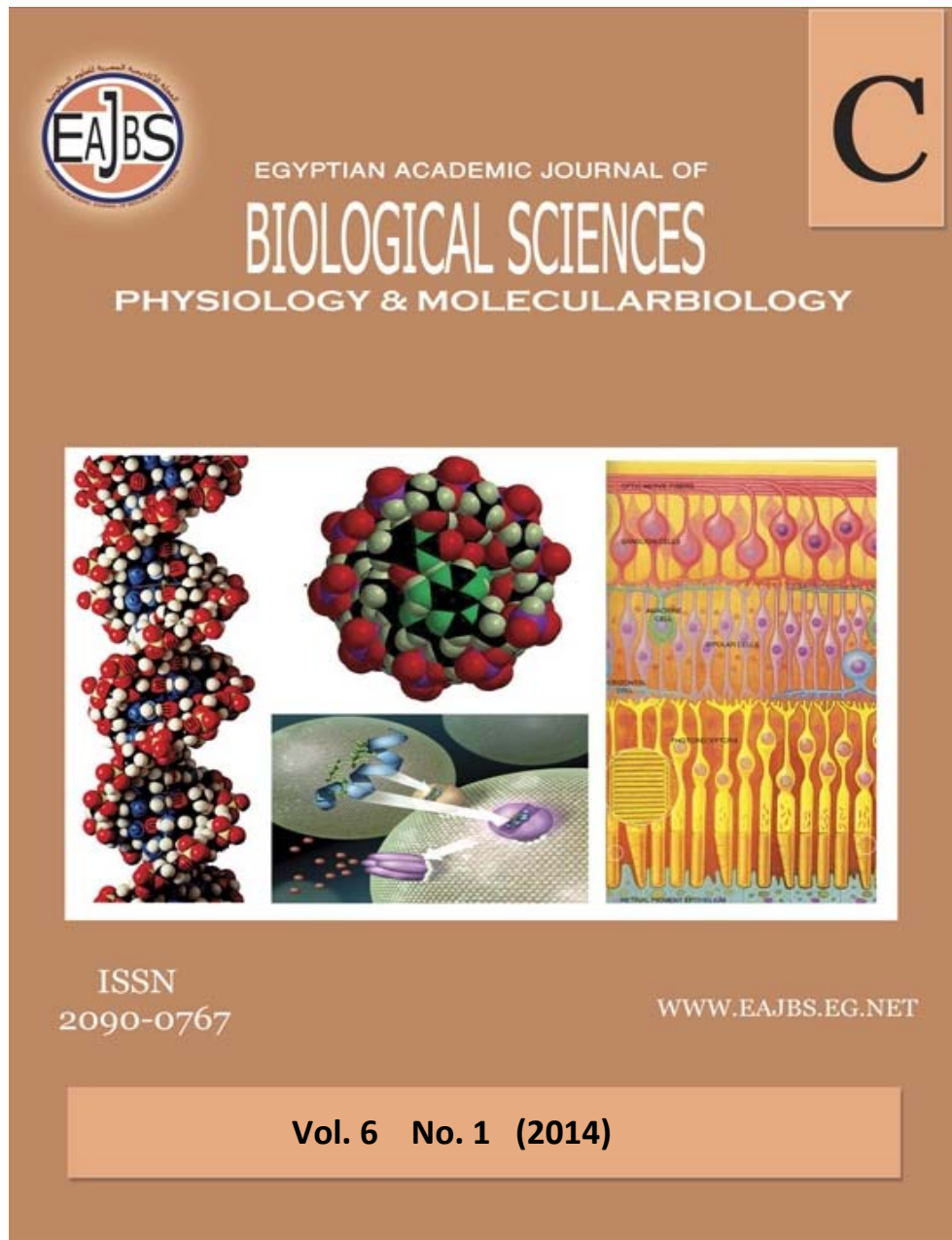


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Phytochemical analysis and inhibitory effects of extract of young fruits of *Ficus palmate* on some pathogenic microbes

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ABSTRACT

The present study was conducted to determine the antimicrobial activity of various extracts of young fruits of *Ficus palmata* Forsk. against some Gram positive and Gram negative bacteria and *Candida* species using the agar well diffusion method. Results showed that the cold water extract had no inhibitory effect against any tested microbes, while the hot water extract inhibited the microbial growth (12.5%). Methanol, chloroform, petroleum ether or acetone extract of the dry fruits inhibited microbial growth (37.5%), while diethyl ether extract inhibition was 12.5%. Methanol extract of fresh fruits inhibited the microbial up to 75%. However, the chloroform and petroleum ether extracts inhibited microbial growth by 62.5% and 37.5%, respectively. Gas Chromatogram- Mass spectrometry (GC-MS) analysis of hexan extract of the young fruits of *Ficus palmata* was carried out to identify its phytochemical constituents. Four chemical constituents have been identified: diethyl phthalate was the major compound (86.19%) (Retention time 16.65), and cyclohexasiloxane, dodecamethyl-, (Rt 12.77), 1,3-dimethyl-2-nitrobenzene (Rt 14.91) and benzamide (Rt 15.07) were the minor constituents (4.47, 7.96 and 1.36%, respectively). It could be concluded that the extract of young fresh fruits of *Ficus palmata* and its bioactive components, in particular diethyl phthalate, could be of phytopharmaceutical importance as an antimicrobial agents.

INTRODUCTION

Ficus palmata Forsk. (Moraceae) is commonly known as 'Fig', deciduous, moderate-sized tree, 6 to 10 meters in height; young branches, tomentose, often becoming glabrous; bark, smooth, dull, ash gray, can be stripped off with the hand, exposing the white to light-yellow wood underneath; wood, moderately hard.

Leaves, alternate, broad, ovate, membranous, 12.92 cm long, 14.16 cm broad, having reticulate pinnate venation and dentate margin; dark green and rough on the upper surface, light green and tomentose on the lower surface. The flowers are monoecious and it is self-fertile, appearing from Jun to September, and the seeds ripen in August (Collenette, 1985; Maghaed 1988). The plant suitable for sandy, loamy and clay soils. The fruit contains about 6% sugars, 1.7% protein, 0.9% ash and 0.2% pectin, low amount in vitamin C (Parmar 1982). The unripe fruits and young growth are cooked and eaten as a vegetable (Manandhar 2002), accordingly, act as a demulcent and laxative. The whole fruit is raw, sweet, succulent and edible, having some astringency and can be used as a poultice (Hedrick 1972, Parmar 1982; Watt, 1890; Kirtikar and Basu, 1935).

Extract from herbal plants represent a continuous effort to find new compounds against pathogens and a substantial number of new drugs introduced on the market are obtained from natural or semisynthetic resources (Mothana and Linclequist, 2005). Numerous studies have been conducted in many countries to prove efficiency of plant extracts against pathogenic microorganism, and most of the studies are restricted with crude extracts (Reddy *et al.*, 2006; Erdo Urul, 2002; Atefl *et al.*, 2003). Sandabe and Kwari (2000) and Wakeel *et al.* (2004) reported the use of extracts of *Ficus* species in Hausa ethno medicine of Northern Nigeria for the treatment of various diseases such as mental illness, dysentery, cough, diarrhoea, chest condition, tuberculosis, convulsive disorder and pain relief. According to Lewis and Ausubel (2006), Adeshokan *et al.* (2007), Oyeleke *et al.* (2008); Udobi *et al.* (2008), Hassan (2005) and Sandabe *et al.* (2006) active

principle constitute is an important source of the pharmaceutical drugs such as tannins, saponins, flavonoids, steroids, anthraquinone glycosides and reducing sugars and many of these compounds have been reported to be present in the *Ficus* species. Therefore, the present study aimed to investigate the *in vitro* antimicrobial activity of the water, chloroform, petroleum ether, methanol diethyl ether, and acetone extract of young fruits of *Ficus palmata* against *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Micrococcus luteus* and *Shigella* sp. and one pathogenic fungus *Candida* sp.

MATERIALS AND METHODS

Experimental materials healthy, fresh young fruits of *Ficus palmata* Forsk. were collected from Abha region, Kingdom of Saudi Arabia. The voucher specimen was stored in the herbarium of the Faculty of Science, King Khalid University.

Preparation of leaves and hips extracts

Forty nine grams of young fruits of *Ficus palmata* were washed thoroughly with distilled water and crushed directly by grinder for 15 minutes, and the solution samples were filtered through 2-layered muslin cloth (Girish and Satish, 2008). The filtrate was divided into a number of aliquots each contains 20 mL, and subjected to fresh extraction by adding 20 mL of water, chloroform, petroleum ether, methanol diethyl ether or acetone. The extraction of dry materials was done by adding 20 ml of water, chloroform, petroleum ether, methanol diethyl ether OR acetone to the half gram (1 g) air-dried and powdered young fruits. All samples were kept for 48 hours in rotary shaker at 99 rpm at 27 °C. After that, the extracts were filtered and placed in an incubator at 39°C until the solvent was completely evaporated.

According to Alamri and Moustafa (2012), each extract was weighed, dissolved in sterile dimethyl sulfoxide (DMSO) and subjected to the antimicrobial activity test.

Test organisms

Six clinical bacterial isolates were used to test the antibacterial activity of the prepared extracts. These isolates were: *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Micrococcus luteus* and *Shigella* sp., and one pathogenic fungus *Candida* sp. The *Candida* sp. was subcultured at 35 °C for 48 h while bacterial strains were subcultured in nutrient broth at 37 °C for 24 hours.

Screening for antimicrobial activity

The antimicrobial activity has been evaluated by using the well diffusion methods (Patel *et al.*, 2007; Alrumman *et al.*, 2012). In each sterile Petri dish, 20 mL of sterilized Mueller Hinton agar (Oxoid, England) media was poured. To the tested bacterium and *Candida* sp. 0.1 mL of standardized inocula was spread equally over the surface of the solidified agar using L-shaped sterile glass. A sterile cork-borer was used to make wells (6 mm in diameter) on the agar and 0.1 mL of the plant extraction was added to each well. All plates were then kept at room temperature for about one hour to allow diffusion of the plant extract into agar. Each experiment was assayed in triplicate and sterile dimethyl sulfoxide (DMSO) served as negative and cefoxitin (30 µg) as positive controls and the plates were incubated at 30 °C for 24 h for bacterial strains, and 48 h for *Candida* sp. The antimicrobial activity was determined by measuring the diameter of the clear zones (Pundir and Jain, 2011).

Gas chromatography-mass spectrometry analysis (GC-MS) of hexan extract of young fruits of *Ficus palmata*

The GC-MS analysis was carried out using a Clarus 500 Perkin-Elmer (Auto system XL) Gas Chromatograph

equipped and coupled to a mass detector Turbo mass gold-Perkin-Elmer Turbo mass 5.1 spectrometer with an Elite – 1 (100% Dimethyl poly siloxane) and TR-V1 column (30 m x 0.32 mm x 1.8 µm) was used. Helium gas (99.999%) was used as a carrier gas at a constant flow rate of 6 mL/min with an injection volume of 2 µL (a split ratio of 5:1) and injector temperature 200°C. The oven temperature was programmed to temperature of 35°C for 4 min hold, 30°C min⁻¹ to 90°C, 30°C min⁻¹ to 110°C with no hold, and then 45°C min⁻¹ to 170°C hold for 1 min. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 40 to 550 Da (Ezhilan and Neelamegam, 2012). Unknown component was compared with the spectrum of the known components stored in the NIST library.

RESULTS AND DISCUSSION

Susceptibility of the microbes to young fruits extract

The results of the antimicrobial activities of water, methanol, chloroform, petroleum ether, diethyl ether or acetone extract of young fruits of *Ficus palmata*, determined by agar well diffusion method, are presented in Fig. 1. The cold-water extract of fresh fruits showed no zones of inhibition against the tested microbes, while the hot water extracts from young fresh fruits inhibited 1 out of the 8 (12.5%) microbes used with average zone diameters ranging from 0.7 cm against *Staphylococcus aureus* (Fig. 1 panel A). Dry extract of young fruits gained from methanol, chloroform, petroleum ether or acetone inhibited 3 out of the 8 (37.5%) microbes used with average zone diameters ranging from 1.6 cm against *M. luteus* to 0.75 cm against *Shigella* sp., while diethyl ether extract inhibited (12.5%) with a zone 0.8 cm against *M. luteus*. The methanol extract of fresh young fruits inhibited 6 out of the 8 (75%) microbes with a zone of inhibition ranged from 1.9 cm against *M.*

luteus and *Candida sp.* to 0.8 cm against *Klebsiella oxytoca*. Chloroform extract of fresh young fruits inhibited 5 out of the 8 (62.5%) microbes with a zone of inhibition ranged from 2.1 cm against *Shigella sp.* to 1.1 cm against *Staphylococcus aureus*. Petroleum ether extract of fresh young fruits inhibited 3 out of the 8 (37.5%) microbes with a zone of inhibition ranged from 3.1 cm against *M. luteus* to 1.2 cm against *Staphylococcus aureus*. Since extraction

method is important as a first step in the analysis of medicinal plants, we used various solvents to check the efficacy of each solvent in extracting various chemical compounds in the young fig fruits. Plant extracts are usually prepared by maceration or percolation of fresh green or dried powdered plant parts in water and/or organic solvent alone, or mixed solvents with different percentage (Alrumman *et al.* 2012; Girish and Satish 2008).

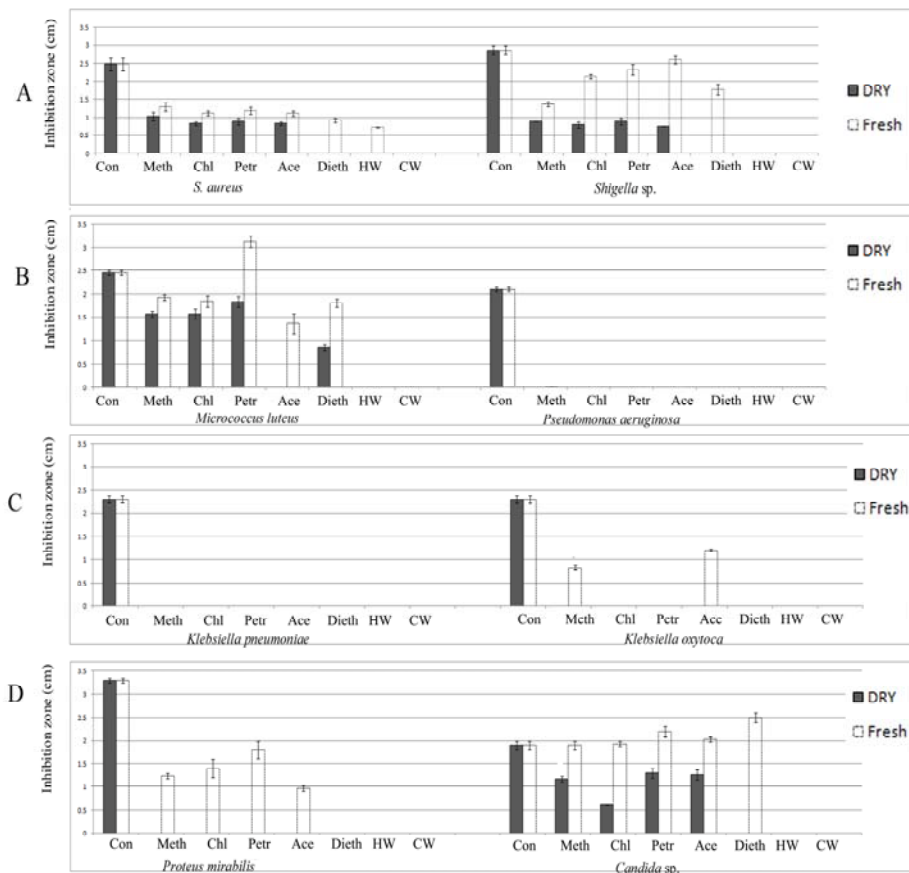


Fig. 1: Antimicrobial yield difference between various extracts from dry and fresh young fruits of *Ficus palmata*. Con (cefoxitin, 30 µg, as positive controls), methanol (Meth), chloroform (Chl), petroleum ether (Petr), acetone (Ace), diethyl ether (Dieth), hot water (HW) and cold water (CW). Bars represent (SE, n = 3) for each means.

It is evident from our results that different extract solvents have different effects to extract active ingredients from the *Ficus palmata* fruits. A comparative study of various methods for extracting antioxidant and antibacterial compounds from seeds of five different plants, using different extracting solvent indicated that each solvent was different from the other

in extraction ability (Kothari *et al.*, 2012), which agrees with our results. Comparing the extracts obtained from fresh and dry young fruits against tested microbes, it turned out that the extracts from the fresh young fruits exhibited more activity than the dry fruits. Our results are in accordance with a previous work of Abeysinghe (2010) who showed

that fresh plant materials from *Lumnitzera racemosa* and *Avicennia marina* could suppress the growth of *Staphylococcus aureus* and *Proteus* sp. more than those of dried plant materials. The finding may be attributable to the notion that temperature of dryness may cause the loss of active antibacterial chemical ingredients from the plant materials. Therefore, we recommend using fresh materials for the extraction to obtain maximum antibacterial compounds. Venskutonis (1997) reported that the concentrations of volatile constituents from various aromatic plants are greatly affected during the drying method. Furthermore, certain monoterpenes such as 1,8-cineole, linalool and geraniol will be lost during drying method (Diaz-Maroto *et al.*, 2002). Differences between the

susceptibility of Gram negative and Gram positive bacteria to different plant extract solvents may be due to the variations in the structure of the bacterial cell walls, because the cell wall outside the plasma membrane of Gram-negative bacterial acts as a barrier to many substances including antibiotics (Tortora *et al.*, 2001).

Phytochemicals in young fruits of Ficus palmata

Investigating the active principles in the *Ficus palmata* young fruits hexan extract by GC-MS analysis clearly showed the presence of four compounds (Table 1). Molecular formula (MF), molecular weight (MW), and concentration (peak area %) in the detected active principles with their retention time (RT) are presented in Table 1.

Table 1: GC-MS analysis of hexan extract of the young fruits *Ficus palmata*.

No.	Compounds	Rt Min.	% Area	M.W.	Chemical Formula
1	Cyclohexasiloxane, dodecamethyl-	12.77	4.47	444.92	C ₁₂ H ₃₆ O ₆ Si ₆
2	1,3-Dimethyl-2-nitrobenzene	14.91	7.96	151.16	(CH ₃) ₂ C ₆ H ₃ NO ₂
3	Benzamide	15.07	1.36	121.14	C ₆ H ₅ CONH ₂
4	Diethyl phthalate	16.65	86.19	222.23	C ₁₂ H ₁₄ O ₄

The GC-MS chromatogram of the four peak of the compounds detected was shown in Fig. 2. The results revealed that diethyl phthalate (86.19%) was the major component in the hexan extract, while

cyclohexasiloxane, dodecamethyl-, (4.47%), 1,3-dimethyl-2-nitrobenzene (7.96%) and benzamide (1.36%) were minor components.

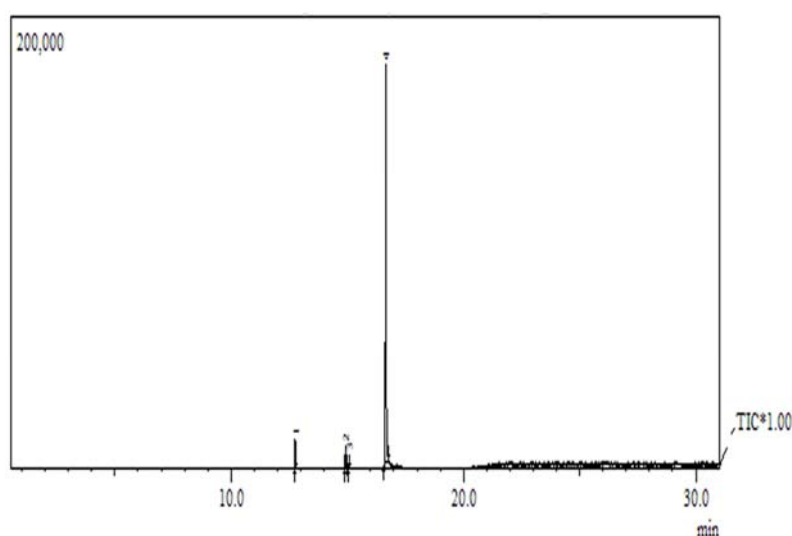


Fig. 2: Gas chromatogram of the hexan extract of young fruits of *Ficus palmata*

It has been reported that diethyl phthalate and its derivatives display a wide range of biological properties such as its inclusion in insecticide sprays, mosquito repellents and camphor substitute (WHO, 2003), and also in soaps, detergents, and skin care preparations (Anonymous, 1985; Kamrin, 1991). Bis- (2-methylheptyl) phthalate isolated from *Pongamia pinnata* Pierre leaves exhibited antiviral activity against White Spot Syndrome Virus of *Penaeus monodon Fabricius* (Rameshthangam and Ramasamy, 2007). In addition, bis-(ethylhexyl) phthalate from *Streptomyces bangladeshiensis* showed antimicrobial activity (Al-Bari *et al.* 2006). Moreover, di-isooctylphthalate from *Nigella glandulifera* Freyn was characterized as an inhibiting melanogenesis factor (Nguyen *et al.* 2007). Di-(2-ethylhexyl) phthalate (DEHP) isolated from marine *Pseudomonas* strain was also used in the treatment of inflammatory joint disease, cancer, and other diseases related to disorder of cathepsin B (Hoang *et al.*, 2008). Benzamide nucleus and its derivatives characterized as an antibacterial compounds such as benzoxazoles (Hisano *et al.*, 1982; Prudhomme *et al.*, 1986; Ersan *et al.*, 1997; Sener *et al.*, 2002; Oren *et al.*, 1997; Temiz *et al.*, 1998; Yalcin *et al.*, 1992; Sener *et al.*, 2000), oxazolidinone (Cui *et al.*, 2006), and oxyclozanide (Hisano *et al.*, 1982). In conclusion, the observable inhibition of tested bacteria by the extracts of young fresh fruits of *Ficus palmata* clearly indicate its potential to be used as an alternative compound for the development of a new antibacterial agents.

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