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Antimicrobial screening of some Egyptian plants and active flavones from *Lagerstroemia indica* leaves

Yasser Diab[1,*], Khaled Atalla[2], Khaled Elbanna[2]

[1] *Department of Biochemistry, Faculty of Agriculture, Fayoum University, Fayoum, Egypt;*
[2] *Department of Agricultural Microbiology, Faculty of Agriculture, Fayoum University, Fayoum, Egypt.*

ABSTRACT: One hundred and twenty four plant extracts were evaluated for their antimicrobial activity against four pathogenic bacteria (*Staphylococcus aureus* (ATCC 8095), *Salmonella enteritides* (ATCC 13076), *Escherichia coli* (ATCC 25922), and *Listeria monocytogenes* (ATCC 15313)) and *Candida albicans* yeast (ATCC 10231) using the disk diffusion and broth microdilution methods. Of the plant extracts, fourteen exhibited antimicrobial activity against two or more of the five microorganisms tested. Only the methanol extract of *Lagerstroemia indica* leaves exhibited antimicrobial activity against all pathogenic bacteria and *C. albicans* yeast that were tested. Purification of the methanol extract of *L. indica* leaves using antimicrobial assay-guided isolation yielded one pure active compound. The chemical structure of the isolated active compound was found to be '4-methoxy apigenin-8-*C*-*β*-D-glucopyranoside; cytisoside according to detailed spectroscopic analysis of its nuclear magnetic resonance and mass spectrometry data. The compound exhibited antimicrobial activity against *C. albicans* (minimum lethal concentration (MLC): 32 µg/mL), *S. aureus* (MLC: 16 µg/mL), *S. enteritides* (MLC: 16 µg/mL), *E. coli* (MLC: 16 µg/mL), and *L. monocytogenes* (MLC: 16 µg/mL). The present study found that the methanol extract of *L. indica* leaves holds great promise as a potential source of beneficial antimicrobial components for different applications.

Keywords: Pathogenic bacteria, *Candida albicans*, *Lagerstroemia indica*, flavones, antimicrobials

**Address correspondence to:*
Dr. Yasser Mohamed Diab, Biochemistry Department, Faculty of Agriculture, Fayoum University, Fayoum, 63514, Egypt.
E-mail: ydiab@hotmail.com; ymd00@fayoum.edu.eg

1. Introduction

Secondary metabolites produced by plants constitute a major source of bioactive substances. Over the past two decades, the scientific interest in these plant metabolites has increased as part of the search for new therapeutic agents, partly due to the increasing resistance of microorganisms to most antimicrobial drugs currently used in medicine and agriculture (*1*). In addition, the need for safer agrochemicals with less environmental and mammalian toxicity is a major concern. In an attempt to discover new chemical classes of antimicrobial drugs that could resolve these problems, a wide range of plant extracts has been examined for antimicrobial properties (*2,3*).

Food products are susceptible to contamination and spoilage by bacteria. More than 90% of the cases of food poisoning or food-related infection each year are caused by Gram-positive bacteria such as *Staphylococcus aureus* and *Listeria monocytogenes* or Gram-negative bacteria such as *Escherichia coli* and *Salmonella enteritidis* (*4-7*). In recent years, the effects of plant extracts and phytochemicals on food pathogenic bacteria have been studied (*8-11*). Candidiasis is an infection caused by *Candida* yeast, especially *Candida albicans*, and is associated with skin infection and other health problems. Treatment of candidiasis can be problematic due to the limited number of effective antifungal drugs, toxicity of the available antifungal drugs, resistance of *Candida* to commonly-used antifungal drugs, relapse of *Candida* infections, and the high cost of antifungal drugs (*12*). The investigation of natural products for activity against *Candida* species has therefore increased in the last 10 years, with approximately 258 plant species from 94 families having been investigated (*13*).

The present study evaluated the chloroform and methanol extracts of 124 plant species grown in Egypt (belonging to 56 plant families) for their antimicrobial activity against *C. albicans* yeast and four food pathogenic bacteria (*S. aureus*, *S. enteritidis*, *E. coli*, and *L. monocytogenes*). As the methanol extract of the leaves of *Lagerstroemia indica* exhibited significant antimicrobial activity against all five microorganisms, bioactivity-guided separation of the methanol extract of *L. indica* leaves was used to isolate and identify the active compound.

2. Materials and Methods

2.1. *Chemicals and plant materials*

All chemicals and reagents used in this study were of analytical grade and were purchased from Sigma Chemical Co. (St. Louis, MO, USA), BDH (Dorset, England) or Fluka Chemie Co. (Buchs, Switzerland).

Leaf samples from a total of 124 plant species belonging to 56 families were collected during April and May 2008 from El-Shrouk farm on the Cairo-Alexandria desert road, 72 km north of Cairo. The botanical identification of the collected specimens was confirmed by Dr. T. Labeb, Herbarium of Orman Garden, Horticulture Research Institute, Giza, Egypt. A voucher specimen of each plant was deposited in the herbarium of the Biochemistry Department, Faculty of Agriculture, Fayoum University. The leaves were cleaned, air-dried in the shade, and then powdered to pass through 24 mesh using a laboratory mill. Powdered material was stored in an air-tight container at room temperature (28 ± 2°C) and protected from light until use.

2.2. *Detection and identification methods*

Analytical thin-layer chromatography (TLC) was carried out on Merck precoated silica gel plates (F245, 0.25 mm thickness). Analytes were visualized under ultraviolet (UV) light (254 and 365 nm) by spraying 30% H_2SO_4 in methanol followed by heating at 105°C for 5 min, by spraying $AlCl_3$ reagent to detect flavonoids, or by spraying naphthoresorcinol/phosphoric acid followed by heating at 105°C for 10 min to detect sugars. The purified compounds were preliminary characterized phytochemically for the presence of saponins, flavonoids, alkaloids, tannins, and glycosides using TLC according to methods described previously (*16*).

^{1}H, ^{13}C nuclear magnetic resonance (NMR) and heteronuclear multiple bond correlation (HMBC) spectra were recorded in deutero-methanol (CD_3OD) on a Bruker DRx 400 MHz spectrometer (Varian Inc., Palo Alto, CA, USA) at 400 MHz for ^{1}H and 100 MHz for ^{13}C. UV spectra were recorded on a Cecil 3000 series spectrophotometer (Cecil Instruments Ltd., Cambridge, UK). Mass spectra were recorded on a GC/MS Qp 100 Ex Shimadzu Mass spectrometer (Shimadzu, Kyoto, Japan) at 70 eV.

2.3. *Preparation of plant extracts*

A known amount of air-dried powdered leaves from each plant was extracted with chloroform at room temperature (28 ± 2°C). This procedure was repeated at least five times and until the organic solvent remained colorless. The same chloroform-extracted samples were then extracted with methanol at least five times or until the solvent remained colorless. The extracts obtained were filtered using Whatman No. 1 filter paper and the combined extract (filtrate) was evaporated to dryness in a rotary evaporator at 45°C. The antimicrobial activity of the dry residue was then assayed.

2.4. *Isolation of bioactive compound(s) from L. indica leaves*

The bioactive methanol extract of *L. indica* leaves was subjected to chromatography to isolate antimicrobial component(s) as follows. Thirteen grams of extract residue in methanol were loaded onto a chromatographic column (5 cm × 100 cm) packed with silica gel (230-400 mesh, 700 g, Merck & Co. Inc., Whitehouse Station, NJ, USA) and eluted with a gradient of chloroform:methanol (85:15, 70:30, 50:50, and 30:70, v/v; 2 L for each eluent). Twenty fractions (100 mL) of each eluent were collected and assayed with TLC. Based on their similarities in TLC, the collected fractions were combined into 11 fractions that were further tested for antimicrobial activity. Two fractions (**8** and **9**) exhibited strong antimicrobial activity. These fractions (**8**, 1.5 g and **9**, 2.9 g) eluted between 400-900 mL and 1,000-2,000 mL of chloroform:methanol (50:50), respectively. Fractions **8** and **9** were subjected to solid phase extraction using a C_{18} cartridge (Waters, Milford, MA, USA) and subsequently chromatographed on a Sephadex LH20 (GE Healthcare-Bio-Sciences, Piscataway, NJ, USA) column (2.7 cm inner diameter × 60 cm, 50 g resin) using methanol as the mobile phase. The eluates were combined into eight fractions (A-H) on the basis of similar TLC profiles. Fraction E (960 mg) contained the most antimicrobial activity and was further purified on a silica gel column (1.5 cm inner diameter × 60 cm; 50 g) with chloroform:methanol:water (60:40:5, v/v) followed by purification on a Sephadex LH20 column (1.6 cm inner diameter × 40 cm; 20 g) with methanol as the eluent to yield 543 mg of pure active compound.

2.5. *Test organisms and the agar disk diffusion method*

The four food pathogenic bacteria (*S. aureus* (ATCC 8095), *S. enteritidis* (ATCC 13076), *E. coli* (ATCC 25922), *L. monocytogenes* (ATCC 15313)) and *C. albicans* (ATCC 10231) were obtained from the Department of Agricultural Microbiology, Faculty of Agriculture, Fayoum University, El-Fayoum, Egypt. Stock cultures of bacteria were maintained on nutrient agar slants at 4°C, and Candida yeast was maintained on potato dextrose agar slants at 4°C. Bacterial and yeast cultures were sub-cultured in Petri dishes prior to testing.

The disk diffusion method (*14*) was used to assay the antimicrobial activity of plant extracts. Three sterilized filter paper discs (6 mm) were soaked with each plant extract (1 g/10 mL) and dried at 40°C. The dried disks were transferred to the surface of the inoculated plates in triplicate. Plates with pathogenic bacteria were then incubated at 37°C for 24-48 h and plates with *Candida* yeast were incubated at 30°C for 48-72 h. Afterwards,

bioactivity was determined by measuring the diameter of inhibition zones (DIZ) around each disk in mm. Solvent without test compounds was used as a control.

2.6. *Determination of minimum lethal concentrations (MLC)*

The MLC of leaf extracts with potential antimicrobial activity and their isolated active constituent(s) were determined using the broth micro-dilution method (*15*). The lowest concentration of a tested extract or compound resulted in a viable count of less than 0.1% of the original inoculum (1×108 colony-forming units per milliliter, cfu/mL, as compared to the turbidity of the McFarland 0.5 standard). Ampicillin and fluconazole served as standards for comparison in antibacterial and antifungal tests, respectively.

3. Results

3.1. *Antimicrobial activity of plant leaf extracts*

The chloroform and methanol extracts of a number of Egyptian plants (124 plant species) belonging to 56 plant families were evaluated for their antimicrobial activity against five pathogenic organisms (*C. albicans* yeast and four food pathogenic bacteria: *S. aureus*, *S. enteritidis*, *E. coli*, and *L. monocytogenes*) using a disk diffusion assay. The methanolic extracts of fourteen plant species exhibited significant antimicrobial activity against two or more of the five pathogenic microorganisms that were tested. The diameter of inhibition zones (DIZ) and the MLC of the promising plant extracts are shown in Table 1. The data indicated that the methanol extracts of these plants exhibited varying levels of antimicrobial activity against the test microorganisms.

The results in Table 1 show that among the fourteen promising plant extracts tested, extracts of *Pelargonium odoratissimum*, *Pelargonium zonale*, and *Rosa spp* were active against *E. coli*, *S. aureus*, *S. enteritidis*, and *L. monocytogenes*. Seven extracts were found to be only active against *E. coli*, *S. enteritidis*, and *L. monocytogenes*. This variation in activity may be due to the intrinsic tolerance of microorganisms and the nature and combination of phytocompounds present in these extracts (*17*). Only a leaf extract of *L. indica* was active against all five of the pathogenic microorganisms tested. Of the bacterial strains tested, *E. coli* was found to be the most sensitive and was inhibited by all plant extracts that exhibited antimicrobial activity.

3.2. *Purification of an antimicrobial compound from methanolic L. indica leaf extract*

Methanolic *L. indica* leaf extract had both bactericidal and fungicidal activity against the five microorganisms tested, with an MLC ranging between 60 and 120 μg/mL. This is similar to the MLC of standard antibiotics (fluconazole and ampicillin ranging from 24 to 128 μg/mL). The results clearly showed that the methanol extract of *L. indica* was the most effective plant extract. It inhibited the growth of both Gram-negative and Gram-positive bacteria that were tested and also of the yeast *C. albicans*, which has a sophisticated mechanism of resistance to many drugs (*18*). Because the methanolic extract of *L. indica* had broad-spectrum antimicrobial action against all five pathogenic organisms tested, this extract was selected for phytochemical analysis and isolation of its bioactive constituent(s).

Table 1. Evaluation of the antimicrobial potential of promising plant extracts and the active compound from *L. indica* (cytisoside) indicated by the diameter of inhibition zones (DIZ, mm) and the minimum lethal concentration (MLC, μg/mL) against pathogenic organisms: *C. albicans* yeast and four bacteria, *i.e. L. monocytogenes*, *S. aureus*, *S. enteritidis*, and *E. coli*

Plant species	Plant family	*L. monocytogenes*		*S. enteritidis*		*S. aureus*		*E. coli*		*C. albicans*	
		DIZ	MLC	DIZ	MLC	DIZ	MLC	DIZ	MLC	DIZ	MLC
Yucca desmettiana	Agavaceae	–	–	–	–	–	–	24	> 120	20	> 120
Dracaena marginata	Agavaceae	–	–	–	–	–	–	21	120	23	> 120
Schinus terebinthifolius	Anacardiaceae	23	> 120	31	120	–	–	27	> 120	–	–
Euonymus japonica	Celastraceae	15	120	15	120	–	–	20	120	–	–
Conocarpus erectus	Combretaceae	20	120	24	60	–	–	34	120	–	–
Acalypha marginata	Euphorbiaceae	17	120	25	30	–	–	30	120	–	–
Pelargonium odoratissimum	Geraniaceae	18	120	20	120	35	120	30	120	–	–
Pelargonium zonale	Geraniaceae	23	120	32	30	32	> 120	30	30	–	–
Asparagus plumosus	Liliaceae	–	–	–	–	–	–	15	> 120	18	> 120
Lagerstroemia indica	Lythraceae	23	120	30	60	30	60	26	120	20	120
Myrtus communis	Myrtaceae	23	> 120	31	120	–	–	20	120	–	–
Punica granatum	Punicaceae	17	120	21	30	–	–	28	120	–	–
Rosa spp	Rosaceae	20	120	29	120	34	120	32	120	–	–
Cestrum diurnum	Solanaceae	12	> 120	–	–	–	–	16	> 120	21	120
Cytisoside (*Control*)		–	16	–	16	–	16	–	16	–	32
Fluconazole (*Control*)		–	–	–	–	–	–	–	–	–	> 128
Ampicillin (*Control*)		–	24	–	24	–	24	–	24	–	–

Phytochemical examination of the extract indicated the presence of phenolic compounds, flavonoids, triterpenoids, alkaloids, and glycoside compounds. One or more of these secondary metabolites may be responsible for antimicrobial activity (*19*).

Bioactivity-guided separation of the methanol extract of the dried leaves of *L. indica* resulted in the isolation of one chromatographically pure compound. The isolated compound had antimicrobial activity against the five human pathogenic microorganisms tested (Table 1). The MLC ranged between 16 and 32 µg/mL, indicating greater activity than that of the standard antibiotics used as positive controls in this experiment. Therefore, this compound was at least in part responsible for the antimicrobial activity of leaves of *L. indica.*

3.3. *Characterization and identification of the active compound*

The purified compound was obtained as a yellow amorphous solid that resulted in a positive color reaction with $AlCl_3$ reagent on TLC, suggesting a flavonoid. Electron ionization mass spectrometry resulted in a molecular ion peak [M^+] at 434 m/z. This finding, together with ^{1}H- and ^{13}C-NMR spectroscopic data, suggested a molecular formula of $C_{21}H_{22}O_{10}$. The UV spectrum had absorption maxima at λ 270 (band II) and 333 nm (band I), which are characteristic absorption bands of the flavone skeleton (*20*). No shift in band I of this compound was observed after the addition of $AlCl_3$/HCl, suggesting the formation of a hydroxyl-keto complex at 5-OH and the absence of an *O*-dihydroxyl group in the β-ring (*21*). The ^{1}H-NMR spectrum of this compound displayed signals characteristic of a methoxy group (δ_H 3.5 (3H)) and six aromatic hydrogens (δ_H 6.4 (H), 6.5 (H), 6.83 (2H), and 7.75 (2H)) in the low field region. The appearance of four aromatic proton signals at δ 6.83 (2H, d, *J* = 8.8 Hz, H-'3, H-'5) and 7.75 (2H, d, *J* = 8.8 Hz, H-'2, H-'6) along with a characteristic pattern for an A2B2 system confirmed the substitution of the β-ring at the '4 position. The proton signals at δ_H 6.4 (1H, s) and 6.5 (1H, s) were attributed to H-3 based on HMBC and compared to reported data (*20,22*). The presence of a free 7-hydroxyl group was noted based on the appearance of a bathochromic shift in the UV spectrum upon the addition of sodium acetate. The position of the methoxy group at the '4-*O*-position of the flavone moiety was confirmed by the heteronuclear correlation according to the HMBC spectrum and the UV spectrum data in the presence of NaOMe. Therefore, the flavone moiety of this compound was determined to be '4-methoxy apigenin (acacetin). The presence of *β*-D-glucopyranose as the sole sugar moiety was noted based on NMR spectra (Table 2) given the appearance of only one anomeric proton signal at δ 4.07 (d, *J* = 7.6 Hz), five glucosyl protons (δ_H 3.08-3.8m) in the ^{1}H-NMR spectrum, and the appearance of one anomeric carbon atom at δ 104.68 ppm and five glucosyl carbons (75.01, 79.76, 71.56, and 62.15 ppm; C-2 to C-6) in the ^{13}C-NMR spectrum. The position of the C-glucose moiety at the C-8-position was noted based on the HMBC spectrum and by comparison to reported data (*20*). Thus, the structure of this compound (Figure 1) was found to be '4-methoxy apigenin (acacetin)-8-*C*-*β*-D-glucopyranose, which was previously reported as cytisoside (*20*).

4. Discussion

The present study identified '4-methoxy apigenin (acacetin)-8-*C*-*β*-D-glucopyranose (cytisoside) as the active antimicrobial component of methanolic *L. indica* leaf extract. This component had both antibacterial and antifungal activity. To the extent known, no previous studies isolated this active compound from the leaves of *L. indica*. Thus, this is the first study to isolate and identify an active flavone glycoside from *L. indica*,

Table 2. NMR data for the active isolated compound in CD_3OD

Atom No.	δ_C	^{13}C	^{1}H
2	C	162.80	–
3	CH	103.50	6.50 (s)
4	CO	184.06	–
5	C	164.88	–
6	CH	95.24	6.40 (s)
7	C	165.04	–
8	C	109.20	–
9	C	158.73	–
10	C	105.20	–
'1	C	123.13	–
'2	CH	129.46	7.75 (d, *J* = 8.8)
'3	CH	117.04	6.83 (d, *J* = 8.8)
'4	C	166.20	–
'5	CH	117.04	6.83 (d, *J* = 8.8)
'6	CH	129.46	7.75 (d, *J* = 8.8)
	OCH_3	57.29	3.5 (s)
"1	CH	104.68	4.07 (d, *J* = 7.6)
"2	CH	75.01	3.26
"3	CH	79.76	3.40
"4	CH	71.32	3.08
"5	CH	77.56	3.21
"6	CH_2	62.15	3.68, 3.80

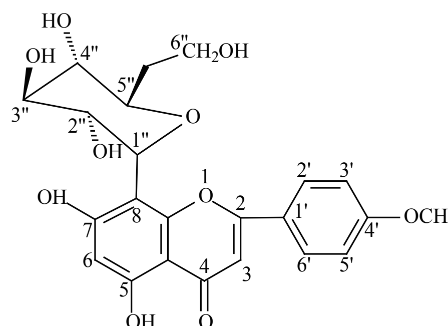

Figure 1. Structural formula of the active isolated compound ('4-methoxy apigenin-8-*C*-*β*-D-glucopyranoside; cytisoside).

i.e. cytisoside, with antimicrobial activity against the five pathogenic microorganisms (*C. albicans* yeast and four bacteria: *S. aureus*, *S. enteritidis*, *E. coli*, and *L. monocytogenes*) tested. Comparison revealed that the bioactive compound had greater antimicrobial activity than both the crude extract of *L. indica* and standard antibiotics (fluconazole and ampicillin).

Flavonoids are ubiquitous in photosynthesizing cells and therefore occur widely in the plant kingdom (*23*). Various flavonoids have been reported to possess a wide range of biological action, such as antimicrobial or antioxidant activity (*19*). Moreover, several groups of researchers have noted a synergy between biologically active flavonoids and existing chemotherapeutics (*24*). Flavonoids isolated from plant extracts are reported to have antimicrobial activity against food pathogenic bacteria and against *C. albicans*; these include compounds such as apigenin (*25,26*), quercetin and quercetin glycosides (*27,28*), luteolin (*29*), kaempferol (*30*), and flavan derivatives. Their remarkable activity is attributed to the inhibition of nucleic acid synthesis (*31*), inhibition of energy metabolism (*32*), and inhibition of cytoplasmic membrane function (*33*). Flavonoids are likely to have minimal toxicity because flavonoids are widely distributed in edible plants and beverages and have previously been used in traditional medicine.

The present study found that the methanol extract of *L. indica* leaves holds great promise as a potential source of beneficial antimicrobial components for different applications (foods and pharmaceuticals) and could resolve the problems of drug resistance and the harmful effects of synthetic compounds. However, further *in vivo* studies are needed to investigate the pharmacological and toxicological properties of *L. indica* extract before it can be considered as a new antimicrobial ingredient for the nutraceutical or functional food market.

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References

1. WHO publication. WHO strategy for containment of antimicrobial resistance. Available at: *www.who.int/drugresistance/WHO_Global_Strategy_English.pdf.* 2001 (accessed March 2012).
2. Cragg GM, Newman DJ, Snader KM. Natural products in drug discovery and development. J Nat Prod. 1997; 60:52-60.
3. De Valk H, Delarocque-Astagneau E, Colomb G, Ple S, Godard E, Vaillant V, Haeghebaet S, Bouvet Ph, Grimont F, Grimont P, Desenclos J-C. A community-wide outbreak of *Salmonella enteritidis* serotype Typhimurium infection associated with eating a raw milk soft cheese in France. Epidemiol Infect. 2000; 124:1-7.
4. Idris S, Ndukwe GI, Gimba CE. Preliminary phytochemical screening and antimicrobial activity of seed extracts of *Persea americana* (Avocado pear). Bayero Journal of Pure and Applied Sciences. 2009; 2: 173-176.
5. Rudol M, Sherer S. High incidence of *Listeria monocytogenes* in Europea red smear cheese. Int J Food Microbiol. 2001; 63:91-98.
6. Araujo VS, Pagliares VA, Queiroz ML, Freitas-Almeida AC. Occurrence of Staphylococcus and enteropathogens in soft cheese commercialized in the city of Rio de Janeiro, Brazil. J Appl Microbiol. 2002; 92:1172-1177.
7. Conedera G, Dalvit P, Martini M, Galiero G, Gramaglia M, Goffredo E, Loffredo G, Morabito S, Ottaviani D, Paterlini F, Pezzotti G, Pisanu M, Semprini P, Caprioli A. Verocytotoxin-producing *Escherichia coli* 0157 in minced beef and dairy products in Italy. Int J Food Microbiol. 2004; 96:67-73.
8. Annan K, Houghton PJ. Antibacterial, antioxidant and fibroblast growth stimulation of aqueous extracts of *Ficus asperifolia* Miq. and *Gossypium arboreum* L., wound-healing plants of Ghana. J Ethnopharmacol. 2008; 119:141-144.
9. Akorum S, bendjeddou D, Satta D, Lalaoui K. Antibacterial activity and acute toxicity effect of flavonoids extracted from *Mentha longifolia*. Am-Euras J Sci Res. 2009; 4:93-99.
10. Mbosso EJT, Ngouela S, Nguedia JCA, Beng VP, Rohmer M, Tsamo E. *In vitro* antimicrobial activity of extracts and compounds of some selected medicinal plants from Cameroon. J Ethnopharmacol. 2010; 128: 476-481.
11. Sakunpak A, Panichayupakaranant P. Antibacterial activity of Thai edible plants against gastrointestinal pathogenic bacteria and isolation of a new broad spectrum antibacterial polyisoprenylated benzophenone, chamuangone. Food Chem. 2012; 130:826-831.
12. Runyoro DK, Matee MI, Ngassapa OD, Joseph CC, Mbwambo ZH. Screening of Tanzanian medicinal plants for anti-Candida activity. BMC Complement Altern Med. 2006; 6:11.
13. Naeini A, Khosravi AR, Chitsaz M, Shokri H, Kamlnejad M. Anti-Candida albicans activity of some Iranian plants used in traditional medicine. J Mycol Med. 2009; 19: 168-172.
14. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol. 1966; 45:493-496.
15. NCCLS. Performance standards for antimicrobial susceptibility testing; ninth informational supplement; NCCLS document M100-S9 2008; (pp. 120-126). Wayne: National Committee for Clinical Laboratory Standard.
16. Farnsworth NR. Biological and phytochemical screening of plants. J Pharm Sci. 1966; 55:225-276.
17. Pathak K, Pathak A, Singl A. Flavonoids as medicinal agents – recent advances. Fitoterapia. LXII, 1991; 5:371-389.
18. Casalinuovo IA, Francesco PDI, Garaci E. Fluconazole

resistance in *Candida albicans*: A review of mechanisms. Eur Rev Med Pharmacol Sci. 2004; 8:69-77.
19. Harborne JB, Baxter H. The handbook of natural flavonoids. John Wiley and Sons, Chichester, UK, 1999.
20. Mabry TJ, Markham KR, Thomas MB. The Systematic Identification of Flavonoids. Springer Verlag, Berlin, Heidelberg, New York, 1970.
21. Markham KR. Flavones, flavonols and their glycosides. In: Methods in plant Biochemistry (Harborne J, ed.). London, UK, 1989; pp. 197-232.
22. Markham KR, Geiger H. ^{1}H nuclear magnetic resonance spectroscopy of flavonoids and their glycosides in hexadeuterodimethylsulphoxide. In: The Flavonoids. Advances in Research since 1986 (Harborne JB, ed.). Chapman and Hall, London, UK, 1993; pp. 441-497.
23. Havsteen B. Flavonoids, a class of natural products of high pharmacological potency. Biochem Pharmacol. 1983; 32:1141-1148.
24. Cushnie TP, Lamb AJ. Antimicrobial activity of flavonoids. Int J Antimicrob Agents. 2005; 26:343-356.
25. Sato Y, Suzaki S, Nishikawa T, Kihara M, Shibata H, Higuti T. Phytochemical flavones isolated from *Scutellaria barbata* and antibacterial activity against methicillin-resistant *Staphylococcus aureus*. J Ethnopharmacol. 2000; 72:483-488.
26. Narayana KR, Reddy SR, Chaluvadi MR, Krishna DR. Bioflavonoids classification, pharmacological, biochemical effects and therapeutic potential. Indian J Pharmacol. 2001; 33:2-16.
27. Ramaswamy AS, Jayaraman S, Sirsi M, Rao KH. Antibacterial action of some naturally occurring citrus bioflavonoids. Indian J Exp Biol. 1972; 10:72-73.
28. Taleb-Contini SH, Salvador MJ, Watanabe E, Ito I, de Oleveira DCR. Antimicrobial activity of flavonoids and steroids isolated from two *Chromolaena* species. Rev Bras Cienc Farm. 2003; 39:403-408.
29. Miski M, Ulubelen A, Johansson C, Mabry TJ. Antibacterial activity studies of flavonoids from *Salvia palaestina*. J Nat Prod. 1983; 46:874-875.
30. Nishino C, Enoki N, Tawata S, Mori A, Kobayashi K, Fukushima M. Antibacterial activity of flavonoids against *Staphylococcus epidermidis*, a skin bacterium. Agric Biol Chem. 1987; 51:139-143.
31. Ohemeng KA, Schwender CF, Fu KP, Barrett JF. DNA gyrase inhibitory and antibacterial activity of some flavones (1). Bioorg Med Chem Lett. 1993; 3:225-230.
32. Haraguchi H, Tanimoto K, Tamura Y, Mizutani K, Kinoshita T. Mode of antibacterial action of retrochalcones from Glycyrrhiza inflata. Phytochemistry. 1998; 48:125-129.
33. Tsuchiya H, Iinuma M. Reduction of membrane fluidity by antibacterial sophoraflavanone G isolated from *Sophora exigua*. Phytomedicine. 2000; 7:161-165.

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