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International Journal of Current Research
Vol. 7, Issue, 07, pp.17706-17712, July, 2015

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

ANTIFUNGAL ACTIVITY OF *ACACIA NILOTICA* EXTRACTS IN CONTROL OF *COLLETOTRICHUM GLOEOSPORIOIDES* [PENZ.] FUNGI CAUSING ANTHRACNOSE OF MANGO FRUITS

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ARTICLE INFO

Article History:
Received 22nd April, 2015
Received in revised form
05th May, 2015
Accepted 19th June, 2015
Published online 28th July, 2015

Key words:

Antifungal,
Acacia nilotica,
Phytopathogen,

ABSTRACT

Antifungal activity of *Acacia nilotica* and a number of other plants extracts was evaluated to control anthracnose disease of mango fruits. During the study leaf extracts of seven plants viz., *Acacia nilotica* (ethyl alcohol), *Citrus aurantifolia* (ethyl acetate), *Murraya koenigii* (ethyl acetate), *Nerium indicum* (ethyl acetate), *Ocimum gratissimum* (petroleum ether, benzene, ethyl acetate, ethyl alcohol,), *Ocimum sanctum* (petroleum ether), *Prunus persica* (ethyl acetate) and bark extract of *Acacia farnesiana* (ethyl acetate) and *Acacia nilotica* (benzene, ethyl acetate, ethyl alcohol, methanol) showed 100 per cent activity against *Colletotrichum gleoesporioides*. The bark of *A. nilotica* showed 100 % activity in four organic solvents viz. benzene, ethyl acetate, methanol, ethyl alcohol. Therefore the bark of *A. nilotica* was selected for further investigations. The maximum inhibitory dilution of ethyl acetate, benzene, methanol and ethyl alcohol extracts of *A. nilotica* were determined to be 1:10,1:2,1:8,1:9 respectively. All the extracts of *A. nilotica* showed broad antifungal activity against ten fruit rotting fungi. The extracts showed significant inhibition in conidia germination of *C. gloeosporioides*. The effect of storage and temperature on fungitoxicity of *A. nilotica* extracts was also evaluated. Antifungal activity of some of the compounds isolated from the bark of *A. nilotica* was also tested. Kaempferol and Kaempferol 3-O-rhamnoside were found to be active against the test fungus and the structure activity relationship amongst kaempferol and kaempferol-3O-rhamnoside was also determined. An *in vivo* trial with the aqueous extract of *A. nilotica* bark showed enhancement of shelf life of mango fruits by preventing them from fungal rotting.

Citation: Pramila Tripathi and Rinki Singh, 2015. "Antifungal activity of *Acacia nilotica* extracts in control of *Colletotrichum gloeosporioides* [Penz.] fungi causing anthracnose of mango fruits", *International Journal of Current Research*, 7, (7), 17706-17712.

INTRODUCTION

Fruits have been an important part of our food from pre-historic times, as they are a chief source of vitamins, minerals, fats, fibers, trace elements etc. Mango has an important place among the national and international fruit market. However, postharvest rots pose one of the biggest problems to commercialize high quality fruits. Anthracnose caused by *Colletotrichum gloeosporides* [(Penz.) Penz. And Sacc] is an important disease of mango (Snowdon, 1990). For many years growers and export industries have relied heavily on synthetic fungicides to control anthracnose. Since the existing chemical control measures being costly and can favour development of resistance in pathogens, there is need to explore alternatives to reduce this important disease. Many natural products have been explored in recent years as alternative chemicals to control postharvest fungal rots of fruits and vegetables (Tripathi and Dubey, 2004).

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Plant extracts have opened a new avenue for the control of plant diseases. Much attention has been given to the use of phenolic rich plant extracts. Substances such as flavonoids, alkaloids, terpenoids are the secondary metabolites that are present in plant extracts and are produced by the plants as chemical defense against pests and disease attacks. The extract of a number of higher plants were proven by previous workers to posses antifungal activities against fruit rotting pathogenic fungi (Sharma *et al*., 2006; Amienyo and Ataga, 2007; Soon-Ok *et al*., 2008; Ijato, 2011; Gatto *et al.*, 2011; Patnaik *et al.,* 2012; Lakshmi *et al*., 2014). However, it is estimated that only 10% of the tropical plants have been investigated for their pesticidal activity. In these studies growth of the fungal pathogens were affected at some stage in their development (mycelia growth, sporulation or conidial germination). The potential fungicidal property of plant extracts prompted this investigation. The objective of study was to screen the various plant extracts in different organic solvents against *C. gloeosporioides* and to propose the fungicidal property in bark of *Acacia nilotica* based on *in vitro* and in *vivo* trials.

MATERIALS AND METHODS

Isolation of fruit rots fungal pathogens

The *Colletotrichum gloeosporioides* and *Botryodiplodia theobromae* were isolated from infected Langra variety of mango fruits (*Mangifera indica* L.), *Aspergillus niger*, *Aspergillus fumigatus, Botrytis cinerea* and *Rhizopus stolonifer* were isolated from infected grapes, *Fusarium roseum* and *Monilinia fructicola* were isolated from infected peach, *Ceratocystis paradoxa* was isolated from infected pine apple, *Penicillium expansum, P. digitatum* and, *Rhizopus stolonifer* were isolated from infected citrus fruits. These fungal pathogens were identified according to Barnett and Hunter (1972). The stock cultures of these fruit rotting fungal pathogens were maintained on potato dextrose-agar (PDA) medium.

Preparation of extracts from fresh leaves

Leaves of twenty four angiospermic plants (*Acacia catechu* Willd (Mimosaceae), *Acacia farnesiana* Linn Willd (Mimosaceae), *Acacia nilotica* (Mimosaceae), *Achyranthus aspera* Linn Del (Amaranthaceae), *Adenocalymna allicea* Mark (Liliaceae), *Adhatoda vasica* Nees (Acanthaceae), *Aegle marmelos* Linn Correa (Rutaceae), *Ageratum conyzoides* Linn (Asteraceae),*Allamanda cathartica* Linn Mant (Apocynaceae), *Antigonon leptopus* Hk and Arny(Polygonaceae), *Asparagus racemosus* Willd (Liliaceae) and bark of *Acacia catechu*, *A.farnesiana* and *A.nilotica* were collected from the locality. Leaves/bark was thoroughly washed in sterilized water and were kept in between filter papers to remove the water. Twenty gm leaves/bark of each plant species were extracted separately in 40 ml of different organic solvents viz. petroleum ether ,benzene, ethyl acetate, methanol and ethyl alcohol by macerating them to pulp in a conical flask stirred vigorously and left to stand for 24h at (27±1ºC). These were filtered through Whatman's filter paper no 1.

Effect of Extract on Radial Mycelia Growth

The filtrates were assayed separately against the test fungus *C.gleosporioides* by the modified paper disc technique as recommended by Conner and Beachut (1984). Two ml of the filtrate of each sample was impregnated separately to assay disc (19 mm diam.from Whatman's filter paper no 1) by repeated addition and evaporation of solvent with the help of hair drier. For control discs were impregnated similarly with the same amount of requisite solvents. The assay discs were aseptically transferred to the centre of the Petri plates (7 cm.diam.) containing 10 ml potato dextrose agar medium/plate. A mycelial disc (5 mm in diam) cut from the periphery of a seven day old culture of the test fungus was aseptically inoculated upside down to the centre of each assay disc in treatment and control sets. The plates were incubated at 27±1ºC for six days and observations were recorded on seventh day. To determine area covered by mycelial growth, with a measuring rule, diameters of the longest and shortest points were taken. The per cent mycelia inhibition was calculated by following equation.

$$IR\ (\%) = \frac{dc - dt}{dc} \times 100$$

where: IR = inhibitory activity to the radial growth
dc = average increase in mycelia growth in control plates
dt = average increase in mycelia growth in treated plates

Determination of maximum inhibitory dilution of the extracts and nature of toxicity

The maximum inhibitory dilution of *A.nilotica* (in benzene, ethyl acetate, ethyl alcohol and methanol) against *C.gloeosporioides* was determined by the usual modified paper disc technique by diluting each extract from 1:2 to 1:10 dilutions. The nature of toxicity (Fungicidal/fungistatic) of the extracts against the test fungus was determined following Thompson (1989).The inhibited fungal discs of the extract-treated sets were re-inoculated into fresh medium and revival of their growth was observed.

Preparation of conidia suspension and conidia germination inhibition

Conidia of *C. gloeosporioides* cultured on PDA plates were taken and conidial suspensions (10^5 /ml) were made separately with *A. nilotica* bark extract in petroleum ether, benzene, ethyl acetate, methanol and ethyl alcohol. These suspensions (1.25 ml) were taken in small sterilized Petri dishes (65 mm) and were kept at 27±1 º C for 5~30 minutes. Drops of treated conidial suspension (from different extracts of A.nilotica) were taken on separate slides at an interval of 5 minutes and were kept at 27± ^{o} C in a moisture chamber for 24 hrs of incubation. Then a drop of lactophenol cotton blue was placed on the conidial suspension on the slides. The slides were examined under high power microscope (40X) for recording conidial germination. The inhibition of conidia germination was calculated following equation

$$Ig\ (\%) = = \frac{dc - dt}{dc} \times 100$$

where Ig =inhibition in conidia germination
dc=average conidial germination in control
dt=average conidia germination in treatment

Effect of storage and temperature on toxicity of extracts of *A. nilotica*

The bark extracts in petroleum ether, benzene, ethyl acetate, methanol and ethyl alcohol were stored in different vials at room temperature and were tested at regular intervals of one month, so as to determine the effect of storage on toxicity of the extracts. The thermostability of the fungitoxicity of the bark was determined by the usual modified paper disc technique by heating the bark at different temperatures viz.20ºC, 40ºC, 60ºC, 80ºC, 100ºC, 120ºC, 140ºC, 180ºC and 200ºC for two hours. The extracts were prepared in petroleum ether, benzene, ethyl acetate, methanol and ethyl acetate.

Range of fungitoxicity of the extracts of *A. nilotica*

The antifungal spectrum of extracts of *A. nilotica* (Bark) (in petroleum ether, benzene, ethyl acetate, methanol and ethyl

alcohol) against ten other fruit rotting fungi viz *Aspergillus niger*, *Aspergillus fumigatus, Botryodiplodia theobromae, Botrytis cinerea, Fusarium roseum, Monilinia fructicola, Ceratocystis fimbriata, Penicillium expansum, P.digitatum* and *Rhizopus stolonifer* were tested by usual modified paper disc technique.

Antifungal activity of compounds from *A. nilotica* bark

Antifungal activity of the compounds sitosterol, amyrin, naringenin-5-methyl ether, Kaempferol, Kaempferol -3-O rhamnoside, myrecetin 3-O-rhamnosoid isolated from the bark extract of *A.nilotica* (Tripathi *et al*., 2002) were examined for radial mycelia growth inhibition of the test fungus *C.gloeosporioides* by using the modified paper disc technique. Stock solutions were prepared by dissolving each compound (10 mg) in 2 ml of DMSO solution (5% in distilled water) separately and then diluting to 10 to 5,000 µg/ml. Single well (8 mm in diameter) was prepared at the center of the plate. A sterile filter paper (8 mm in diameter) was placed on the bottom of the well and 100 µl of the diluted solutions of the compounds or 5% DMSO solution (control) was dripped into the well. An agar plug (8 mm in diameter) cut from the margin of actively growing cultures of the *C. gloeosporioids* was placed on the filter paper in the well. Inhibition of fungal growth was examined by measuring the length of mycelium mat from the center of the plate after 7 days incubation at 27±1 ^{o}C.

***In vivo* applicability of the *A.nilotica* aqueous extract in control *C.gloeosporioides* causing anthracnose of mango fruits**

Freshlly harvested, half ripen healthy fruits of mango (langra variety) were washed with water, surface sterilized with 1% sodium hypochlorite solution and rinsed in five changes of sterile distilled water. The fruits were soaked in aqueous bark extract (1:1w/v) and allowed to stand in the extract for 5min, 15min and 30min separately. In the control, fruits were soaked in sterile distilled water. The fruits were removed from the extract and water (for control) and incubated at room temperature for 24 h. Using a 1.1 cm cork borer, discs were removed from the extract treated and water treated control fruits, and replaced with 1.1 cm discs of a 5 day old culture of test fungus *C. gloeosporioides*. Vaseline jelly was used to completely seal each hole. The inoculated mango fruits were placed in sterile sealed containers and incubated at room temperature (27± 1 ^{o} C) .The initiation of rotting of the fruits were observed. Eight replicates were kept for treatment and control sets.

RESULTS

Results of present investigation indicate that most of the plant species showed either poor (below 50%) or moderate (above 50 % and below 100%) activity against *C. gloeosporioides* (Table 1). The antifungal activity of the plant extracts also found varied with in solvents used for extraction. Leaf extracts of seven plants viz. *Acacia nilotica* (ethyl alcohol), *Citrus aurantifolia* (ethyl acetate), *Murraya koenigii* (ethyl acetate), *Nerium indicum* (ethyl acetate), *Ocimum gratissimum* (petroleum ether, benzene, ethyl acetate, ethyl alcohol), *Ocimum sanctum* (petroleum ether), *Prunus persica* (ethyl acetate) and bark extract of *Acacia farnesiana* (ethyl acetate) and *Acacia nilotica* bark (benzene, ethyl acetate, methanol, ethyl alcohol) showed 100 per cent activity against test fungus. Leaf extracts of *Achyratnthus aspera* and *Hyptis suaveolens* showed poor activity. The bark extract of *A. nilotica* showed 100% activity in four organic solvents viz. benzene, ethyl acetate, ethyl alcohol and methanol. Therefore, on account of strong fungitoxicity, *Acacia nilotica* bark extracts were selected for detailed investigations in the present study.

The maximum inhibitory dilutions of all the extracts of *A. nilotica* were determined against the test fungus *C. gloeosporioides* (Table 2). It was found that the *A. nilotica* bark extract in ethyl acetate showed 100% inhibition in all the dilutions starting from 1:2 to 1:10. Therefore its Maximum inhibitory dilution was determined to be 1:10. Methanolic and ethyl alcoholic extracts of *A. nilotica* were found showed 100% inhibitory at 1:8 and 1:9 dilutions respectively. Therefore their MID were determined as 1:8 and 1:9 respectively. Benzene extract showed 100% inhibition at 1:2 dilution therefore it's MID was determined to be 1:2 dilution. Petroleum ether extract did not show 100% inhibition in any of the dilution. The benzene extract was found to be fungistatic at 1:2 dilutions. The ethyl acetate extract showed fungicidal nature at 1:2, 1:3, 1:4, 1:5 dilutions while its activity was turned static at further dilutions of 1:6, 1:7, 1:8, 1:9 and 1:10 dilutions. Similarly the methanol and ethanol extracts showed fungicidal nature up to 1:4 dilutions after that at each dilution both the extracts showed fungistatic nature.

Extracts of *A. nilotica* bark in benzene, ethyl acetate ,methanol and ethyl acetate showed strong fungitoxicity at their respective MID against all the fruit rotting fungi viz *Aspergillus niger*, *Aspergillus fumigatus, Botryodiplodia theobromae, Botrytis cinerea, Fusarium roseum, Monilinia fructicola, Ceratocystis fimbriata, Penicillium expansum, P.digitatum* and *Rhizopus stolonifer* fungi. While petroleum ether extract did not show fungitoxicity against all the tested fruit rotting fungi. All the extracts of *A. nilotica* showed inhibitory activity of conidia germination (Table 3). Ethyl acetate extract showed maxmum inhibition at each treatment period. It showed 99 % inhibition at 30 min treatment. Benzene, Methanol and ethyl alcohol extracts also showed conidial germination inhibition of 94, 95 and 96 % respectively on 30 min treatment interval.

The effect of storage on the fungitoxicity of the extracts was determined. The petroleum ether extract lost its fungitoxic activity after one month. The benzene extract showed activity for four months and Extract in ethyl acetate, methanol and ethyl alcohol retained fungitoxic activity up to 24 months. The effect of temperature on fungitoxicity of the bark extract was also determined. At 200ºC ethyl acetate, ethyl alcohol and methanol extracts retained their fungitoxicity. Benzene extract showed fungitoxicity only up tp 80ºC while petroleum ether extract did not showed fungitoicity even at 60ºC. The antifungal activity of the compounds reported (Tripathi *et al*., 2002) from the bark of *A. nilotica* was also tested against the test fungus *C. gloeosporioides*. Kaempferol showed 100% inhibition of radial mycelial growth of *C. gloeosporioides* at 2000µg/ml.

Table 1. Evaluation of plant extracts for their fungitoxicity against *Colletotrichum gloeosporioides*

% inhibition of growth of *C.gloeosporioides*							
Plant species	Family	Source	Petroleum ether	Benzene	Ethyle acetate	Methanol	Ethyl alcohol (90%)
Acacia catechu Willd	Mimosaceae	Leaf	10.5	25.0	80.5	75.8	84.4
Acacia catechu Willd	Mimosaceae	Bark	00.0	74.0	48.0	89.0	86.0
A.farnesiana Linn Willd	Mimosaceae	Leaf	48.2	10.0	52.7	25.2	35.9
A.farnesiana Linn Willd	Mimosaceae	Bark	00.0	36.2	100.0	95.0	75.0
A.nilotica Linn Del	Mimosaceae	leaf	35.0	12.0	90.0	55.2	100.0
A.nilotica Linn Del	Mimosaceae	bark	32.1	100.0	100.0	100.0	100.0
Achyranthus aspera Linn Del	Amaranthaceae	Leaf	00.0	00.0	50.0	00.0	00.0
Adenocalymna allicea Mark ex Meissn	Liliaceae	Leaf	45.6	65.5	72.1	80.0	40.2
Adhatoda vasica Nees	Acanthaceae	Leaf	35.5	50.0	75.3	45.2	35.0
Aegle marmelos Linn Correa	Rutaceae	Leaf	65.4	90.2	95.0	48.8	57.0
Ageratum conyzoides Linn	Asteraceae	Leaf	24.4	42.4	50.0	65.7	75.0
Allamanda cathartica Linn Mant	Apocynaceae	Leaf	50.0	30.0	90.0	95.0	95.0
Antigonon leptopus Hk and Arnu	Polygonaceae	Leaf	00.0	45.0	65.0	60.0	35.0
Asparagas racemosus Willd	Liliaceae	Leaf	50.0	56.4	48.9	25.4	48.0
Caesulia axillaris Roxb	Asteraceae	Leaf	72.4	62.4	8.0	00.0	10.0
Citrus aurantifolia Christum Swingle	Rutaceae	Leaf	90.0	30.0	100.0	10.0	45.0
Clerodendrum inerme Gaerth	Verbenaceae	Leaf	88.0	84.0	83.0	80.0	85.6
Hyptis suaveolens Linn Poir	Lamiaceae	Leaf	00.0	30.0	38.5	10.0	00.0
Lantana indica Roxb	Verbenaceae	Leaf	25.4	54.0	70.0	55.0	00.0
Lippia alba Mill	Verbenaceae	Leaf	23.4	34.4	50.0	44.2	55.0
Murraya koenigii Linn Stooks	Rutaceae	Leaf	00.0	87.2	100.0	70.0	63.9
Nerium indicum Mill Gard	Apocynaceae	Leaf	74.0	70.0	100.0	25.0	78.0
Ocimum gratissimum Linn	Lamiaceae	Leaf	100.0	100.0	100.0	28.0	100.0
O.sanctum Linn Mant	Lamiaceae	Leaf	100.0	96.0	85.0	10.0	00.0
Pothos scandens Linn	Araceae	Leaf	00.0	55.6	10.2	40.0	10.0
Prunus persica Linn Stooks	Rosaceae	Leaf	00.0	79.0	100.0	70.0	65.0
Spilanthus oleracea Linn	Asteraceae	Leaf	10.5	0.0	25.0	00.0	00.0
Control							

Table 2. Maximum inhibitory dilution of the *A. nilotica* extracts

Percent inhibition of radial colony growth of *C.gloeosporioides*									
Dilution	1:2	1:3	1:4	1:5	1:6	1:7	1:8	1:9	1:10
Petroleum ether	10.0	00.00	00.0	00.0	00.0	00.0	00.0	00.0	00.0
Benzene	100	90.00	85.00	70.00	50.00	35.00	10.00	0.00	0.00
Ethyl acetate	100	100	100	100	100	100	100	100	100
Methanol	100	100	100	100	100	100	100	95.00	80.00
Ethyl alcohol	100	100	100	100	100	100	100	100	96.00

Table 3. Effect of *A.nilotica* bark extracts in different organic solvents on conidial germination of C. *gleosporioides*

% of conidia germination inhibition after immersed in *A.nilotica* bark extracts (minutes)						
	5	10	15	20	25	30
Petroleum ether extract	2	5	6	10	12	15
Benzene extract	50	57	66	70	85	94
Ethyl acetate extract	66	69	73	80	90	99
Methanol extract	60	62	70	79	83	95
Ethyl alcohol 90% extract	56	68	72	76	85	96

Table 4. Efficacy of plant extracts by Dip method under *in vivo* condition against pathogenic fungi

Dip treatment (min)	Initiation of rotting of fruits	Enhancement of storage life (in days)
Control	3	
5	6	3
15	7	4
30	9	6

Kaempferol 3O-rhamnosoid showed 94% inhibition at the same concentration. Sitosterol inhibited only 30%mycelial growth and amyrin, narengenin 5 methyl ether and myrecetin 3-Orhamnosoide showed inhibition of 45, 50 and 65% respectively at 2000µg/ml.

The *in vivo* experiment with the aqueous extract of *A. nilotica* was also evaluated (Table 4). The aqueous extract treated mango fruits showed enhancement of storage life of 3 to 6 days depending on the dip treatment duration. 5min dip enhanced shelf life of 3 days while 15 min dip enhanced shelf life for 4 days and 30 min dip treatment enhanced shelf life for 6 days. In general, fruit quality was not significantly affected when fruits were dipped in aqueous extract.

DISCUSSION

Plant extracts are one of several non chemical control measures currently being intensively researched for control of plant diseases. In recent years, plant extracts have been specially reported to have antimicrobial activity against a wide range of fungi (Doubrava *et al*., 1998; Kumar and Tripathi, 1991; Gujar and Talwankar, 2012; Lakshmi *et al*., 2014). Screening of the plants of the locality was carried out against the test fungus *C gloeosporioides* the causal organism of anthracnose of mango. In the present investigation (Table1) 24 angiospermic plant species belonging to 12 different families were screened. Most of the earlier workers have screened plants against different storage fungi using only one organic solvent or water for extraction. But this practice would not give complete idea of fungitoxicity of a plant, as the concentration of the fungitoxic principle of a plant will vary in different organic extracts. Therefore, in the present study five organic solvents viz. petroleum ether, benzene, ethyl acetate, methanol and ethyl alcohol were taken for extraction of the plants in order to get clear picture of fungitoxicity. Ethyl acetate extract of leaves of *Citrus aurantifolia*, *Murraya koenigii*, *Nerium indicum* and *Prunus persica* showed 100 per cent activity. Ethyl alcoholic extract of *Acacia nilotica* leaves and petroleum ether extract of *Ocimum sanctum* were found to exhibit 100 percent antifungal activity. *Ocimum gratissimum* exhibited 100 percent activity in four organic solvents viz petroleum ether.benzene, ethyl acetate and ethyl alcohol against the test pathogen. The ethyl acetate was found to be effective solvent for screening as in this solvent seven (viz., *A. farnesiana, A. nilotica, C. aurantifolia, M. koenigii, N. indicum, O. gratissimum* and *P. persica*) plants have shown fungitoxicity. While extract in petroleum ether solvent was not found effective as in this solvent extract only a single plant (*O. sanctum*) has shown its activity. In methanol only *A. nilotica* leaf has shown its 100% activity while in ethyl alcohol (*A. nilotica* leaf and bark) and *O. gratissimum* has shown 100% activity. Therefore it is evident that the fungitoxicity of the plants has been found to vary with the solvents used for the extraction. Raghvendra *et al*. (2009) also reported the antifungal activity of aqueous, petroleum ether, benzene, chloroform, methanol and ethanol extracts of *Prosopis juliflora* against *Alternaria alternata* the causal organism of brown spot of tobacco.

Although the organic solvent extracts of *Acacia farnesiana* (ethyl acetate), *Acacia nilotica* (ethyl alcohol), *Citrus aurantifolia* (ethyl acetate), *Murraya koenigii* (ethyl acetate), *Nerium indicum* (ethyl acetate), *Ocimum gratissimum* (petroleum ether, ethyl acetate, benzene), *O.sanctum* (petroleum ether) and *Prunus persica* (ethyl acetate) showed absolute fungitoxicity inhibiting complete growth of the test fungus. Only *Acacia nilotica* was taken as active plant for further investigation as its bark showed antifungal activity in four different organic solvents (benzene, ethyl acetate, ethyl alcohol, methanol). The plant grows luxuriantly as waste land tree in different parts of India and used traditionally in Indian system of medicine.

The maximum inhibitory dilution (MID) of a fungitoxicant should be determined in order to prescribe appropriate dosage for its exploitation, as higher concentration of dosage may cause side effects and involve wastage. The ethyl acetate extract of *A. nilotica* bark (Table 2) showed MID of 1:10 dilution. While the MID of benzene, methanol and ethanol extracts were found to be 1:2, 1:8 and 1:9 dilutions respectively. The determination of nature of a fungitoxicant (fungicide/fungistatic) is of great practical value as it helps in regulating the time intervals of treatment. A fungitoxicant may inhibit the growth of a fungus temporarily or permanently. In the former, the fungitoxicant is referred to as fungistatic while in later, as fungicidal. The benzene extract was found to be fungistatic at 1:2 dilutions. Ethyl acetate extracts (up to 1:8 dilutions), Methanol extracts (up to 1:4 dilutions) ethyl alcohol extracts (up to1:6 dilutions), remained cidal and they turned static at higher dilutions viz. ethyl acetate (above 1:9) methanol (above 1:5) ethyl alcohol (above 1:7) dilutions.

The Fungitoxic spectrum of a plant product should be worked out to know its range of fungitocicity. A fungitoxicant may exhibit broad fungitoxic spectrum inhibiting many fungi or may be effective against some specific one only. A fungitoxicant possessing a narrow range of toxicity cannot be employed successfully in controlling fungal infestation of perishables during storage and transit, because a variety of fungi usually infest the perishables at the same time. On the other hand fungitoxicants with broad fungitoxic spectrum offer greatest promise to control fungal infestation of perishables. Antifungal activity of aqueous and ethanol extracts of *Tridax procumbens*, *Venonia amygdalina*, *Chromolaena odorata* and *Azadirachta indica* were determined *in vitro* against *A. niger, F. oxysporum, R. stolonifer* and *Geotrichum candidum*. All the four extracts showed good efficiency in controlling the post harvest rot of tomato (Ijato *et al*., 2011) showing the broad range of fungitoxicity. The ethyl acetate extract, methanolic extract and ethanolic extracts showed a broad spectrum of activity inhibiting all the ten fruit rotting fungi viz., *Aspergillus niger*, *Aspergillus fumigatus, Botryodiplodia theobromae, Botrytis cinerea, Fusarium roseum, Monilinia fructicola, Ceratocystis fimbriata, Penicillium expansum, P.digitatum* and *Rhizopus stolonifer.*

In previous studies, the antimicrobial properties of plant extracts from various species have been proven to affect fungal development (Thangavelu *et al*., 2004; Gujar and Talwankar, 2012). Spore formation and germination, mycelial growth *in vitro*, and infection of plants could be inhibited by plant extracts (Kyu Kyu Win *et al*., 2007; Lee *et al*., 2007; Plaza *et al*., 2004). Alam *et al*. (2002) tested the effect of ten plant extract as fungicides on conidial germination of *C. gloeosporioides* and found *Tagetus erecta* (leaf) and *Azadirachta indica* (bark) extracts to be most effective in inhibition of conidial germination after immersing 5~30 min in 5:1.5 (w/v)concentration. Extracts from Sanguisorba minor and Orobanche crenata showed the strong efficacy in vitro in reducing fungal conidial germination and germ tube elongation of Monilinia laxa, Penicillium digitatum, P. italicum, Aspergillus niger, and Botritys cinerea (Gatto *et al*., 2011). In the present investigation it has been found that there was significant different role of plant extracts and immersion period on conidia germination (Table 3). About 99% conidia germination inhibition was observed with ethyl

acetate extract of A. nilotica at 30 min immersion period. Benzene, methanol and ethyl alcohol extracts inhibited conidia germination up to 94,95 and 96 % respectively at the 30 min immersion period. With increasing the immersion period there was increased % of inhibition of conidia germination.

The storage of plant parts and their products for different periods may affect their fungitoxicity. The fungitoxicity may expire after a short period or it may persist for longer duration .This aspect should be recorded for the exploitation of plants and their products in effective forms. The ethyl acetate, methanol and ethyl alcohol extracts retained their fungitoxicity up 24 months. A fungicide must also retain its fungitoxicity at extremes of temperature. The present study indicates that the fungitoxicity of the ethyl acetate extract, methanol and ethanol extract was thermostable. While the toxicity in benzene is thermolabile as it was not active even at 60ºC. A lot of phytochemical investigation with different parts of *A. nilotica* have been done and the presence of tannins, saponins, caumarins, carbohydrates, glycosides, sterols, triterpenes, alkaloids, nitrogenous bases flavonoids and cynogenic compounds are identified (Wassel *et al*., 1992). The strong antifungal activity shown by the extracts of *A. nilotica* may be attributed due to the synergistic action of all these phytochemicals. A study to test the fungitoxicity of the some compounds viz., sitosterol, α-amyrin, narengenin5-methyl ether, kaempferol, kaempferol 3-o rhamnoside, myrcetin 3-o rhamnoside isolated (Tripathi *et al.,* 2002) from the bark of *A. nilotica* was carried out in the present investigation and Kaempferol (Fig.1) was found to exhibit absolute toxicity against the test fungus at 2000µg/ml. Kaempferol-3-o-rhamnoside (Fig.2) also exhibited 95 percent inhibition of growth of test fungus *C. gloeosporioides*.

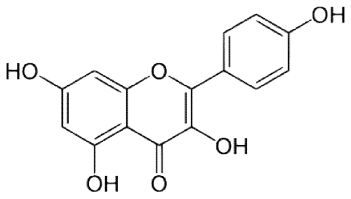

Fig. 1. Kaempferol

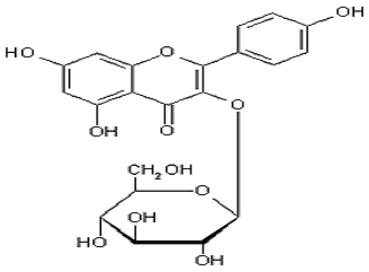

Fig.2 Kaempferol 3-O-rhamnoside

The study also reveals the structure activity relationship amongst the compounds viz., Kaempferol and Kaempferol-3-o-rhamnoside of *A. nilotica*. In Kaempferol-3-o rhamnoside the rhamnose unit is attached at C3 position of Kaempferol (Fig.1 and 2). This rhamnose unit is held responsible for slight reduction in the fungitoxic potency of kaempferol. Soon-okoh *et al.* (2008) isolated gallic acid, 3,4, dihydroxybenzoic acid, quercetin, quercetin-3-O-α L rhamnoside, quercetin 3-O-β –D glucoside from *Eukalyptus darympleana*. Among them only gallic acid was found to be effective in mycelia growth and spore germination inhibition of *B.cinerea*. The results of present investigations suggest that kaempferol can be safer and more acceptable alternative to current synthetic fungicides controlling anthracnose of mango fruits during post harvest storage.

Although plant extracts with antifungal potential in *in vitro* tests are not always effective under *in vivo* conditions, several works showed the effectiveness of plant extracts in controlling plant diseases *in vivo* trial. The application of *Alpinia galanga* root extract and *Carica papaya* leaf extract effectively suppressed the development of *Ceratocystis* fruit rot of *Salacca edulis* (Suprapta *et al*., 2001). Treatment with leaf powders and water extracts of the *Cassia lata* and *Dennetia tripetata* leaf significantly reduced the radial growth of the *Sclerotia rolfsii* causing cocoyam cormet rot in *in vitro* and in *in vivo* conditions (Nwachukwu and Osuji, 2008). Nahed (2007) reported the inhibition of Fusarium *oxysporum*, cause of rot in cucumber using cold aqueous extract of Azadirachta indica. Fokunang *et al*. (2000) used the extract of *Vernonia amygdalina* and *A. indica* against cassava anthracnose disease caused by *C. gloesporides*. Yam rot caused by *Rhizopus stolonifer* was controlled using V. *amygdalina* by (Hycenth, 2008). In the present investigation the aqueous extract of bark of *A.nilotica* was tested for its *in vivo* efficacy in control of anthracnose of mangoes (Table 4). Enhancement of shelf life was observed in different dipping periods. The 5 min, 15min and 30min dipping enhanced shelf life for 3days, 6 days and 9days respectively. By extending the dipping period there was enhancement of shelf life was observed. Sanguisorba minor extract completely inhibited brown rot on apricots and nectarines and Orobanche crenata extract strongly reduced grey mould, brown rot, and green mould on table grapes, apricots and nectarines, and oranges (Gatto *et al*., 2011).

The fungitoxic inhibition of *C. gloeosporioides* by *A.nilotica* organic extracts (*in vitro*) and aqueous extract (*in vivo*) indicates the possibility of their use as a postharvest treatment. However, further investigation should be undertaken to determine the effects of *A. nilotica* fungitoxicity on other fruit rotting fungi which cause post harvest diseases in mangoes for further formulation.

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