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Article

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Lantana camara: Phytochemical Analysis and Antifungal Prospective

Lantana camara: *Análise Fitoquímica e Prospecção Antifúngica*

ABSTRACT - In the current study phytochemical analysis and *in vitro* antifungal potential of fruits, leaves and stem of *Lantana camara* L. were studied. The phytochemical analysis indicated the presence of alkaloids, flavonoids, tannins, saponins, glycosides and terpenoids in fruit, stem and leaves of *L. camara*. The *in vitro* antifungal activity of fruit, stem and leaves of *L. camara* was tested against *Colletotrichum gloeosporioides* Penz. Different concentrations (1-5%) of methanolic extract of all the selected parts of *L. camara* were applied *in vitro* against the test fungus. The results of *in vitro* experiment revealed that higher concentration of methanolic fruit extract (5%) significantly reduced the biomass *C. gloeosporioides* up to 66%. This effective extract of *L. camara* was partitioned with *n*-hexane, chloroform, ethyl acetate and *n*-butanol. The bioactivity of these fractions was tested against *C. gloeosporioides*. The trials showed that 0.5% concentration of *n*-hexane fraction of methanolic fruit extract caused the highest reduction (45%) in the radial colony growth of the test fungus. This effective *n*-hexane fraction was selected for GC-MS analysis to identify various possible antifungal compounds. Cyclopropane, carboxylic acid, 5-heptonic acid, 2,2-dimethyl1-4-pentenoate and 2-Propyloctahydro-1-benzothiophene were identified as major compounds. This study can be concluded that *L. camara* fruit comprised of bioactive compounds which possess antifungal activity against *C. gloeosporioides.*

Keywords: anthracnose, bioactivity, *Colletotrichum gleosporioides*, GC-MS, phytochemicals.

RESUMO - Neste estudo, foi feita uma análise fitoquímica e do potencial antifúngico in vitro de frutos, folhas e caule de ***Lantana camara*** *L. A análise fitoquímica indicou a presença de alcaloides, flavonoides, taninos, saponinas, glicosídeos e terpenoides em frutos, caule e folhas de* ***L. camara****. A atividade antifúngica in vitro de frutos, caule e folhas de* ***L. camara*** *foi testada contra* ***Colletotrichum gloeosporioides*** *Penz. Diferentes concentrações (1-5%) do extrato metanólico de todas as partes selecionadas de* ***L. camara*** *foram aplicadas in vitro contra o fungo de teste. Os resultados do experimento in vitro revelaram que a maior concentração do extrato metanólico da fruta (5%) reduziu significativamente a biomassa de* ***C. gloeosporioides*** *em até 66%. Esse extrato eficaz de* ***L. camara*** *foi dividido em seções com n-hexano, clorofórmio, acetato de etila e n-butanol. A bioatividade dessas frações foi testada contra* ***C. gloeosporioides****. Os testes mostraram que a concentração de 0,5% da fração de n-hexano do extrato metanólico da fruta causou a maior redução (45%) no crescimento de colônias radial do fungo de teste. Essa fração eficaz de n-heaxane foi selecionada por análise GC-MS para identificar vários compostos antifúngicos possíveis. Ciclopropano, ácido carboxílico, ácido 5-heptônico, 2,2-dimetil-1-4-pentenoato e 2-Propilocta-hidro-1-benzotiofeno foram*

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identificados com os principais compostos. Esse estudo pode concluir que o fruto de L.camara compreendido de compostos de bioativos que possuem atividade antifúngica contra C. gloeosporioides.

Palavras-chave: antracnose, bioatividade, *Colletotrichum gleosporioides*, GC-MS, fitoquímicos.

INTRODUCTION

Mango (*Mangifera indica* L.) is an excellent and one of the most desirable fruits worldwide because of its delicious taste (Diedhiou et al., 2007). Mango is affected by a number of diseases at all stages of its development, right from the plants in the nursery to the fruits in storage or transit. The mango tree especially its fruit is the host of a large number of pathogens among which, fungi could be a major agent of fruit rot (Akem, 2006). Anthracnose disease, caused by *Colletotrichum gloeosporioides* Penz. belonging to Ascomycota is among the most destructive field and postharvest fungal pathogen of mango in the world (Arauz, 2000; Cannon et al., 2012). Synthetic fungicides are currently used as the primary means for the control of postharvest mango anthracnose. However, increasing public concern over the indiscriminate use of pesticides that leads to environmental hazards, as well as the occurrence of fungicide-resistant pathogen strains has stimulated research on alternative methods to control postharvest diseases (Yao and Tian, 2005). The plant world comprises a rich storehouse of biochemicals to be used as natural fungicides. Plant-based natural products emerging as safe alternatives to conventional fungicides for the control of plant diseases due to their ability to decompose rapidly (Tripathi and Shukla, 2007; Jabeen and Javaid, 2010; Karim et al., 2017; Hanif et al., 2017).

Lantana camara belongs to family Verbenaceae is an ornamental flowering plant and six hundred species of *L. camara* are available worldwide (Thakur et al., 1992). This plant is also known as, wild sage, tea plant and spanish flag. *L. camara* grows in un-shaded regions like wastelands, rainforest edges, beachfront and forests. Phytochemical investigation of *L. camara* showed the presences of flavones, isoflavones, flavonoids, anthocyanins, coumarins, lignans, catechins, isocatechins, alkaloids, tannin, saponins and triterpenoids (Ganjewala et al., 2009; Saraf et al., 2011; Singh and Srivastava, 2012; Mariajancyrani et al., 2014). The present study was therefore designed to study antifungal potential of *L. camara* against *C. gloeosporioides*.

MATERIALS AND METHODS

Phytochemical analysis of fruits leaves and stem of *L. camara* was carried out by using the protocols available to identify the categories of secondary metabolites present in the test plant species (Edeoga et al., 2005; Parekh & Chanda, 2007). In this analysis presence and absence of tannins, saponins, terpenoids, phenolics, alkaloids, glycoside & coumarin was tested.

In vitro antifungal activity of the experimental plant, *L. camara* against targeted fungus was performed by using the method of Waheed et al., (2016). Twenty grams of leaves, stem and fruit of test plant *L. camara* were soaked in 100 mL methanol separately. After 7 days soaked material was filtered with an autoclaved muslin cloth in pre-weighed beakers and the filtrate was evaporated at room temperature. The stock solutions for each test plant part were prepared by taking 5.144 g of each extract and diluted with 25.75 mL of distilled water to make 20% of the 60 mL stock solution. These stock solutions were stored at 4 °C. *C. gloeosporioides* was isolated from the infected inflorescence of mango plant, cultured and maintained on 2% MEA (Malt Extract Agar) medium and then re-cultured on 2% MEA and stored at 4 °C. The test fungus was identified on morphological basis using macroscopic and microscopic characters. *In vitro* antifungal activity of *L. camara* against *C. gloeosporioides* was tested. Various concentration viz. 1%, 2%, 3%, 4% and 5% of methanolic extract were prepared in 2% MEA medium (Sherazi et al., 2016). The control treatment was without any plant extract. Chloromycetin (anti-bacterial) capsules were added in each flask to avoid bacterial contamination. Each concentration (20 mL) was poured into 9 × 9 cm sterilized Petri plates. Three replicates were made for each treatment. Mycelia discs (5 mm) were taken from 7 days old culture as inoculum and were inoculated in all Petri plates with a sterilized cork borer. All these plates were sealed with parafilm strips and incubated at 25 °C for 7 days. After 7 days fungal growth was measured by using the formula:

$$Growth\ Inhibition\ \% = \frac{Growth\ in\ control - Growth\ in\ treatment}{Growth\ in\ control} \times 100$$

As the methanolic fruit extract exhibited maximum inhibition of fungal growth in initial screering therefore this extract was used for fractionation. For this purpose the fruit of *L. camara* (50 g) was extracted with 200 mL of methanol and 0.05 g gummy mass was obtained after evaporation. This gummy mass of methanolic fruit extract of *L. camara* (0.05 g) was partitioned with *n*-hexane, chloroform, ethyl acetate and *n*-butanol in separating funnel. These organic fractions were then evaporated in rotary evaporated at 40 °C. The *in vitro* antifungal activity of various isolated organic fractions and fungicide (Metalaxyl + Mancozeb 72% WP) was checked. Two concentrations (0.05% and 0.1%) for each fraction were prepared in MEA medium. Control media was without plant extract. Three replicates were made for each concentration and fungal inoculums were added in each plate of each concentration (Jabeen et al., 2013).

n-hexane fraction was selected for GC-MS analysis due to significant antifungal potential in the previous assay. *n*-hexane fraction was filtered with a nylon membrane filter (0.22 µm pore size and 47 mm diameter). Sample was analyzed by a GC-MS-QP2010 chromatograph and was separated on an DM-5MS capillary column (30 m, 0.25 mm, 0.25 µm) by applying the following temperature program 50 °C for 5 min, 40-70 °C at 2 °C min^{-1}, 70 °C for 2 min, 70-120 °C at 3 °C min^{-1}, 120-150 °C at 5 °C min^{-1}, 150-220 °C at 10 °C min^{-1} and then 220 °C for 2 min. Helium was used as a carrier gas. The injector and detector temperatures were 200 °C and 250 °C respectively. Mass detector conditions were: ionization voltage 70 eV mass scanning range m/z 29-540 and source temperature 230 °C. The percentage composition of volatile compounds was computed from GC peak areas. Qualitative analysis was based on a comparison of retention times, indices and mass spectra with the corresponding data in the literature (NIST Library 2010 word software) (Sherazi et al., 2016)

All the data were statistically analyzed by using analysis of variance (ANOVA) followed by Duncan's Multiple Range test (DMR) at Pd"0.05 (Steel et al., 1997).

RESULT AND DISCUSSION

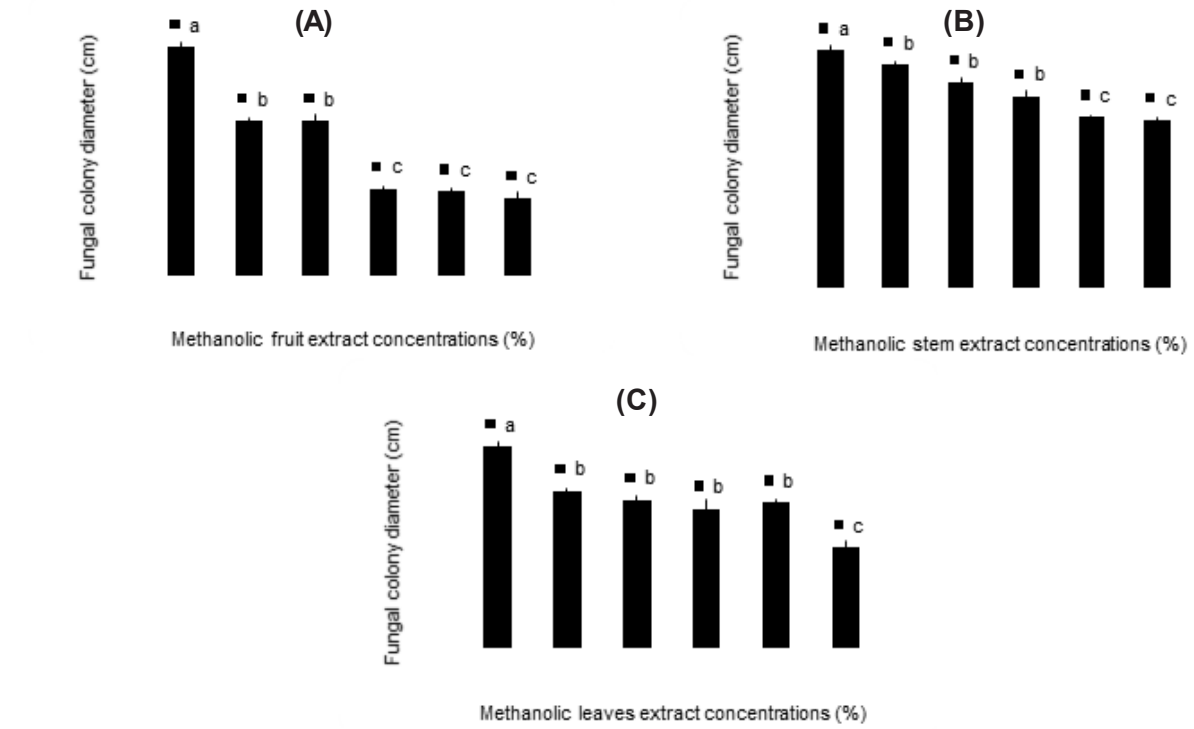
In the present study, fruits, leaves and stem of *L. camara* were examined as a natural alternative of synthetic fungicide for the control of *C. gleosporioides*. Phytochemical analysis of *L. camara* was also performed instead of evaluated. The phytochemical analysis of *L. camara* extracts showed the presence of different secondary metabolites, like alkaloids, phenols, flavonoids, glycosides, tannins and terpenoids (Table 1). Bhakta and Ganjewala (2009) reported that flavonoids, carbohydrates, proteins, alkaloids, glycosides, saponins, steroids, triterpenes and tannin are the major phytochemicals groups present in *L. camara*. The presence of these compounds might be responsible for its antifungal activity as strengthened by literature. The flavones extracted from the methanol extract of dried leaves of *L. camara* also showed the antibacterial and antifungal properties (Boughalleb et al., 2005; Gujar and Talwankar, 2012).

Antifungal potential of fruits, stem and leaves of *L. camara* was assessed against *C. gloeosporioides*. Among all the applied extracts methanolic fruit extract of the test plant significantly inhibited the radial growth of *C. gloeosporioides*. Results showed that 5% concentration of methanolic fruit extract exhibited maximum inhibition (66%). While other concentrations 1%, 2% 3% and 4% also significantly reduced the test fungal growth up to 32-63% (Figure 1A). Stem methanolic extract of *L. camara* also significantly reduced the radial diameter of *C. gloeosporioides*. However, maximum inhibition was observed in 5% extract i.e. 29% as compared to control. Other applied concentration 1%, 2%, 3% and

Table 1 - Phytochemical analysis of leaves, stem and fruit of *Lantana camara*

Phytochemical tests	*L. camara* seaves	*L. camara* stem	*L. camara* fruit
Tannins	+ve	+ve	+ve
Phlobtannins	-ve	-ve	-ve
Saponin	+ve	+ve	+ve
Flavonoids	-ve	+ve	+ve
Terpenoids	+ve	+ve	+ve
Glycoside	+ve	-ve	-ve
Alkaloids	+ve	+ve	-ve
Coumarins	-ve	-ve	-ve

(+ve) present (-ve) absent.



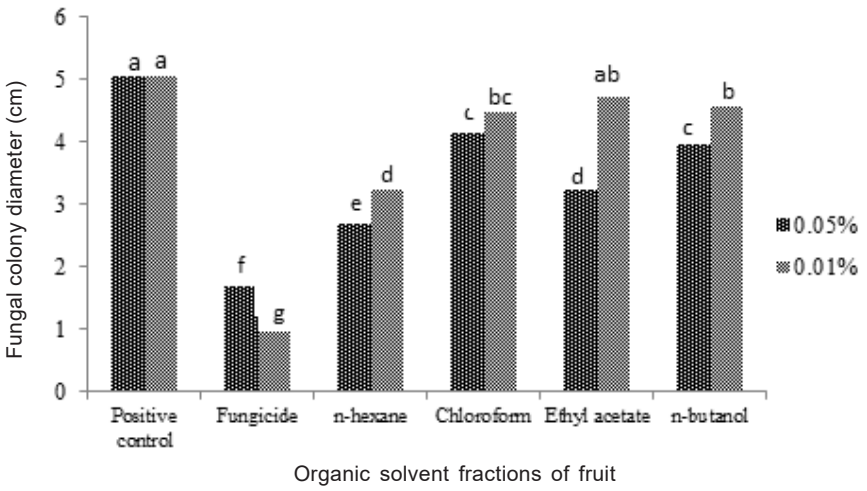
Vertical lines show the standard error while letters (a-c) show a significant difference.

Figure 1 - *In vitro* effect of methanolic fruit, stem and leaf extracts of *Lantan camara* on growth of *Colletotrichum gloeosporioides*.

4% were found comparatively less effective and retarded the growth of *C. gloeosporioides* up to 13-28% (Figure 1B). All the applied concentrations (1-5%) of methanolic extract of leaves of *L. camara* significantly retarded the growth of *C. gloeosporioides*. The maximum antifungal activity was shown by 5% leaves extract which caused 50% inhibition in the radial growth of test fungus as compared to control treatment. Other concentrations 1% - 4% caused 22% - 31% decline in the test fungal colony growth (Figure 1C). Similarly, Singh and Srivastava (2012) reported that *L. camara* leaves, likely possess biofungicidal activity against *Alternaria alternata.* Various literature reports also suggested that *L. camara* leaves displayed substantial antifungal properties (Boughalleb et al., 2005; Saraf et al., 2011; Gujar and Talwankar, 2012).

The fruit methanolic extract was found to be highly effective in antifungal bioassay so this plant part was used in bioassay guided fractionation and further partitioned by using various organic solvents. Four organic fractions viz. *n*-hexane, chloroform, ethyl acetate and *n*-butanol were isolated. The bioactivity of all the isolated organic fractions and fungicide metalaxyl mancozeb was tested against *C. gloeosporioides.* The *n*-hexane fraction of fruit of *L. camara* showed the best results as its lowest applied concentration 0.05% inhibited the growth of test fungus 45% (Figure 2).

The GC-MS analysis of *n*-hexane fraction of *L. camara* was performed and 16 bioactive compounds were identified. The identified compounds were Glutaraldehyde, 2- Decenal (E), 2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R), 2-pentadecyn-1-ol, cis-α-Bergamotene, Benzene, (1,1-dimethyl butyl)-(1,1-dimethyl butyl)benzene, 2-Methyl-2-phenylpentane, 4-Hexadecen-6-yne, (z), Turmerone 2-Methyl-6-(4-methyl-1,3-cyclohexadien-1-yl)-2-hepten-4-one, Curlone, Hexadecanoic acid, 15-methyl-,methyl ester, Octadecanoic acid, 6-Methyl-2-tridecanone, Heptadecanoic acid, Nonadecane, 2-methyl-, Tetradecanoic acid and Pentadecanoic acid, 14-methyl-,methyl ester (Table 2). One of the major compound cyclopropane identified in the present study has known fungicidal activity against the rice blast disease caused by *P. oryzae* (Cartwright et al., 1977). A 5-Heptenoic acid compound also contains antifungal activity against *Myrothecium verrucaria* and *Trichoderma viride* (Gershon and Shanks, 1978). Similarly, the compound Glutaraldehyde i.e. is a dialdehyde also displayed bactericidal, fungicidal, mycobactericidal and



Vertical lines show the standard error while letters show significant difference.

Figure 2 - *In vitro* effect of different organic solvent fractions of fruit of *Lantana camara* extract on growth of *Colletotrichum gloeosporioides*.

Table 2 - GC-MS analysis of n-Hexane fraction of *Lantana camara* fruit

Sr#	R. time	Compound	Molecular formula	Mol. weight	Peak area
1.	6.650	Glutaraldehyde	$C_5H_8O_2$	100	4.02
2.	29.120	2- Decenal (E)	$C_{10}H_{18}O$	154	0.89
3.	40.880	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl, (R)	$C_{11}H_{16}O_2$	180	2.23
4.	42.525	2-pentadecyn-1-ol	$C_{15}H_{28}$	224	2.67
5.	42.658	cis-α-Bergamotene	$C_{15}H_{24}$	204	2.23
6.	43.092	Benzene, (1,1-dimethylbutyl)-(1,1-dimethylbutyl)benzene,2-Methyl-2-phenylpentane	$C_{12}H_{18}$	162	8.03
7.	44.258	4-Hexadecen-6-yne, (z)	$C_{16}H_{28}$	220	3.57
8.	44.792	Turmerone 2-Methyl-6-(4-methyl-1,3-cyclohexadien-1-yl)-2-hepten-4-one	$C_{15}H_{22}O$	218	8.48
9.	45.517	Curlone	$C_{15}H_{22}O$	218	3.12
10.	45.925	Hexadecanoic acid, 15-methyl-,methyl ester	$C_{18}H_{36}O_2$	284	6.25
11.	46.750	Octadecanoic acid	$C_{18}H_{36}O_2$	284	8.03
12.	47.770	6-Methyl-2-tridecanone	$C_{14}H_{28}O$	212	3.13
13.	48.090	Heptadeanoic acid	$C_{17}H_{34}O_2$	270	3.13
14.	48.500	Nonadecane, 2-methyl-	$C_{20}H_{42}$	282	2.23
15.	49.850	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	21.00
16.	48.870	Pentadecanoic acid, 14-methyl-,methyl ester	$C_{17}H_{34}O_2$	270	21.00

sporicidal activity (Russell, 1994). Glutaraldehyde, a fungicide, is proved to be effective against the saprophytic fungi has been identified in GC-MS analysis of *n*-hexane fraction in the present study. 2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7atrimethyl, a bioactive compound also possessed antifungal properties has also been identified in this GC-MS analysis. Curlone, a sesquiterpene compound and essential oil of turmeric, has been found in the current study of *n*-hexane fraction during GC-MS analysis. Previously Kumar et al. (2016) studied the antifungal properties of curlone (essential oil). Omoruyi et al., 2014 reported that *n*-hexadecanoic acid (Palmitic acid) which was identified in GC-MS analysis of *n*-hexane of *L. camara* fruit possesses antimicrobial properties. Heptadecanoic acid is an unsaturated fatty acid which has also been identified in the present study has antifungal potential against many fungal strains (Agoramoorthy et al., 2007). However, in the present study, the *n*-hexane extract of the fruit of *L. camara* is specifically tested against the anthracnose causing fungus *C. gloeosporioides*. So, the results of the present study depicted that *L. camara* fruit contains potential antifungal constituents against *C. gloeosporioides*. Any of these identified compounds alone or in combination might be responsible for the strong fungicidal potential of *Lantana camara*. These compounds can be exploited in future for the synthesis of nature-friendly antifungal compounds against *C. gloeosporioides*.

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