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ANTIFUNGAL AND ANTIMICROBIAL ACTIVITY OF CRUDE METHANOLIC EXTRACT AND ITS FRACTIONS OF *JATROPHA CURCAS* L., LEAVES

(With 3 Tables and 5 Figures)

By

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تأثير المستخلص الميثانولى الخام ومكوناته لنبات الجاتروفا على الفطريات

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يهدف هذا البحث فحص المكونات الكيميائية للمستخلص الميثانولى لأوراق الجاتروفا . وقد أفادت المعاملات التحليلية لهذا المركب احتوائه على ٧ مركبات فلافونويدية وهى:

Biapigenin-(8,8″)-methylene-6,6″-di-C-*β*-D-glucopyranoside (1), Apigenin 7-*O*-*β*-D-neohesperidoside (2), Apigenin 7-*O*-*β*-D-galactoside (3), Orientin (4), Apigenin (5), Isovitexin (6) and Vitexin (7).

وتم اختبار هذه المركبات كلها من حيث تأثيرها على نمو الفطريات *Botrytis cinerea, Rhizoctonia solani, Sclerotinia sclerotiorum and Fusarium solani* . أوضحت النتائج أن للمركب ٧ تأثير قوى فى تثبيط كافة أنواع الفطريات المستخدمة. وكان لباقى المركبات تأثير ضعيف على نمو الفطريات فى المقابل كان المستخلص الميثانولى الخام لأوراق الجاتروفا التأثير المانع تماما لنمو كل أنواع الفطريات. وكذلك تم اختبار مستخلص الميثانول الخام والمركبات الفلافونودية النقية على نمو البكتريا الموجبة لجرام وهي الميكروب العنقودى الذهبى والبكتريا السالبة لجرام وهي الميكروب القولوني وكانت النتائج متشابهة حيث كان التاثير الكامل لمنع نمو البكتريا بفعل المستخلص الخام ثم الجرعات الخفيفة عند ٥٠ ميكروجرام من كل جزيء فلافونويدي على كل انواع البكتريا بصفة عامة

SUMMARY

In this paper the polyphenolic compounds of methanolic extract of *Jatropha curcas* leaves have been investigated. The analytical procedures revealed 7 flavonoid compounds: biapigenin-(8,8″)-methylene-6,6″-di-C-β-D-glucopyranoside (1), apigenin-7-*O*-β-D-neohesperidoside (2), apigenin 7-*O*-β-D-galactoside (3), orientin (4), apigenin (5), isovitexin (6) and vitexin (7). The crude methanol extract and the 7 purified compounds were tested against fungi *Botrytis cinerea, Rhizoctonia solani, Sclerotinia sclerotiorum and Fusarium solani*. The crude extracts absolutely inhibit the fungi growth. On the other hand, vitexin was similar to crude extract as well. The other compounds were moderate in their antifungal effects in comparison to control ones. In-vitro antimicrobial activity of the methanolic leaves extract was carried out by using the agar-well diffusion method on Gram-positive bacteria *Staphylococcus aureus*. The Gram-negative included *Escherichia coli* was compared with standard drug Streptomycin and Ampicillin.

Key words: *Jatrpha, Staph. aureus, E.coli, Methanolic, Leave extract, Fractions, Fungus, Latex, antibacterial, antifungal*

INTRODUCTION

Jatropha species are all members of the Euphorbiaceae family. Plants are believed to be native of tropical America but they are distributed throughout tropical and subtropical Asia and Africa. It is now widely distributed throughout the tropics occurring in south West Africa, India, Cape Verde islands, Madagascar, Java, Malaysia, Thailand, Burma and Philippines (Cano-Asseleih, 1986; Cano-Asseleih *et al.,* 1989). Botanist Carl von Linnaeus first classified the plant in 1753; he gave it the botanical name "*Jatropha curcas*" from the Greek word "Jatros" meaning a "Doctor" and "Trophe" meaning "Nutrition" (Agnote, 1998; Dorth, 2003). *Jatropha* is commonly known as Physic nut or purging nut but in different countries and in different languages it has different common or vernacular names: Physic nut, Purging nut (English); Pourghere, Pignon d'Inde (French); Purgier-nub, Brechnab (German); Pinoncillo (Mexico); Coquillo, Tempate (Costa Rica); Tartago (Puerto Rico); Mundubi-assu (Brazil); Pinol (Peru); Pinon (Guatemala); Bagbherenda, Jangliarandi, (Hindi); Sabudam (Thailand); Tubangbakod (Philippines), Jarak budge (Indonesia); Bagani (Cote D' Ivoire); Tabanani (Senegal); Butuje, Lapalapa (Nigeria); Makaen

(Tanzania); Purgeer boontjie (South Africa); Dand barri, Habel melak (Arab); Yu-la-Tzu (Chinese); Fagiala d' India (Italian) and Purgueira (Portuguese) (Heller, 1996; Becker and Francis, 2003). *Jatropha curcas* or Physic nut is a multipurpose and drought resistant, perennial plant (Jones and Miller 1992; Openshaw 1986). It can be grown in low to high rainfall areas either in the farm as a commercial crop or on the boundaries as a hedge to protect fields from grazing animals and to prevent erosion (Henning 1996; Gübitz *et al.,* 1999). The leaves are remedy for jaundice, applied by rectal injection (Okujagu *et al.,* 2006). The by-products are press cake; good organic fertilizer and oil contains also insecticides (Openshaw, 1986). Traditionally it is used to cure diseases like cancer, piles, snakes bites, paralysis and dropsy (Okujagu *et al.,* 2006). Medically, the heated leaves are placed on the breast as a lactagogue in Guatemala (Morton, 1981). The leaves are regarded as antiparasitic and applied in scabies. They are also used as rubefacients in paralysis, in rheumatism and also applied to tumors (Perry, 1980; Duke, Atchley 1984). *J curcas* leaves extract is a component of formulation orally used in the treatment of malaria (Ankrah *et al.,* 2003). These suggest that the plant leaves may not be as toxic as the seeds. *J curcas* leaves possess antiseptic, purgative, cicatrizant effects (Faria *et al.,* 2006). Phytochemical analysis of *J curcas* revealed the presence of alkaloids, glycosides, saponins, tannins, flavonoids, resins, sterols, terpenoids and carbohydrates in the methanol extract that have implicated in the antimicrobial activities as well as free radical scavenging activities of some plants. The leaves also demonstrated antioxidant and haemostatic activities as well as acceleration of wound healing and antimicrobial activity against microbial wound contamination (Okoli *et al.,* 2008). Flavonoids have free radical scavenging and antioxidation properties, which are useful for their pharmacological activities including anticancer and anti-aging properties. Many flavonoids show antibacterial, antiviral and antifungal activities (Ng *et al.,* 1996; Sharma 2006). The studies of chemistry, biochemistry and pharmacological properties of flavonoids may be important (Cody *et al.,* 1984; Cody *et al.,* 1989; Havsteen 2002; Winkel-Shirely 2002). They also possess antimicrobial properties (Hernandez *et al.,* 2000; Sohn *et al.,* 2004). Previous works have shown that many *Jatropha* species possess antimicrobial activity (Aiyelaagbe *et al.,* 2000 and Aiyelaagbe *et al.,* 2007). Several studies have confirmed the antimicrobial efficacy of different *Jatropha* species; however, there is insufficient information regarding the antimicrobial activities of *J. curcas*. The aim of this work is the evaluation of *Jatropha curcas* L. leaves constituents and its antifungal and antibacterial activities.

MATERIALS and METHODS

Sample Preparation:

Jatropha curcas L. leaves were collected at Sept. 20, 2009 from *Jatropha curcas* plants which planted on March 15, 2009 at Faculty of Agriculture Farm, AL-Azhar University, Assiut branch, Assiut, Egypt. Leaves were air dried at laboratory of Chemistry Department conditions for one month. The leaves were crushed and grinded to fine powder by plant grinder.

Extraction and Fractionation:

One kilogram of the fine leaves powder was macerated in 4 liters of 80 % methanol for 24 hours at room temperature and extracted again by 3L x 3 times of 80 % methanol at room temperature. The extracts were combined and concentrated by rotary evaporator under reduced pressure at low temperature. The residue (95 g) was defatted by (3L x 3 times) petroleum ether (bp. 40-60°C). The petroleum ether fraction (oil) was discarded. Crude methanol extract were tested against fungi. The defatted residue (89 g) was preliminary fractionated on silica gel column (3 x 70 cm) using a step gradient of solvent system 1 (Hexane: EtOAc, 100:0, 0: 100 v/v) then, solvent system 2 (methanol: H_2O, 100:0, 0:100 v/v) was used for elution to give 35 fractions. Then, collected and divided by U.V. light into seven (I-VII) major collective fractions. Some of other fractions are still unknown. The homogeneity of the compounds was purified by silica gel TLC plates. These fractions were subjected to further purification by column chromatography each as below. Fraction I (180mg) was fractionated by Sephadex LH_{20} column chromatography and eluted by methanol. Fraction II chromatographed similar and gave pure compound 2 (130mg). Carboxy methyl cellulose column of fraction III further purified using {Butanol: isopropanol: H_2O, 4:1:5 v/v, : methanol (50:50)} as eluent to give purified 28mg of compound 3 and 25 mg of compound 4. Fractions IV, V and VI exposed to Sephadex LH_{20} column chromatography with methanol: water as eluent solvent system to gain 30mg of pure compound 5, 14 mg of compound 6 and 11 mg of compound 7. Fraction VII eluted by butanol: isopropanol: water to give compound 1. All of these fractions and others (unknowns) were subjected to analysis by H^1-NMR and GC-MS instruments as follows: ^{1}H-NMR data were measured in $CDCl_3$ or DMSO-d_6 on a Varian XL 200, 300 MHz instruments using TMS as internal standard. Chemical shifts were reported in ppm (δ) downfield from internal TMS and coupling constants are expressed in hertz. Mass spectra were recorded on GC-MS QP-1000 EX. Shimadzu Instrument consisted of a Varian 3400 gas chromatography equipped with a fused-silica column

(30m x 0.25 mm, film thickness 0.25 mm). The operating conditions were: oven temperature 40-210 °C with the rate of 5°C / min; injector mode: split injection; with the carrier gas, He; flow rate 1ml / min; ionization current, 750mA; scan range, 40-300µ., Faculty of Science, Cairo University.

Fungal Experiment:

Preparation of crude antibiotic discs: sterile Whatman No. 1 paper was punched into 5 mm diameter disc sizes. The Whatman discs were placed in Mac Cartney bottles and sterilized in an autoclave at 120°C for 15 min. the bottles was transferred into a hot air oven at 60°C to dry for 30 min. an aqueous extract of leaves (1.0 ml) was transferred into a sterile bottle containing sterile discs. The sterile crude discs were allows to soak in extract of each fraction (I-VII) for 4 hr for proper absorption, after which they removed and allowed to dry. The control disc was immersed in distilled water instead of fractions (Cheesebrough, 2000). Potato dextrose agar (PDA) media (200g of potato, 20g of dextrose and 20g of agar) was thawed at 45°C and poured onto 9 cm Petri dishes. After cooling at room temperature, the dishes were fertilized with uniform discs of the fungus (Barnette and Hunter, 1972). Four tested fungi genus; *Botrytis cinerea, Rhizoctonia solani, Sclerotinia sclerotiorum and Fusarium solani* against the crude extract and the seven purified fractions. The experiment was made in triplicate for each fraction. The dishes were incubated for 7 days at 25° C ± 3, then, the inhibition zone was measured.

Bacterial Experiment:

Preparation of microorganism for the experiment

The following typed cultures and locally isolated organisms obtained from culture collection\of lab Ministry of heath were used for the experiment. These bacteria isolates include Gram-positive *Staphylococcus aureus* (NCIB 8588). The Gram-negative include *Escherichia coli* (NCIB 86). The bacterial isolates were first subcultured in a nutrient broth (Oxoid) and incubated at 37°C for 18 h.

Antibacterial activity

The antibacterial activity of the crude extracts was determined in accordance with the agar-well diffusion method described by Irobi *et al.* (1994). The bacterial isolates were first grown in a nutrient broth for 18 h before use and standardized to 0.5 McFarland standards (106 cfu / ml^{-1}). Two hundred microliter of the standardized cell suspensions were spread on a Mueller-Hinton agar (Oxoid). Wells were then bored into the agar using a sterile 6 mm diameter cork borer. Approximately 100 µl of the crude extract at 10 mg/ ml^{-1} were introduced into the wells, allowed to stand at room temperature for about 2 h and then incubated at 37°C.

Controls were set up in parallel using the solvents that were used to reconstitute the extract. The plates were observed for zones of inhibition after 24 h. The effects were compared with those of streptomycin and ampicilin at a concentration of 1 mg / ml and 10 μg / ml^{-1} respectively.

Minimum inhibitory concentration (MIC)

The estimation of MIC of the crude extracts was carried out using the method of Akinpelu and Kolawole (2004). Two-fold dilutions of the crude extract was prepared and 2 ml aliquots of different concentrations of the solution were added to 18 ml of pre-sterilized molten nutrient agar at 40°C to give final concentration regimes of 0.050 and 10 mg/ml. The medium was then poured into sterile Petri dishes and allowed to set. The surface of the medium was allowed to dry under laminar flow before streaking with 18 h old bacterial cultures. The plates were later incubated at 37°C for 24 h for up to 72 h for bacteria after which they were examined for the presence or absence of growth. The MIC was taken as the lowest concentration that prevented the growth of the test microorganism.

Minimum bactericidal concentration (MBC)

The MBC of the plant extracts was determined by a modification of the method of Spencer and Spencer (2004). Samples were taken from plates with no visible growth in the MIC assay and subcultured on freshly prepared nutrient agar plates and incubated at 37°C for 48 h. The MBC was taken as the concentration of the extract that did not show any growth on a new set of agar plates.

RESULTS

Table 1: Antifungal activity of *J. curcas* crude methanolic extract and its flavonoids.

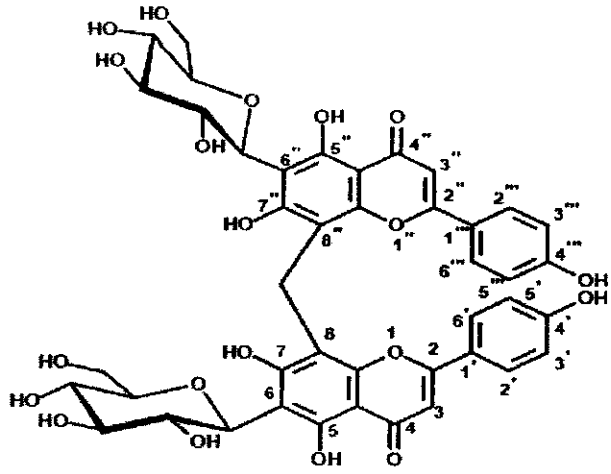
Fraction No.	Fungi species			
	B. cinerea	*R. solani*	*S. sclerotiorum*	*F. solani*
I	4.6	6.1	4.8	5.8
II	5.2	6.0	5.0	6.2
III	8.2	8.2	8.2	8.8
IV	7.6	9.0	8.4	9.0
V	4.9	6.5	5.4	6.3
VI	5.2	5.6	4.8	5.8
VII	0.9	1.9	0.7	1.7
Crude Ext.	0.0	0.0	0.0	0.0
control	9.0	9.0	9.0	9.0

Table 2: Antibacterial activity of *jatropha* leaves crude mathanolic extract and its fractions.

Organism	Fraction	Concentration (µg \ ml)			
		50	100	150	200
		Inhibition Zone (mm)			
Escherichia coli (NCIB 86)	Crude	0	0	0	0
	1	8.25	11.50	13.00	16.75
	2	6.75	8.75	12.50	16.75
	3	7.00	9.25	12.00	16.00
	4	5.75	8.75	13.00	15.25
	5	8.00	3.66	13.00	16.66
	6	8.75	11.00	13.33	16.66
	7	7.00	9.66	12.00	15.33
	Strept.	0	0	0	0
	Amp.	18	18	18	18
Staphylococcus aureus (NCIB 8588)	Crude	0	0	0	0
	1	9.25	12.00	15.25	16.50
	2	8.50	10.75	12.25	15.00
	3	8.50	11.50	14.00	16.25
	4	9.00	12.25	14.75	16.75
	5	8.33	12.00	15.33	17.00
	6	9.00	11.25	14.00	16.00
	7	7.33	10.00	11.66	12.66
	Strept.	22	22	22	22
	Amp.	0	0	0	0

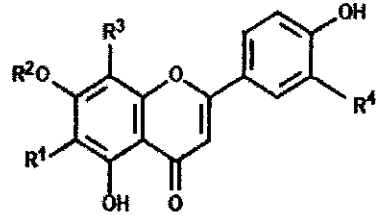
Table 3: Minimum inhibitory concentration (MIC) and minimum bactericidal activity (MBC)

Test organisms	The minimum inhibitory concentration (MIC)	The minimum bactericidal activity (MBC)
Escherichia coli (NCIB 86)	50 µg / ml	15.33 mm.
Staphylococcus aureus(NCIB 8588)	100 µg / ml	7 mm.



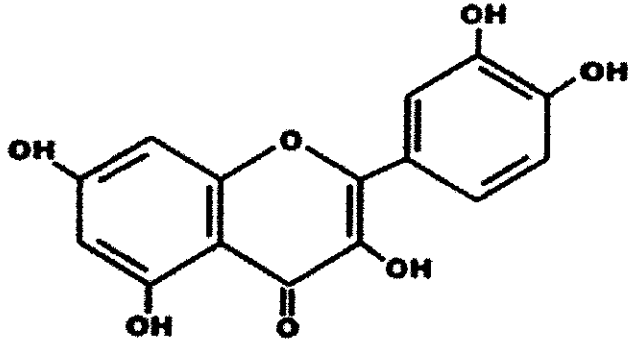
Biapigenin-(8,8″)-methylene-6,6″-di-C-β-D-glucopyranoside (1)

(Fig. 1)



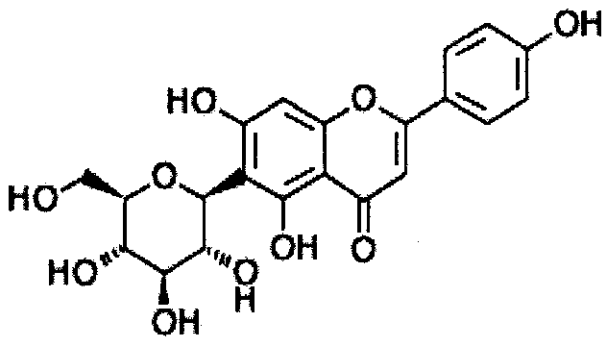
Apigenin-7-*O*-β-D-neohesperidoside (2) $R^1 = R^3 = R^4 = H$, R^2 = β-D-neohesperidoside
Apigenin 7-*O*-β-D-galactoside (3) $R^1 = R^3 = R^4 = H$, R^2 = β-D-galactopyranoside
Orientin (4) $R^1 = R^2 = H$, R^3 = β-D-glucopyranoside, R^3 = OH

(Fig. 2)



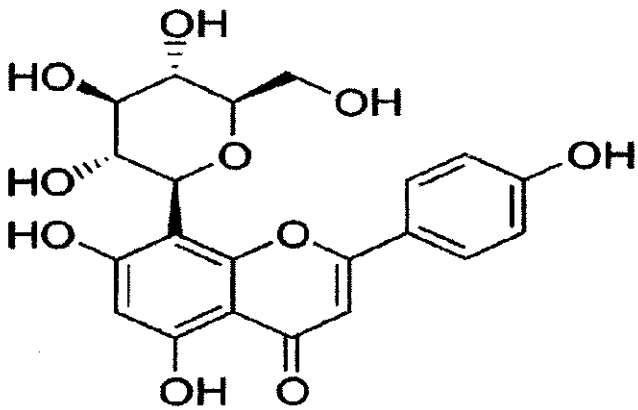
Apigenin (5)

(Fig. 3)



Isovitexin (6)

(Fig. 4)



Vitexin (7)

(Fig. 5)

DISCUSSION

The obtained analytical data revealed that the major polyphenolic compounds are flavonoids and biflavonaids. The identified compounds were authenticated according to its H^1-NMR and GC/MS analytical data which matched to computer library. Compounds are biapigenin-(8,8″)-methylene-6,6″-di-C-*β*-D-glucopyranoside (1), apigenin 7-*O*-*β*-D-neohesperidoside (2), apigenin 7-*O*-*β*-D-galactoside (3), orientin (4), apigenin (5), isovitexin (6) and vitexin (7). Table (1) shows the obtained results. Crude methanolic extract showed absolutely inhibition zones and have zero growth up to one month. While, the purified fractions showed a variance in growth zones from 0.95 cm to 9 cm after one week since

inoculation. Compound VII (vitexin) seems to be the most antifungal compounds. The compounds I, II, V and VI have moderate effect as fungicides. On the other hand, compounds III and IV showed no significant inhibition zone of fungi species (Sharma 2006 and Ng *et al.*, 1996). These polyphenolic compounds have health benefits. So *J. curcas* leaves consider as important source of these compounds and not be toxic as the seeds or in the native *J. curcas* countries (Masaoud *et al.*, 1995; Okuyama *et al.*, 1996; Aiyelaagbe *et al.*, 1998). The obtained data is agreed with that of Abd-Alla *et al.* (2010) on the same plant species.

Antibacterial activity

Data in Table (2) reveals that methanolic leaves extract possess potential antimicrobial activity. The obtained results indicates that the methanol leaf extract had shown maximum zone of inhibition at 200 µg/ml on the 7 flavonoid compounds. All extracts of the plant tested showed varying degree of antibacterial activities against the test bacterial; *Staphylococcus aureus* and on *Escherichia coli* species. The antibacterial activities of the methanolic extracts compared favorably with that of two standard antibiotics (streptomycin and ampicillin) and appeared to have a broad spectrum according to its activities on gram reaction. The inhibition zone of *Staphylococcus aureus* and *Escherichia coli* was compared to the minimum inhibitory concentration (MIC) of the methanolic fractions (I-VII) ranged between 100 µg / ml and 50 µg / ml for Gram positive and negative bacteria, respectively. The minimum bactericidal activity (MBC) of the extract for Gram positive and negative bacteria ranged between 7-15.33 mm. (Table 3) Generally, the methanol extract was more active. This may be attributed to the presence of soluble phenolic and polyphenolic compounds (Kowalski and Kedzia, 2007). The inhibitory effect of the extract of *J. curcas* against pathogenic bacterial strains can introduce the plant as a potential candidate for drug development for the treatment of ailments caused by these pathogens investigated in this study is in agreement with (Koduru *et al.*, 2006; Aliero *et al.*, 2006; Ashafa *et al.*, 2008; Aiyegoro *et al.*, 2008). Mujumdar *et al.* (2001) also reported that the crude methanol extract from the root of *J. curcas* exhibited anti-diarrhea activity in mice through the inhibition of prostaglandin biosynthesis and the reduction of osmotic pressure. Recently, Aiyelaagbe *et al.* (2007) reported that the presence of some secondary metabolites in the root extract of *J. curcas* inhibited some microorganisms isolated from sexually transmitted infections. In future, it seems that this plant still need some research on its chemical composition to be evaluated under Egyptian conditions.

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