* and *A. paniculata* at 5000 pm exhibited inhibition of spore germination (80.40% and 79.00% inhibition, respectively) of *C. capsici* at 18 hrs (Table 4). Perusal of literature have shown no reports on the effect of *A. vasica* against *C. capsici* so far. However, there are reports on the effect of diethyl ether solvent extracted metabolites of *A. vasica* against different bacterial clinical pathogens *Klebsiella pneumoniae*, *Staphylococcus aureus, Proteus vulgaris, Pseudomonas aeruginosa* and *Streptococcus pyogenes* (Sheeba and Mohan, 2012) and the ethanolic extracts of Ocimum sanctum and *A. vasica* against Vibrio cholerae (Mishra and Rai, 2021). The effect chloroform extract of *A. paniculata* against *S. aureus* and *Escherichia coli*, methanol and chloroform extracts against *E. coli, Aeromonas hydrophila, P. vulgaris, P. aeruginosa, S. aureus, S. pyogenes, Bacillus subtilis, K. pneumonia* and *Salmonella typhi* have been reported (Geetha, 2017). It was observed that ethyl acetate solvent extracted polar

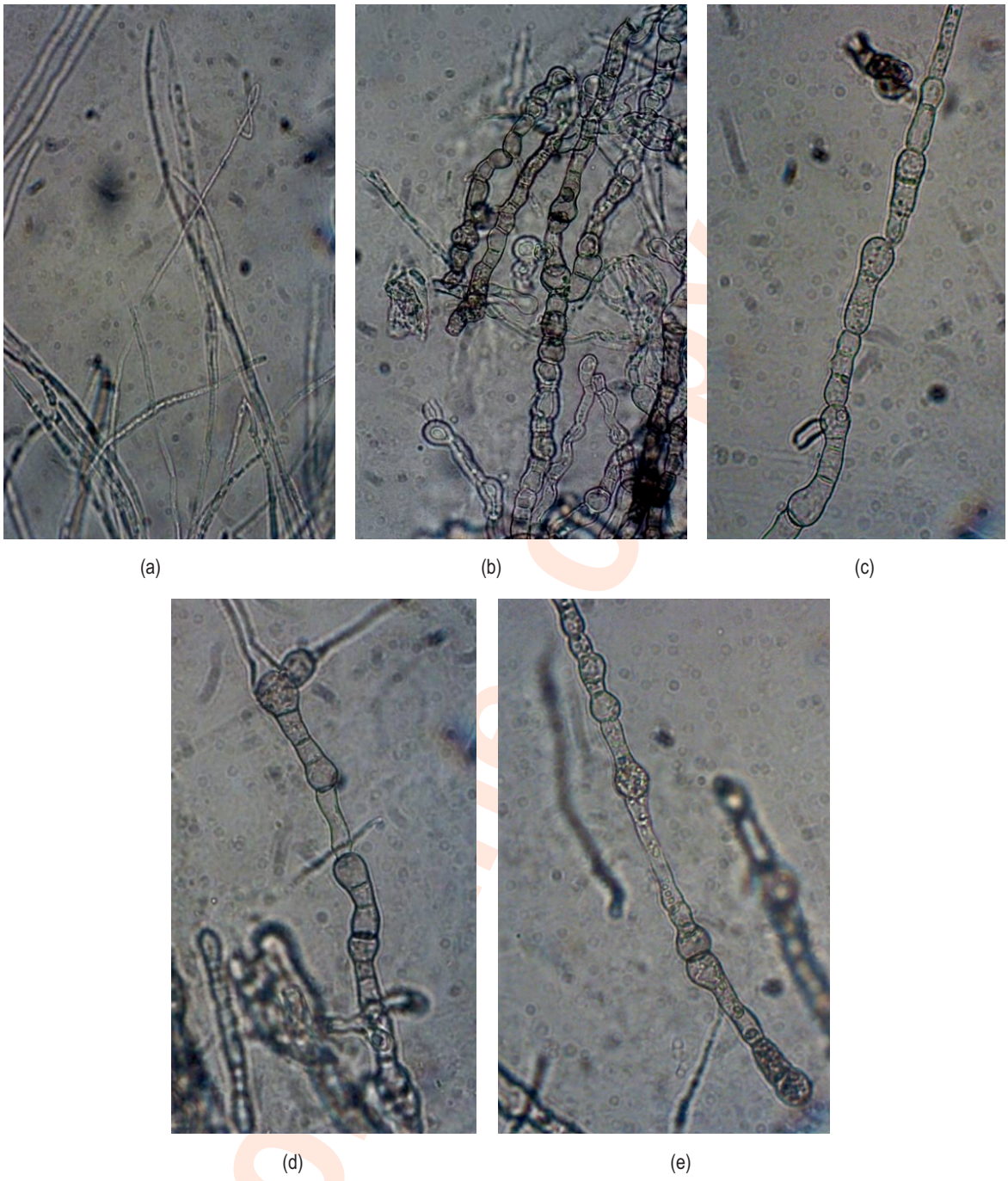

Fig. 1: Microscopic observation of mycelial modifications in plant extracts treated plate of *C. capsici.* (a) Control); (b) *A. vasica* treated; (c) *A. paniculata* treated; (d) Neem seed kernel extract treated and (e) *Tribulus terrestris* treated.

compounds of antimicrobial nature from *A. vasica* and *A. paniculata* leaf extracts and contributed to more significant inhibition per cent of mycelial growth and spore germination of *C. capsici* when compared to other solvents. Other researchers also opined that polar solvents like ethyl acetate have the ability to extract organic and inorganic materials from plants (Campos *et al.,* 2002). Many plant species have been reported to possess natural substances that are toxic to a variety of plant pathogenic fungi, and identifying compounds from plant products offers wide scope for developing fungicidal formulations. In the present study, characterization of compounds from ethyl acetate fraction of *A. vasica* leaf extract through GC-MS indicated the presence of 11 different compounds and among them 1-Octen-3-0l, 2,4-Di-tert-butylphenol, 1H-Pyrrolo [2,1-b] quinazolin-9-one,3 hydroxy-2,3-

dihydro-, Phytol and Octadecenoic acid possessed antimicrobial activities as mentioned by many researchers.

Among these compounds, 1H-Pyrrolo[2,1-b] quinazolin-9-one,3 hydroxy-2,3-dihydro- exhibited maximum peak area per cent with the probability of 93.9% at the retention time of 21.64. Serveral researchers have reported the antimicrobial activities of 1-Octen-3-ol against *B. subtilis*, *Streptomyces* spp., *Fusarium tincinctum* (Xiong *et al.,* 2017), 2,4-Di-tert-butylphenol against *F. oxysporum*, *Aspergillus niger, Candida albicans* (Varsha *et al.,* 2015), 1H-Pyrrolo[2,1-b]quinazolin-9-one, 3hydroxy-2,3-dihydro, Phytol and Octadecanoic acid against fungi and bacteria (Srinivasan *et al.,* 2014; Ghaneian *et al.,* 2015; Zhong hui *et al.,* 2010). The presence of these compounds in *A. vasica* leaf extract would have been responsible for the inhibition of mycelial growth of *C. capsici.* Similarly, 10 different compounds were detected in the ethyl acetate solvent fraction of *A. paniculata* leaf extract and among them Decane, Hexadecanoic acid, Phytol, Oleic acid and Docosahexaenoic acid possessed antimicrobial activities which has been reported earlier (Nahar *et al.,* 2016; Agoramoorthy *et al.,* 2007; Ghaneian *et al.,* 2015; Walters *et al.,* 2004; Bajpai *et al.,* 2009). Among the compounds detected, docosahexaenoic acid and oleic acid recorded maximum peak area at the retention time of 25.758 and 23.73 respectively. Indeed, the presence of these compounds in *A. paniculata* leaf extract would have been responsible for the inhibition of mycelial growth of *C. capsici* Supporting to this, reports show that n-Hexadecanoic acid, Phytol and Octadecenoic acid produced from chloroform extracts of *A. vasica* leaves (Jayashree and Gopukumar, 2019) and Octadecenoic acid, Oleic acid, n-Hexadecanoic acid present in the ethanolic extracts of *A. paniculata* (Kalaivani *et al.,* 2012; Vasantha *et al.,* 2013) to posses antimicrobial activities. It is also thought that like fungicides, the plant extracts also possess antimicrobial constituents with multifaceted action. The mechanism of plant-based parts in inhibiting enterotoxigenic bacterial strains has been well explained as interference with the phospholipoidal cell membranes, damage of genetic material and disturbance of the cytoplasmic membrane, disrupting the proton motive force, leading to coagulation of cell composition (Kotzekidou *et al*., 2008).

In the present study, the ethyl acetate solvent fraction of *A. vasica* evaluated by FT-IR analysis revealed that the leading bands in the regions between 624.823 and 3309.25 cm^{-1} belonged to aliphatic primary amine, amine salt, thiol, conjugated aldehydes, alkane, alcohol, sulfoxide and halo compounds with weak to strong range of intensity. The group of compounds falls under C=C=C stretching, N-H stretching, C=O stretching, O-H bending and S=O stretching (Table 5a). Different functional groups from ethyl acetate solvent fraction of *A. paniculata* belonged to aliphatic primary amine, amine salt, thiol, conjugated ketone, allene, carboxylic acid alcohol and halo compounds with the absorption ranging between 622.895 and 3309.25 cm^{-1}. The band's intensity ranged from weak to strong with C-O stretching, C=C stretching, N-H stretching, N-O stretching (Table 5b). The presence of different functional groups revealed that *A. vasica* and. *A. paniculata* consists of several important bioactive compounds in the aerial parts. Similar to our study, Srinivasan *et al.* (2014) identified the compound 1H-Pyrrolo[2,1-b] quinazolin-9-one,3 hydroxy-2,3-dihydro to possess antimicrobial, anti-inflammatory activity against fungal pathogens. Similar to our results, FTIR spectrum analysis of *Caralluma fimbriyata* indicated the presence of phenols, alkanes, aromatic amines (Packialakshmi and Naziya, 2014).

Detection of specific compounds from the metabolites of ethyl acetate solvent extracts of *A. vasica* and *A. paniculata* analyzed through TLC showed detection of two bands under UV lamp at Rf value of 0.17 and 0.57 for *A. vasica* and a single band for *A. paniculata* at the Rf value of 0.41. The bands eluted from *A. vasica* and *A. paniculata* spotted TCL plate when tested against mycelial growth of *C. capsici* showed that band 1 from *A. vasica* recorded maximum inhibition mycelial growth (60%) when compared to band 2 with inhibition percent of about 55.77%. The compounds eluted from band of *A. paniculata* showed 63.33% inhibition of mycelial growth (Table 6). The compounds eluted from TLC plate spotted with *A. vasica* metabolites were non volatile compounds and were not detected through GC-MS analysis. *A. paniculata* spotted TLC plates detected campesterol, a phytosterol compound at RT of 29.73 with the area percentage of 59.93 at probability of 58.52 through GC- MS analysis. The antibacterial and antifungal activity of phytosterols of Norway spruce bark extracts has been studied by Burcova *et al*.(2018) which add information that the campesterol detected in our study also possess antifungal activity. Further purification of campesterol through LC-MS will be attempted in future to elucidate the exact mode of action. Similar to present study, Taleb-Contini *et al.* (2003) reported that ethanolic and dichloromethane extract of *Chromolaena* species produce campesterol (phytosterol compound) with antimicrobial activity against Gram positive bacteria. The present study also depicts the extraction and elucidation of metabolites from *A. vasica* and *A. paniculata* with antimicrobial activity against *C. capsici* causing fruit rot of chilli, Finding more natural compounds from medicinal plants to control plant diseases will lead to a sustainable disease management approach and a potent alternative to synthetic chemicals. Future studies are directed towards developing spray dried/ nano based formulations of *A. paniculata* for field application to get desired effect on the management of chilli fruit rot pathogen. Also ongoing studies aim for developing formulations to treat chilli seeds to contain the seed borne nature of pathogen .

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Add-on Information

Authors' contribution: K. Priya: Performed the research

experiments on antimicrobial screening of plant extracts and microscopic documentation of pathogen. **G. Thiribhuvanamala:** Formulated the project, provided overall guidance and prepared the manuscript. **C. Sangeetha:** Performed the FTIR analysis. **A. Kamalakannan:** Provided guidance in testing of plant extracts against pathogen. **S. Haripriya:** Provided guidance on the biochemical characterisation of antimicrobial compounds from plant extracts. **S. Parthasarathy:** Executed the microscopic documentation of conidial and mycelial morphostructural changes in pathogen.

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