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**-Short Communication**

# *IN VITRO* SCREENING OF TWO FLAVONOID COMPOUNDS ISOLATED FROM *CASSIA ALATA* L. LEAVES FOR FUNGICIDAL ACTIVITIES

**M S Rahman[1*], M Y Ali and M U Ali**

*Department of Chemistry, University of Rajshahi, Rajshahi-6205, Bangladesh*
*[1]BCSIR Laboratories, Rajshahi, Bangladesh*

**Key words:** *Cassia alata*, flavonoids, fungicidal activities.

*Cassia alata* L. is rich in flavonoids which is one of the most diverse and wide spread groups of natural products. The flavonoids are a heterogeneous group of ubiquitous plant polyphenols that abound in the human diet and are endowed with several biological activities, including immunomodulating and antioxidant activities (Lelpo *et al*. 2000). The bioactivity of flavonoids is tightly correlated with their chemical structure and action mechanisms, mostly inhibitory on enzymatic systems involved in cellular activation. Many flavonoids, purified from medicinal plants and herbs used in the practice of traditional medicine in many countries are endowed with biological effects. The anti-inflammatory, anti-allergic, antimicrobial action of flavonoids is well documented. Cytotoxic and carcinogenic effects have also been reported (Kandaswami *et al.* 1994, Havsteen 1983).

Because of their multiple bioactivities, the flavonoids are included among the natural 'biological response modifiers' (Middleton 1998). Moreover, the effects of different flavonoids may be antigonistic; in some cases they are immunosuppressive and in others, immunostimulating (Di Carlo *et al.* 1999).

Different classes of flavonoids have been investigated for various physiological activities. No universal function for the flavones in plants has yet been established, in spite of their being the most common and widely distributed flavonoids. However, many functions in individual plants of plant groups have either been demonstrated or proposed (Harborne 1982).

Despite several works are done on the biological activities of different flavonoids isolated from different plants, no reports on the fungicidal activities of any specific flavonoids isolated from *C. alata* have been found. So, the aim of the study was to evaluate the fungicidal effects of two flavonoid compounds isolated from *C. alata.*

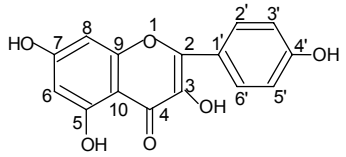
## Materials and Methods

*Extraction of plant materials:* Fresh leaves of *C. alata* were collected from the plants grown in the adjoining area of BCSIR Laboratories, Rajshahi Campus during August-September period. The leaves were washed with water to remove extraneous materials and then dried in shade. The dried materials were crushed to powder. The dried leaf powder (6.5kg) was soaked in 80% ethanol for a week. The ethanolic extract was then filtered and the solvent was removed under reduced pressure to obtain a viscous residue (475g). The crude ethanolic extract was then defatted with n-hexane. The n-hexane solvent was then removed under reduced pressure to yield the residue (150g).

* Corresponding author

*Compound isolation and characterization:* The defatted extract was then treated with water, shaken well to resolve into water soluble and water insoluble parts. The water soluble part was extracted with ethyl acetate. The ethyl acetate soluble part was chromatographed over a silica gel (70-230mesh) column and successively eluted with increasing polarities of n-hexane and ethyl acetate. Elution of the column with n-hexane: ethyl acetate (40:60) afforded two compounds ($R_f$0.49 and 0.60) along with minor impurities. The compounds were then applied to a PTLC card of silica gel $60G_{254}$ (thickness 0.1mm) and eluted with n-hexane: ethyl acetate (8:1). Two distinct light yellow bands ($R_f$ 0.49 and 0.60) were observed on the PTLC card. The bands were collected separately and washed out with ethyl acetate to obtain a yellow solid (compound-1, 20.5mg, m.p 276-278$^o$C, $R_f$ 0.49) and another light yellow solid (compound-2, 15.4mg, m.p 272-274$^o$C, $R_f$ 0.60).

On the basis of the spectral evidences, compound-1 and 2 were characterized as 3,5,7,4´- tetrahydroxy flavone (Rahman *et al* 2006) and 2,5,7,4´- tetrahydroxy isoflavone.




Structure of the compound-1

Spectral data of the compound-2

UV$\lambda_{max}$ ( MeOH ) nm: 287, 243, 228, 204, 198, 193.

IR$\nu_{max}$ (KBr) $cm^{-1}$: 3490 ( O-H ), 2980 ( C-H ), 1650 ( C=O ), 1600 ( C=C ).

EIMS m/z ( rel.int% ): 286(100), 257(3), 244(1), 229(1), 194(4), 165(2), 153(33), 134(25), 106(11), 93(7), 69(20).

Peak matching m/z (formula): 286.06261 ($C_{15}H_{10}O_6$)

$^1$H-NMR(500 $MH_z$) $\delta_{TMS}$ ($CD_3OD$):

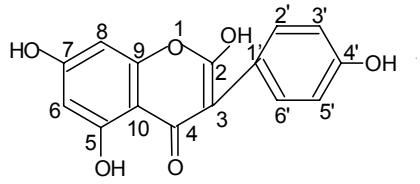
δ 6.22 [ H-6, IH, d, $J_{(H-6, H-8)}$ 2.1 $H_z$]

δ 6.95 [ H-8, IH, d, $J_{(H-8, H-6)}$ 2.1 $H_z$]

δ 7.13 [H-2´, H-6´, 2H, dd, $J_{(H-2',H-3')}$ 8.95 $H_z$ , $J_{(H-2',H-6')}$ 2.1 $H_z$]

δ 6.86 [H-3´, H-5´, 2H, dd, $J_{(H-5',H-6')}$ 8.95 $H_z$ , $J_{(H-3',H-5')}$ 2.1 $H_z$]

$^{13}$C-NMR ($CD_3OD$, 100 $MH_z$): 143.3, 129.7, 183.2, 161.5, 99.5, 164.1, 94.1, 156.2, 1.0.0, 122.4, 122.0, 116.7, 168.1, 116.7, 122.0.




Structure of the compound-2

*Test organisms:* Human pathogens were *Epidermophyton floccosum, Trichophyton schoenleinii, Trichophyton longifurus, Pseudallescheria boydii, Candida albicans, Aspergillus niger.* Animal pathogens were *Microsporum canis, Trichophyton mentagrophytes.* Plant pathogens were *Fusarium oxysporum* var. *lycopersici, Fusarium solani var. lycopersici, Macrophomina phaseolina, Rizoctonia solani.* Standard drugs were Miconazole for Human and Animal pathogens; Amphoterein B for plant pathogens.

*Preparation of the test plates and discs:* The test organisms were transferred from the subcultures to the Petri-dishes with the help of an inoculating loop in an aseptic area. The transferred organisms were then seeded in petri-dishes with the spreader agitated clock wise, anti-clock wise,, right to left and left to right to assure homogeneous distribution of the test organisms. Three types of discs were used for the fungicidal test. Sample disc with 20 µl of sample solution containing 100µg of the pure compound was applied on the disc by a micropipette and air dried. Standard disc with 10µl each of standard miconazole (for human and animal) and amphoterein B (for plant) containing 10µg drugs of each was applied separately on the disc with the help of a micropipette. Control disc was made on which only the solvent was applied. The impregnated sample discs and standard fungicidal disc were placed gently on the solidified agar plates, freshly seeded with the test organisms by the help of a sterile forceps to assure complete contact with medium surface. The arrangements of the discs were such that discs were no closer than 15 mm to the edge of the plate and enough apart to prevent over- lapping the zones of inhibition. The plates were then turned upside down and kept in a refrigerator for 12 hours at 4°C temperature. Sufficient time was allowed to diffuse the material to a considerable area of the medium. Finally the plates were incubated at 27°C for 7 days.

Measurement and determination of the inhibition zone: After incubation, the plates were observed for antifungal activity. The compounds of the discs diffused through the medium and created a concentration gradient. Thus the compounds of the discs resulted a clear round zone of inhibition for particular organisms and were measured by naked eyes using a transparent scale.

**Results and Discussion**

Compound 2,5,7,4´-tetrahydroxy isoflavone (100 µg/disc) showed inhibition against most of the fungi used (Table 1) but showed no activities against *Epidermophyton floccosum.* The compound was found active against *Trichophyton longifurus* and *Pseudallescheria boydii*. Compound 3,5,7,4´- tetrahydroxy flavone (100 µg/disc) also showed inhibition against most of the fungi excepting the *Epidermophyton floccosum* against which no activity was observed. Moderate activities were found for the compound 3,5,7,4´- tetrahydroxy flavone against *Trichophyton longifurus* and *pseudallescheria boydii*. The standard Miconazole (fungicide) also showed comparable activities with those of the compounds. The fungicidal activities of the compounds against three animal pathogens were depicted in Table-1.

**Table 1.** Fungicidal activities of two compounds of *Cassia alata* against six human and three plant pathogens.

| Fungi | Name of the compounds | | | | | | Name of standard drug | | |
|---|---|---|---|---|---|---|---|---|---|
| | 2,5,7,4´- tetrahydroxy isoflavone | | | 3,5,7,4´- tetrahydroxy flavone | | | Miconazole/ Amphoterein B | | |
| | 100 µg/disc | | | 100 µg/disc | | | 10 µg/disc | | |
| | ZI (mm) | | (mm) Inhibition% | ZI (mm) | | (mm) Inhibition% | ZI (mm) | | (mm) Inhibition% |
| | Sample | Control | | Sample | Control | | Sample | Control | |
| **Human pathogen** | | | | | | | | | |
| *Epidermophyton floccosum* | - | - | - | - | - | - | - | - | - |
| *Trichophyton schoenleinii* | 45 | 70 | 35.7 | 42 | 64 | 34.3 | 36 | 68 | 47.0 |
| *Trichophyton longifurus* | 23 | 50 | 54.0 | 30 | 55 | 45.4 | 22 | 57 | 61.4 |
| *Pseudallescheria boydii* | 22 | 44 | 50.0 | 27 | 48 | 43.7 | 24 | 50 | 52.0 |
| *Candida albicans* | 51 | 65 | 21.5 | 56 | 67 | 16.4 | 50 | 70 | 28.5 |
| *Aspergillus niger* | 54 | 80 | 32.5 | 60 | 75 | 20.0 | 48 | 75 | 36.0 |
| **Animal pathogen** | | | | | | | | | |
| *Microsporum canis* | 22 | 48 | 54.1 | 28 | 54 | 48.1 | 32 | 67 | 52.2 |
| *Trichophyton mentagrophytes* | 20 | 38 | 47.3 | 29 | 48 | 39.5 | 32 | 62 | 48.3 |
| **Plant pathogen** | | | | | | | | | |
| *Fusarium oxysoporum* | - | - | - | - | - | - | - | - | - |
| *Fusarium solani* | 35 | 65 | 46.1 | 30 | 50 | 40 | - | - | - |
| *Macrophomina phaseolina* | - | - | - | - | - | - | - | - | - |
| *Rhizoctonia solani* | - | - | - | - | - | - | - | - | - |

- = No activity

Compound 2,5,7,4´- tetrahydroxy isofalvone was found active against *Microsporum canis* and moderately active against *Trichophyton mentagrophytes*. But compound 3,5,7,4´- tetrahydroxy flavone showed moderate activity against the same pathogens. Here the standard drug Miconazole showed also the comparable activity with those of the compounds used for the test. Both the compounds 2,5,7,4´- tetrahydroxy isoflavone and 3,5,7,4´- tetrahydroxy flavone showed moderate activity against plant pathogens *Fusarium solani var. lycopersici* but showed no activities against other three pathogens. The standard amphoterein B was completely resistant to four of the plant pathogens.

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