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# Antifungal activity of flavonoids isolated from mango (*Mangifera indica* L.) leaves

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Five flavonoids, namely (–)-epicatechin-3-*O*-*β*-glucopyranoside (**1**), 5-hydroxy-3-(4-hydroxylphenyl)pyrano[3,2-g]chromene-4(8H)-one (**2**), 6-(*p*-hydroxybenzyl)taxifolin-7-*O*-*β*-D-glucoside (tricuspid) (**3**), quercetin-3-*O*-*α*-glucopyranosyl-(1 → 2)-*β*-D-glucopyranoside (**4**) and (–)-epicatechin(2-(3,4-dihydroxyphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol (**5**), were isolated from the leaves of mango (*Mangifera indica* L.). Antifungal activity of these compounds was evaluated against five fungal species, namely *Alternaria alternata* (Fr.) Keissler, *Aspergillus fumigatus* Fresenius, *Aspergillus niger* van Tieghem, *Macrophomina phaseolina* (Tassi) Goid. and *Penicillium citrii*. Six concentrations, namely 100, 300, 500, 700, 900 and 1000 ppm of each of the five flavonoids were employed by means of the poisoned medium technique. All concentrations of the five test flavonoids significantly suppressed fungal growth. However, the specificity of different test compounds was evident against different fungal species. In general, antifungal activity of the flavonoids was gradually increased by increasing their concentrations. The highest concentration (of 1000 ppm) of compounds **1**–**5** reduced the growth of different target fungal species by 63–97%, 56–96%, 76–99%, 76–98% and 82–96%, respectively.

**Keywords:** antifungal; flavonoids; *Mangifera indica*; mango

## 1. Introduction

Flavonoids are secondary metabolites of plants and are generally located in the vacuoles of the epidermal cells of leaves as water soluble glycosides (Harborne & Williams, 2000). They occur widely in the plant kingdom and represent a common constituent of the human diet (Havsteen, 1983; Middleton & Kandaswami, 1994). The daily dietary intake of mixed flavonoids is estimated to be in the range of 500–1000 mg (Skibola & Smith, 2000). The basic structural feature of flavonoids is the 2-phenyl-benzopyrane or flavan nucleus, which consists of two benzene rings linked through a heterocyclic pyran ring (Brown, 1980). These compounds have broad biological and pharmacological activities (Cushnie & Lamb, 2005), including

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antifungal activities (Galeotti, Barile, Curir, Dolci, & Lanzotti, 2008; Sathiamoorthy et al., 2007). They are not only present in plants as constitutive agents but are also formed in plant tissues in response to microbial attack (Harborne, 1999).

Fungi are an important group of heterotrophic organisms that cause many diseases in plants such as root rot, blight, wilt, etc. (Das, Fakrudin, & Arora, 2007; Porta-Pugilia, Infantino, Crino, & Venora, 1997; Zhang et al., 2008). Fungal diseases of plants are generally controlled by synthetic fungicides. However, this approach is not safe, as chemicals used in crop protection pollute the environment and cause health hazards (Cuthbertson & Murchie, 2003). Increasing public concern on environmental issues requires alternative disease management systems which are less pesticide-dependent or are based on naturally-occurring compounds (Cuthbertson & Murchie, 2005). The plant world comprises a rich storehouse of biochemicals to be used as pesticides. Botanical derivatives are more environmentally safe than synthetic chemicals (Hashim & Devi, 2003). Natural products have been the source of many fungicides, either used directly as crude preparations or as pure compounds. Instead of being used directly, more often they have been used as the structural lead for the discovery and development of natural product-based fungicides (Duke, 2002). Several antifungal compounds present in certain plant species have been used for controlling fungal pathogens (Bajwa et al., 2008; Riaz, Khan, & Javaid, 2007; Serrano, Martínez-Romero, Castillo, Guillén, & Valero, 2005; Tripathi & Dubey, 2004). This study was undertaken to evaluate the antifungal potential of flavonoids isolated from mango leaves against some pathogenic fungal species.



## 2. Results and discussion

### 2.1. *Identification of the isolated compounds*

The identification of the five isolated flavonoids was undertaken by comparing the spectroscopic data of these compounds with those published earlier. Compound **1** was identified as (−)-epicatechin-3-*O*-*β*-glucopyranoside. Earlier, this compound was isolated from *Davalia mariesii* (Cui et al., 1992). Compound **2** was recognised as 5-hydroxy-3-(4-hydroxylphenyl)pyrano[3,2-g]chromene-4(8H)-one and was previously reported to be isolated from *Erythrina lysistemon* (Juma & Majinda, 2005). Compound **3** was found to be 6-*p*-hydroxybenzyltaxifolin-7-*O*-*β*-D-glucoside (tricuspid). This compound was originally reported in *Cudrania tricuspidata* (Zheng, Liang, & Hu, 2006). Compound **4** was documented as quercetin-3-*O*-*α*-glucopyranosyl-(1 → 2)-*β*-D-glucopyranoside with reference to Gohar (2002), who isolated it from *Cadaba glandulosa*. Compound **5** was found to be (−)-epicatechin(2-(3,4-dihydroxyphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol and was previously reported from *Adansonia digitata* (Shahat, 2006). The structures of the isolated compounds are given in Figure 1.

### 2.2. *Antifungal bioassay*

Analysis of variance revealed the highly significant ($p \leq 0.001$) effect of flavonoids, fungal species and concentrations as well as the interactive effects of these variables for fungal growth (Table 1). In general, all the concentrations of test flavonoids significantly suppressed the growth of all the five target fungal pathogens. Generally,

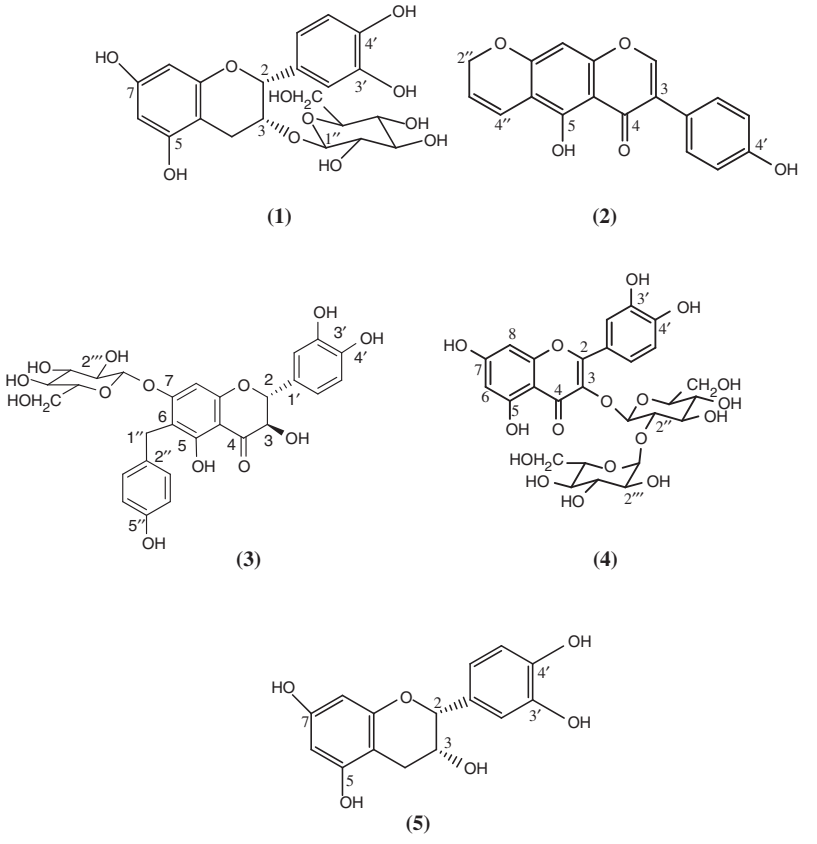


Figure 1. Structures of antifungal flavonoids isolated from mango leaves.

Table 1. Analysis of variance for the effect of different concentrations of the five flavonoids isolated from mango leaves against five fungal species.

| Sources of variation | df | SS | MS | *F*-value |
|---|---|---|---|---|
| Treatments | 174 | 337,781 | 1941 | 207* |
| Flavonoids (F) | 4 | 9839 | 2460 | 262* |
| Fungal species (S) | 4 | 15,390 | 3847 | 410* |
| Concentration (C) | 6 | 282,918 | 47,153 | 5023* |
| F × S | 16 | 17,566 | 1098 | 117* |
| F × C | 24 | 2405 | 100 | 10.7* |
| S × C | 24 | 3582 | 149 | 15.9* |
| F × S × C | 96 | 6081 | 63 | 6.7* |
| Error | 350 | 3285 | 9.4 | |
| Total | 525 | 874,779 | | |

Notes: df, degree of freedom; SS, sum of squares; MS, mean square. *Significant at $p \leq 0.001$.

Table 2. Effect of different concentrations of mango leaf flavonoids (compounds **1**–**5**) on the colony diameter of different fungal species.

| Compounds | Concentration (ppm) | Diameter of fungal colony (mm) | | | | |
|---|---|---|---|---|---|---|
| | | *A. alternata* | *A. fumigatus* | *A. niger* | *M. phaseolina* | *P. citrii* |
| **1** | 0 | 88 a | 89 a | 87 a | 90 a | 88 a |
| | 100 | 48 e | 35 c | 78 b | 44 de | 32 b |
| | 300 | 32 h | 31 cd | 67 c | 33 f–h | 23 de |
| | 500 | 25 j–l | 27 d–f | 57 d | 29 g–i | 13 f–h |
| | 700 | 18 m–o | 22 e–h | 50 de | 25 h–l | 9 h–j |
| | 900 | 11 pq | 18 g–i | 43 e–g | 18 k–o | 6 jk |
| | 1000 | 9 qr | 14 i–k | 32 g–i | 13 m–o | 3 kl |
| **2** | 100 | 78 b | 41 b | 47 d–f | 71 b | 24 cd |
| | 300 | 68 c | 35 c | 43 e–g | 26 h–l | 16 f |
| | 500 | 57 d | 26 d–f | 36 f–i | 44 de | 13 fg |
| | 700 | 50 e | 21 f–i | 28 h–l | 34 f–h | 8 ij |
| | 900 | 43 f | 10 k–m | 24 l–m | 25 h–l | 6 jk |
| | 1000 | 39 g | 4 m–o | 20 j–n | 22 i–m | 5 j–l |
| **3** | 100 | 25 j–l | 36 c | 54 de | 46 d | 28 c |
| | 300 | 14 m–o | 29 de | 35 g–i | 38 ef | 26 c |
| | 500 | 5 rs | 25 d–f | 31 g–k | 28 g–j | 16 f |
| | 700 | 4 rs | 9 k–n | 26 h–m | 21 i–n | 12 f–h |
| | 900 | 3 s | 6 m–o | 20 j–n | 14 m–o | 5 j–l |
| | 1000 | 2 s | 1 o | 16 l–n | 11 o | 21 |
| **4** | 100 | 34 gh | 27 d–f | 38 f–h | 38 ef | 27 c |
| | 300 | 27 ij | 23 e–g | 32 g–j | 31 f–h | 22 de |
| | 500 | 20 l–m | 13 j–l | 25 h–m | 25 h–l | 17 f |
| | 700 | 16 n–p | 9 k–n | 19 j–n | 21 l–n | 11 g–i |
| | 900 | 9 q–r | 6 l–o | 13 mn | 18 j–o | 5 j–l |
| | 1000 | 4 rs | 2 no | 9 n | 15 m–o | 21 |
| **5** | 100 | 34 gh | 35 c | 32 g–j | 56 c | 33 b |
| | 300 | 27 ij | 28 d–f | 32 g–j | 35 fg | 27 c |
| | 500 | 20 lm | 21 f–i | 28 h–l | 31 f–h | 23 de |
| | 700 | 16 n–p | 16 h–k | 24 h–m | 27 g–k | 13 f–h |
| | 900 | 9 qr | 9 k–n | 18 k–n | 21 i–n | 9 h–j |
| | 1000 | 4 rs | 4 m–o | 9 n | 16 l–o | 6 jk |

Note: In each column, values with different letters are significantly different ($p \leq 0.05$) according to the Student–Newman–Keuls test.



the antifungal activity of the compounds was gradually increased by increasing their concentration. However, variation in antifungal activity of the different flavonoids against various fungal species was evident (Table 2). Similar specificity has also been reported for the compounds of other plant species against fungal growth (Bajwa et al., 2008; Galeotti et al., 2008). Toxicity is assumed to be associated with the presence of strong electrophilic or nucleophilic systems. Action by such systems on specific positions of proteins or enzymes would alter their configuration and affect their activity (Macías, Galindoadoand, & Massanet, 1992).

Compound **1** was most effective against *P. citrii*, where its different concentrations caused a 64–97% reduction in fungal growth. This compound also suppressed the growth of *A. alternata*, *A. fumigatus, A. niger* and *M. phaseolina* by 45–87, 61–84,

10–63 and 51–86%, respectively (Table 2). Compound **2** was highly effective against *A. fumigatus* and *P. citrii*, where a 54–96% and 73–94% decline in fungal colony growth, respectively, was recorded due to various employed concentrations of this compound. *Aspergillus niger* and *M. phaseolina* were found to be comparatively less sensitive to compound **2**, where the highest concentration of 1000 ppm resulted up to a 77% and 76% suppression in fungal colony growth, respectively. This compound was found to be least effective against *A. alternata*, where only a 56% reduction in fungal colony growth was recorded following application of the highest concentration of 1000 ppm (Table 2). Compounds **3**–**5** were found to be highly toxic to *A. alternata* and *A. fumigatus*. The highest concentration of 1000 ppm of these compounds resulted in a 95–99% suppression in the colony growth of these two fungal species. These compounds also exhibited considerable antifungal potential against the three other tested fungal species (Table 2). Similar antifungal activity of the flavonoids isolated from carnation (*Dianthus caryophyllus*) and *Vitex negundo* have also been reported against other fungal species such as *Verticillium alba-atrum*, *Trichophyton mentagrophytes* and *Cryptococcus neoformans* (Galeotti et al., 2008; Sathiamoorthy et al., 2007).

This study concludes that all five tested flavonoids from mango leaves possess antifungal activities against pathogenic fungi. All the compounds except **1** were highly toxic to *A. fumigatus*, which is responsible for pulmonary aspergillosis, aspergilloma, and numerous forms of hypersensitivity diseases (Latgé, 1999). Since flavonoids are available at low cost and are less toxic to humans (Galeotti et al., 2008), they are highly suitable for treating such human diseases. Although all the concentrations of the test flavonoids significantly reduced the growth of *M. phaseolina*, this fungus was, however, comparatively less susceptible to these compounds when compared to the rest of the test fungal species. This soil-borne phytopathogenic fungal species attacks about 400 plant species, including crops such as soyabean, sunflower, maize and sorghum (Das et al., 2007), but there is no registered fungicide to control this pathogen. The antifungal activities of these natural compounds may be further enhanced against *M. phaseolina* by some structural modifications, to use these compounds as commercial fungicides for the management of this noxious pathogen. The mechanism of action of flavonoids against fungi is still unknown.

## 3. Experimental

### 3.1. *General procedure*

All the reagents and the solvents used in this study were procured from E. Merck Germany, Fluka Switzerland, BDH Chemicals, England and Sigma–Aldrich Chemicals, USA. The solvents used were of analytical grade. For column chromatography, silica gel 60 (Merck 230–400 mesh) was used and TLC was performed on silica gel (Merck, Keiselgel 60F256). Melting points were determined by the sealed capillary method using Gallenkamp melting point apparatus. The melting points were uncorrected. The optical rotation was measured by polarimeter (model wxg-4 disc polarimeter).

The IR spectra of the compounds in KBr discs were recorded on a Shimazdu 4200 Fourier transform apparatus. $^{1}$H- and $^{13}$C-NMR spectra were recorded on

Bruker 14.1 TNMR spectrometer, operating at a frequency of 600 MHz DEPT. Experiments were performed using polarisation transfer pulses of 90° and 135°. The FABMS (glycerol matrix) and EI-MS (at an ionisation voltage of 70 ev) spectra were measured with a Jeol JMS-AX 505 HAD mass spectrometer.

### 3.2. *Isolation*

Five hundred grams of fresh leaves of mango (equivalent to 220 g dry weight) were collected from Quaid-e-Azam Campus, University of the Punjab, Lahore, Pakistan, in May 2007. The specimen was deposited in the Herbarium of the University of the Punjab, Lahore, Pakistan (LHR) with voucher no. AJ 102. The leaves were washed with distilled water and dried in the shade. The leaves were soaked in 1 L methanol for 15 min to remove the chlorophyll. The leaves were then blended with 1.5 L methanol, left overnight, filtered with Whatman No. 1 filter paper under vacuum, centrifuged at 2000 rpm for 5 min and the supernatant was concentrated under vacuum at 50°C to 100 mL. The concentrated solution was diluted with water (1 : 1), to enable precipitation to occur. The precipitates were filtered, washed with ether and dried in a vacuum dessicator to yield compound **1** (215 mg). The filtrate was then concentrated to reduce the volume to 100 mL, extracted with 100 mL of acetone and filtered, and the residue was removed. The residue was purified with the help of preparative TLC (MeOH : $CHCl_3$, 1 : 99) and recrystallised in $CHCl_3$ : MeOH (4:1) to yield compound **2** (323 mg). The remaining filtrate was successively extracted with 150 mL each of $CHCl_3$ and *n*-butanol. The $CHCl_3$ extract was subjected to silica gel column chromatography using a solvent system of ethyl acetate : MeOH : $H_2O$ (4 : 1 : 1). From this column, compound **3** (1.75 g) was isolated and subsequently purified by preparative TLC using a solvent system of EtOAc : MeOH (1 : 4). The butanolic extract was fractionated by silica gel column (90 × 4 cm) chromatography using an isocratic solvent system of MeOH : $CHCl_3$ : $H_2O$ (3 : 1 : 1) to yield compounds **4** (720 mg) and **5** (1.1 g) (Kanwal, Hussain, Siddiqui, & Javaid, 2009).

Each flavonoid glycoside (3 mg) was refluxed with 2 N HCl (3 mL) for 1 h. A glycon part was extracted with EtOAC and identified with the help of IR, UV and NMR spectral analyses. The sugar part was isolated from aqueous layer and identified by co-TLC with authentic samples, using the solvent system *n*-BuOH : EtOAc : i-PrOH : HOAc : H2O (7 : 20 : 12 : 7 : 6). The spots were visualised with aniline phthalate reagent (Gohar, 2002; Imperato & Nazzaro, 1996).

### 3.3. *Antifungal bioassays*

Five fungal strains, namely *Alternaria alternata*, *Aspergillus fumigatus*, *Aspergillus niger*, *Macrophomina phaseolina* and *Penicillium citrii* were obtained from the Fungal Culture Bank of the Institute of Mycology and Plant Pathology, University of the Punjab, Lahore, Pakistan. The antifungal activity of flavonoids **1**–**5** was evaluated by means of the poisoned medium technique. Two percent malt extract agar medium was prepared by autoclaving at 121°C for 30 min. Weighed quantities of compounds **1**–**5** were dissolved in 500 µL of sterilised distilled water, and added to flasks containing 59.5 mL of malt extract agar medium, when still molten, to obtain 100, 300, 500, 700, 900 and 1000 ppm final concentrations. The control received the same

quantity of distilled water. Twenty millilitres of each medium were poured in each 90 mm diameter sterilised Petri plate and the medium was allowed to solidify. Mycelial discs of 5 mm diameter were prepared from the tips of 5–7 day-old culture of the five test fungal species with the help of a sterilised cork borer and placed in the centre of each Petri plate. Each treatment was replicated three times. Plates were incubated in an incubator at $25 \pm 2^{\circ}C$ for seven days. Fungal growth was measured by averaging the three diameters, taken at right angles, for each colony.



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