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Phytochemical and Antimicrobial Studies on *Acacia saligna* Leaves

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THE THERAPY of the medicinal plants has always been a part of our environment as they have several therapeutic virtues. These properties are generally attributed to secondary metabolites such as polyphenols. In this study we investigated the three extracts; ethyl acetate, methanolic and water extract of the leaves of *Acacia saligna* (Wendl H. L.**)** which is used traditionally in the treatment of various diseases. They were assayed against Gram-positive bacteria (*Staphylococcus aureus, Staphylococcus pyogens, Bacillus cereus, Bacillus subtilis*), Gram-negative bacteria (*Escherichia coli*), fungi and yeast (*Candida albicans*) using the diffusion and serial methods. The susceptibility of the microorganisms to the extracts of these plants was compared with each other and with selected antibiotics whereby the ethyl acetate extract being the most effective. The active ethyl acetate extract was phytochemicaly studied as well for their polyphenolic constituents, where they afforded sixteen polyphenolic compounds among which two different phenolic acids, two catechins and seven flavonoids were isolated for the first time from *A. saligna*. The structure of all the isolated compounds was elucidated on the basis of spectral analysis.

Keywords: *Acacia saligna* leaves, *Fabaceae*, Phenolic compounds and Antimicrobial assay.

Acacia is a genus of shrubs and trees of Gondwanian origin belonging to the Subfamily *Mimosoideae* of the Pea Family (Family *Fabaceae*), there are roughly 1300 species of *Acacia* worldwide, 13 species of *Acacia* in Egypt are known to grow wild in the Eastern Desert, Sinai Peninsula and the Oases of the Western Desert[(1)]. A number of secondary metabolites have been reported from various *Acacia* species including hydrolyzable tannins, condensed tannins and flavonoids[(2)]. In a review, discussed by Harborne and Williams[(3)], many studies have suggested that flavonoids exhibit antioxidant, anti-inflamatory, antimicrobial, vascular activities and others medicinal properties.

The identification of *Acacia* species is difficult and their taxonomic relationships are not clear[(4)] which led to lack of information given in the current literature about the phenolic constituents of the leaves of *Acacia saligna* and as many species of *Acacia* have been described to have astringent, aphroldisia,

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antioxidant, antidiabetic, anti-ulcer and antisyphilic properties[5-8], we therefore subjected this species for a comprehensive investigation due to its economic importance and the lack of literature concerning their constituents. Phytochemical investigation resulted in the isolation of gallic acid, methyl gallate, catechin, 7-*O*-galloylcatechin, quercetin-3-*O*-α-L-rhamnopyranoside, myricetin-3-*O*-α-L-rhamnopyranoside, myricetin-3-*O*-α-L-arabinopyranoside, quercetin-3-*O*-α-L-arabinopyranoside, myricetin-3-*O*-β-D-glucopyranoside, apigenin-7-*O*-β-D-glucopyranoside, luteolin- 7-*O*-β-D-glucopyranoside, apigenin, luteolin, quercetin and myricetin from its ethyl acetate extract. The structure of the isolated compounds was elucidated on the basis of spectral analysis as UV, MS, ^{1}H NMR, ^{13}C NMR.

Experimental

General

^{1}H and ^{13}C NMR: were measured relative to DMSO-d_6 at 500 and 75 MHz, respectively on Bruker AMS spectrometer-500 MHz where values are given in ppm; ESI-MS: on a Finnigan MAT 4600 spectrometer; UV: Shimadzu spectrophotometer model UV-240; CC: Polyamide 6S and Sephadex LH-20; PC: carried out on Whatman No.1 and 3MM using solvent systems (1) BAW (*n*-BuOH: HOAc: H_2O, 4:1:5, upper layer); (2) H_2O; (3) 15 % AcOH (AcOH :H_2O).

Plant material

Acacia saligna leaves were collected from the Orman Botanical Garden, Giza, in September 2005. Identification of the plant was confirmed by the Department of Flora, Agricultural Museum, Ministry of Agriculture and Herbarium of the Department of Botany, Faculty of Science, Cairo University. Voucher specimen (number: As 30) was kept in Herbarium, National Research Centre, Cairo, Egypt.

Extraction and isolation

The comminuted air-dried leaves material (1.5 kg) was defatted with chloroform (2x3L) and exhaustively extracted with MeOH:H_2O (7:3) under reflux over a boiling water bath for 10 hr. The extract was then filtered and the solvent was removed in vacuo at ≈40^{o}C. The dried extract was then fractionated by successive maceration with ethyl acetate, methanol and water in a soxhlet extractor at room temperature till exhaustion (5L each). The extracts were concentrated to dryness under reduced pressure and controlled temperature ≈40°C to yield the crude extract of each. The respective yields of the ethyl acetate, methanol and water extracts (w/w) were found to be 150, 75 and 100g, respectively.

A sample (100 g) of ethyl acetate extract was applied to the top of a column (150 x 3.5 cm) containing 500 gm of polyamide 6S. Gradient elution started with water followed by H_2O/EtOH mixtures of decreasing polarities at a flow rate 1ml/minute was then carried out. Eight fractions were then arised, individually collected, dried in vacuo at ≈ 40^{o}C and subjected to detailed investigations. Application of 100 mg of the material of fraction II to Sephadex LH-20 column

(80 x 1.5 cm) and elution with 10 % EtOH led to the desorption of two successive bands. These bands were concentrated to afford two pure samples of gallic acid (A_1) (27 mg) and methyl gallate (A_2) (30 mg). 100 mg of fraction III were carefully applied on a column (80 x 2 cm) containing ≈ 60 gm of Polyamide 6S. Elution with 50 % EtOH and inspection of the chromatographic process under UV light led to desorption of two bands which migrated successively along the column as fluorescent bands of characteristic color. Crystallization of the received crude samples of subfraction afforded a 25 mg of catechin (A_3) and 29 mg of 7-*O*-galloycatechin (A_4). The fraction IV (170 mg) obtained after the evaporation of the eluent (60% EtOH) was applied on a polyamide column and eluted by the solvent system n-Butanol-water saturated which gave rise to two successive sub-fractions. The first sub-fraction was further applied on smaller Sephadex LH-20 column and eluted by 40 % EtOH to afford the purified samples of quercetin-3-*O*-α-L-rhamnopyranoside (A_5) (30 mg) and myricetin-3-*O*-α-L-rhamnopyranoside (A_6) (34 mg) while, the second sub-fraction, afforded a pure sample of myricetin-3-*O*-α-L-arabinopyranoside (A_7) (28 mg). The column chromatography technique was applied for the resolution of (142 mg) of the material of fraction V. The process was carried out on polyamide column, using n-Butanol-water saturated as the mobile phase led to the separation of two distinct sub-fractions of dark brown color under UV light, which were individually collected and dried under vacuo. The first sub-fraction afforded a pure sample of quercetin-3-*O*-β-D-glucopyranoside (A_8) (21 mg). Application of the obtained material of the second sub-fraction on Sephadex LH-20 column and eluted with 60% EtOH, led to the separation of pure samples of quercetin-3-*O*-α-L-arabinopyranoside (A_9) (30 mg) and myricetin-3-*O*-β-D-glucopyranoside (A_{10}) (29 mg). 90 mg of fraction VI were applied on a Sephadex LH-20 (60 x 2 cm) and eluted with 70 % EtOH. Two successive major bands were individually desorbed from the column. Removal of the eluent in vacuo at ≈ 40°C and crystallization of the received dry material from 70 % EtOH, gave pure samples of each of compounds; apigenin-7-*O*-β-D-glucopyranoside (A_{11}) (30 mg) and Luteolin-7-*O*-β-D-glucopyranoside (A_{12}) (27 mg). Sephadex LH-20 column chromatography of the material of fraction VII (82.5 mg) using the solvent system n-BUOH-water saturated, was carried out whereby two major bands were detected under UV light. Both bands individually collected, dried and crystallized to give pure samples of apigenin (A_{13}) (30 mg) and Luteolin (A_{14}) (29 mg). Finally, a cellulose column chromatography of the material of fraction VIII (97.5 mg) using ethanol as eluent, was carried out whereby two major bands were detected under UV light. Both bands were individually collected, dried and crystallized to give pure samples of quercetin (A_{15}) (25 mg) and myricetin (A_{16}) (23 mg).

Characterisation of some natural compounds

7-O-Galloyl-Catechin, (A_4)

Colourless needles, m.p.: 165-168°C; R_f-values (x100): 53 (HOAc-15), 67 (BAW); UV λ_{max} nm (MeOH): 230 sh, 278; ^{1}H NMR δ (ppm): 7.27 (*s*, galloyl), 6.95 (*d*, *J* =1.8 Hz, H-2'), 6.85 (*d*, *J* = 7.8 Hz, H-5'), 6.84 (*dd*, *J* = 7.8 Hz, and *J* = 1.8 Hz, H-6'), 6.38 (*d*, *J* = 2.1 Hz, H-6), 6.28 (*d*, *J* = 2.1 Hz, H-8), 4.71 (*d*, *J* = 7.5 Hz, H-2), 3.02 (*m*, H-3), 2.70 (eq., *dd*, *J* = 16.5 Hz and *J* = 8.1 Hz, H-4) or 2.65

(ax., *dd*, *J* = 16.5 Hz and *J* = 8.1 Hz, H-4); ^{13}C NMR δ (ppm): 82.21 (C-2), 67.23 (C-3), 28.14 (C-4), 156.31 (C-5), 101.47 (C-6), 150.82 (C-7), 101.19 (C-8), 156.00 (C-9), 106.25 (C-10), 131.22 (C-1'), 114.53 (C-2'),145.15 (C-3'), 145.04 (C-4'), 115.13 (C-5'), 119.38 (C-5'), 120.43 (galloyl C-1), 109.68 (galloyl C-2), 145.59 (galloyl C-3), 138.76 (galloyl C-4), 145.59 (galloyl C-5), 109.68 (galloyl C-6), 164.63 (-COO-).

Quercetin-3-O-α-L-rhamnopyranoside, (A_5)

R_f-values (x100):20 (H_2O), 50 (HOAc-15), 71 (BAW); UV λ_{max} nm (MeOH): 253, 263sh, 344; + NaOMe: 272, 322sh, 372; + NaOAc: 260, 300sh, 367; + NaOAc/H_3BO_3: 272, 382; +$AlCl_3$: 272, 304sh, 333sh, 430; + $AlCl_3$/HCl: 272, 303sh, 353, 401; m.p.: 186^{o}C; Negative ESI-Mass: $[M-H]^-$ m/z 447.1; ^{1}H NMR: δ (ppm) 7.33 (*d*, *J* = 2 Hz, H-2'), 7.28 (*dd*, *J* = 2 Hz, and *J* = 8.5 Hz, H-6'), 6.90 (*d*, *J* = 8.5 Hz, H-5'), 6.42 (*d*, *J* = 2 Hz, H-8), 6.23 (*d*, *J* = 2 Hz, H-6); 5.29 (*d*, *J* = 1.41 Hz, H-1" of rhamnose), 3.16-3.56 (*m*, rest of rhamnose protons), 0.85 (*d*,J = 6.07 Hz, CH_3 of rhamnose); ^{13}C NMR: δ (ppm) 157.34 (C-2), 134.27 (C-3), 177.79 (C-4), 161.35 (C-5), 98.78 (C-6), 164.34 (C-7), 93.70 (C-8), 156.86 (C-9), 104.16 (C-10), 121.17 (C-1'), 115.52 (C-2'), 145.25 (C-3'), 148.50 (C-4'), 115.72 (C-5'), 120.80 (C-6'); 101.87 (C-1"), 70.43 (C-2"), 70.63 (C-3"), 71.25 (C-4"), 70.11 (C-5"), 17.54 (C-6").

Myricetin-3-O-α-L-rhamnopyranoside, (A_6)

R_f-values (x100) :15 (H_2O), 45 (HOAc-15), 72 (BAW); m.p.: 199-200^{o}C; UV λ_{max} nm (MeOH): 250 sh, 262, 298 sh, 349; + NaOMe: 272, 324, 392; + NaOAc : 270, 318, 366; +NaOAc/H_3BO_3: 260, 300, 374; +$AlCl_3$: 272, 312, 428; + $AlCl_3$/HCl: 270, 310, 404; Negative ESI-Mass: $[M-H]^-$ = m/z 463.1; ^{1}H NMR: δ (ppm) 6.90 (*s*, H-2' and H-6'), 6.38 (*d*, *J* = 1.92 Hz, H-8), 6.21 (*d*, *J* = 1.92 Hz, H6); 5.21 (*d*, *J* =1.44 Hz, H-1"), 3.15–4.00 (*m*, protons of rhamnose), 0.85 (*d*, *J*= 6.15 Hz, CH_3–rhamnose); ^{13}C NMR: δ (ppm) 157.49 (C-2), 134.29 (C-3), 177.91 (C-4), 161.31 (C-5), 98.67 (C-6), 164.23 (C-7), 93.53 (C-8), 156.24 (C-9), 104.03 (C-10), 119.63 (C-1'), 107.92 (C-2'), 145.78 (C-3'), 136.46 (C-4'), 145.78 (C-5'), 107.92 (C-6'); 101.94 (C-1"), 70.40 (C-2"), 70.55 (C-3"), 71.29 (C-4"), 70.23 (C-5"), 17.52 (C-6").

Myricetin-3-O-α-L-arabinopyranoside, (A_7)

R_f-values (x100): 07 (H_2O), 24 (HOAc-15), 64 (BAW); UV λ_{max} (nm) (MeOH): 265, 360; +NaOMe: 275, (dec.); +NaOAc: 270, 385; +NaOAc /H_3BO_3: 260, 381; +$AlCl_3$: 270, 402; Negative ESI-Mass: $[M-H]^-$= m/z 449.1; ^{1}H NMR: δ (ppm) 7.15 (*s*, H-2' and H-6'), 6.38 (*d*, *J* = 1.8 Hz, H-8), 6.19 (*d*, *J* = 1.8 Hz, H-6); 5.26 (*d*, *J* = 5.13 Hz, H-1" of arabinose), 3.12-3.63 (*m*, rest of arabinose protons); ^{13}C NMR: δ (ppm) 156.59 (C-2), 134.13 (C-3), 177.77 (C-4), 161.44 (C-5), 99.05 (C-6), 164.50 (C-7), 93.88 (C-8), 156.76 (C-9), 104.20 (C-10), 120.08 (C-1'), 108.74 (C-2'), 145.78 (C-3'), 137.09 (C-4'), 145.78 (C-5'), 108.74 (C-6'); 102.03 (C-1"), 72.13 (C-2"), 71.03 (C- 3"), 66.75 (C- 4"), 65.09 (C-5").

Quercetin-3-O-α-L-arabinopyranoside, (A_9)

R_f-values (x100): 07 (H_2O), 30 (HOAc-15), 70 (BAW); m.p.: 218 ^oC; UV λ_{max} (nm) (MeOH): 260, 300^{sh}, 362; + NaOMe: 277, 333, 412; + NaOAc: 276, 325, 388; + NaOAc /H_3BO_3: 262, 300sh, 382; +$AlCl_3$: 276, 303, 338sh, 438; + $AlCl_3$/HCl: 276, 303sh, 367, 405; Negative FAB-Mass: $[M-H]^-$ = m/z 433; ^{1}H NMR: δ (ppm) 7.64 (*d*, *J* = 2.1 Hz, H-2'), 7.49 (*dd*, *J* = 2.1 Hz and *J* = 8.46 Hz, H-6'), 6.82 (*d*, *J* = 8.46 Hz, H-5'), 6.39 (*d*, *J* = 1.8 Hz, H-8), 6.18 (*d*, *J* = 1.8 Hz, H-6); 5.26 (*d*, *J* = 5.13 Hz, H-1" of arabinose), 3.12-3.63 (*m*, rest of arabinose protons); ^{13}C NMR: δ (ppm) 156.63 (C-2), 134.05 (C-3), 177.84 (C-4), 161.53 (C-5), 99.05 (C-6), 164.59 (C-7), 93.90 (C-8), 156.63 (C-9), 104.22 (C-10), 121.22 (C-1'), 115.71 (C-2'), 145.31 (C-3'), 148.94 (C-4'), 116.10 (C-5'), 122.36 (C-6'); 101.76 (C-1"), 71.07 (C-2"), 72.00 (C-3"), 66.46 (C-4"), 64.70 (C-5").

Myricetin-3-O-β-D-glucopyranoside, (A_{10})

R_f-values (x100): 05 (H_2O), 19 (HOAc-15), 47 (BAW); UV λ_{max} (nm) (MeOH): 258, 365; + NaOMe: 266, 395; + NaOAc: 266, 340. (dec.); + NaOAc/H_3BO_3: 257, 305sh, 395; +$AlCl_3$: 269, 310sh, 405; + $AlCl_3$/HCl: 270, 360sh, 400; ^{1}H NMR: δ (ppm): 7.28 (*s*, H-2' and H-6'), 6.44 (*d*, *J* =1.8 Hz, H-8), 6.25 (*d*, *J* = 1.8 Hz, H-6); 5.40 (*d*, *J* = 7.77 Hz, H-1" of glucose), 3.36-3.73 (*m*, rest of glucose protons). ^{13}C-NMR δ (ppm): 156.47 (C-2), 133.99 (C-3), 177.64 (C-4), 161.44 (C-5), 98.91 (C-6), 164.36 (C-7), 93.62 (C-8), 156.36 (C-9), 104.14 (C-10), 120.23 (C-1'), 108.80 (C-2'), 145.58 (C-3'), 136.89 (C-4'), 145.58 (C-5'), 108.80 (C-6').

Antimicrobial activity assay

Diffusion method

0.1 ml (10 mg) of samples extract was spotted on filter paper disc (whatman 3 MM, 8mm diameter) and transferred to the surface of agar plates freshly inoculated with test organisms. The diameter of the clear zones of inhibition was determined after 24 and 48 hr of incubation at 30^{o}C for bacteria, yeast and fungi, respectively[9]. Disks (8.0 mm in diameter) impregnated with each extract at a concentration of 10.0 mg/ml were placed on the inoculated plates. Similarly, each plate carried antibiotic disks (8.0 mm in diameter) of 20 *m*g/ml *Ciprofl-oxacin* (for bacteria), and *Fungican fluconazol* (for fungi) were also used as positive controls.

Serial dilution method

Sets of test tubes, each containing 2 ml of nutrient broth were prepared and sterilized. Twenty milligrams of plant extracts in 1 ml sterilized water were aseptically transferred to the first tube and after thorough shaking; one ml of the mixture was aseptically transferred to a second tube. Such process was repeated from the second till the last tube of the series. Each tube was then inoculated with one drop of freshly prepared spoor suspension of the test organism and the whole series were incubated for 24 hr for bacteria and 48 hr for yeast and fungi at 30^{o}C.

The tubes showing no growth of the test organism (no turbid tubes) were counted and the minimum dilution of the fermented broth, which caused the

inhibition of the test organism, was calculated[10]. All tests were performed in duplicate and the antimicrobial activity was expressed as the mean of inhibition zone diameters in mm produced by the sample.

Determination of MIC

Minimum inhibitory concentration (MIC) is defined as the lowest concentration of the antimicrobial agent in the liquid medium resulting in complete inhibition of visible growth. The lowest concentration of the tube or plate that did not show any visible growth by microscopic evaluation was considered as the MIC. Determination of MIC of samples was carried out by Serial dilution method[9].

Results and Discussion

The Antimicrobial profile of *Acacia saligna* leaves occurred using the diffusion and serial methods whereby it indicated that the crude extracts containing most of polyphenols (ethyl acetate extract) has significant activity against various strains of bacteria (gram positive and gram negative), fungi and yeast.

Plant phenolics represent a huge number of natural compounds that are of great interest and importance nowadays due to their various pharmacological activities. The leaves of *Acacia saligna* were air-dried, grounded and the phenolic constituents were extracted with 70% aqueous methanol for several times. This crude extract was further fractionated using ethyl acetate, methanol and water.

As there are growing interests in using natural antimicrobial compounds, especially plant extracts[11]. The biological assay of the ethyl acetate extract (EA) together with the methanolic extract (M) and water extract (H) showed anti-microbial activity as shown in Table 1 whereby the Ciprofloxacin was used as antibiotic standard drug for bacteria and yeast while, fungican fluconazol was used as antibiotic standard for mould.

Among the three extracts, it was found that the ethyl acetate extract (EA) exhibited moderate activity against *Staphylococcus aureus, Streptococcus pyogens* and *Bacillus cereus* (20 mm diameter) and low activity against *Bacillus subtilis* (16 mm diameter) compared with that exerted by antibiotics (MIC, 1.020–3.05 mg/ml), and it was found inactive against *Bacillus subtilis*. Besides, the three extracts exhibited an anti-candidal activity.

The antifungal activity of the three extracts was studied against seven pathogenic fungi. In contrast, the inhibition zones of the three extracts were almost zero or below 8 mm, so that they were not active to all tested fungi microorganisms. Though no activity was observed against fungi, these results may provide scientific support for some uses of the plant in traditional medicine.

TABLE 1. Antimicrobial activities (mm in diameter) of samples against microbes by diffusion method.

Test organism	Zone of inhibition (mm diameter)			Control Antibiotic standard (Ciprofloxacin) (Fungican fluconazol) 20mg/ml
	EA ethyl acetate extract	M Methyl alcohol extract	H water extract (residue)	
Gram-negative Bacteria				
1- *Escherichia coli* (NRRLB-3704)	0.0	0.0	0.0	40.0
Gram-positive Bacteria				
1- *Staphylococcus aureus*	15	0.0	0.0	44.0
2- *Streptococcus pyogens*	16	0.0	0.0	50.0
3- *Bacillus cereus*	16	0.0	0.0	36.0
4- *Bacillus subtilis* (NRRLB-941)	14	0.0	0.0	36.0
Yeast				
1-*Candida albicans* (NRRLY-12983)	21	17	15	50.0
2-*Sacchromyces cerevisiae* (NRRLY-12632)	±	±	0.0	60.0
Fungi				
1- *Aspergillus niger* (NRRLA-326)	0.0	0.0	0.0	30.0
2- *Aspergillus flavos* (NRRLA-1957)	0.0	0.0	0.0	36.0
3- *Macrophomina Phaseali* (NRRLA-62743)	0.0	0.0	0.0	35.0
4- *Fusarium oxysporium* (NRRLA-2014)	0.0	0.0	0.0	35.0
5- *Vas infectum*	0.0	0.0	0.0	30.0
6- *Diplodia oryzae*	0.0	0.0	0.0	40.0
7- *Tricoderma viride* (NRRLA-63065)	0.0	0.0	0.0	35.0

Finally, MIC determination was carried out to calculate the lowest concentration of the antimicrobial agent for inhibition of different microbes, especially *Staphylococcus aureus*, *Streptococcus pyogens*, *Bacillus cereus*, *Bacillus subtilis* and *Candida albicans*. The MIC values of the two extracts (EA and M) are generally within the range of 0.1- 0.4 μg/ml against evaluated strains, as shown in Table 2. The ethyl acetate extract (1) was found to be more effective against *Streptococcus pyogens* and *Candida albicans*, MIC values of 0.41μg/ml and 0.46μg/ml, respectively were attained.

For these reasons, the ethyl acetae extract was subjected to qualitative tests which indicated the presence of compounds of strong phenolic and flavonoid nature.

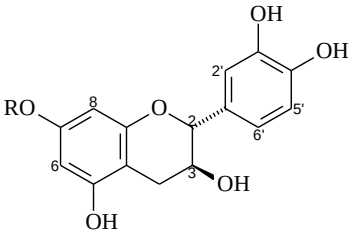
TABLE 2. MIC (minimum inhibition concentration) of two extracts against different microbes.

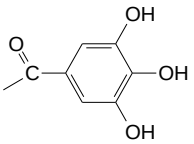
Test Organism	MIC(µg/ml)	
	Extract EA	Extract M
Bacteria		
1- *Staphylococcus aureus*	0.41	-
2- *Streptococcus pyogens*	0.46	-
3- *Bacillus cereus*	0.41	-
4- *Bacillus subtilis (* NRRLB-941)	0.14	-
Yeast		
1- *Candida albicans* (NRRLY-12983)	3.7	0.41
2- *Sacchromyces cerevisiae* (NRRLY-12632)	-	-

The phytochemical investigation of the ethyl acetate extract was then occurred by applying onto Polyamide 6S column chromatography and eluted with water and water/ethanol mixtures with gradual increase in concentration. A series of further fractionation on Sephadex LH-20 and polyamide 6S columns afforded the isolation of sixteen phenolic compounds. The structure of the isolated compounds was established through chromatography, as well as conventional chemical and spectroscopic methods of analysis (*e.g.* UV, ESI, 1/2 D NMR) where among them; catechin, 7-*O*-galloylcatechin, myricetin-3-*O*-α-L-arabinopyranoside, quercetin-3-*O*-β-D-glucopyranoside, quercetin-3-*O*-α-L-arabinopyranoside, apigenin-7-*O*-β-D-glucopyranoside and luteolin-7-*O*-β-D-glucopyranoside as shown in Fig. 1, were isolated for the first time from *Acacia saligna* leaves.

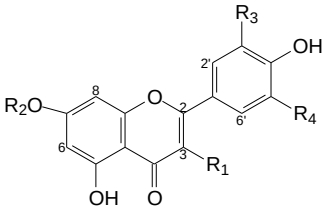
Compound (A_4) was strongly positive (a dark blue color) to the ferric chloride reagent and exhibited R_f-values and UV spectral data similar to those of Catechin derivatives. The ^{1}H-NMR spectrum of (A_4) was similar to that of Catechin except for the additional signal at δ 7.27 (2H, *s*), due to the presence of a galloyl group. The proton resonances of H-6 and H-8 appeared at δ 6.38 and 6.28 ppm more downfield than observed in Catechin[12], besides the ^{13}C NMR spectrum ensured esterification at position C-7 followed from the upfield shift of C-7 to δ 150.83, while the recognizable downfield shift at C-6 and C-8 resonances to 101.47 and 101.19, respectively. Thus, compound (A_4) was identified as 7-*O*-Galloyl-Catechin[13]. Compound (A_5) obtained as yellow amorphous powder of chromatographic properties and color reactions similar to those reported for quercetin 3-*O*-glycoside. This assumption was primarily supported by UV spectral data of (A_5) in methanol and different diagnostic shift reagents[14]. Complete acid hydrolysis of (A_5) yielded quercetin and rhamnose, identified by CoPC with authentic samples in different solvents. Compound (A_5) exhibited a Molecular weight of 448 in Negative ESI-MS spectrum analysis which showed $[M-H]^-$ at m/z: 447.1. Confirmation of the structure of (A_5) was achieved through ^{1}H NMR spectroscopic analysis whereby the spectrum revealed the characteristic

pattern of quercetin proton resonances besides the anomeric proton resonance appearing as doublet signal of coupling constant (J =1.41 Hz), at δ 5.29 ppm assignable to the rhamnoside proton H-1"; with the methyl rhamnose proton resonance revealed at δ 0.85 ppm (d, J = 6.07 Hz). Finally, from the above data conpound (A_5) is quercetin 3-O α-L rhamnopyranside.





A_3: R= H **A_4**: R=



A_7: R_1= *O*-α-L-arabinopyranoside; R_2= H R_3= OH; R_4= OH
A_8: R_1= *O*- β-D-glucopyranoside; R_2= H R_3= OH; R_4= H
A_9: R_1= *O*-α-L-arabinopyranoside; R_2= H R_3= OH; R_4= H
A_{11}: R_1=H R_2= *O*-β-D-glucopyranoside; R_3= R_4= H
A_{12}: R_1=H R_2= *O*-β-D-glucopyranoside R_3= OH; R_4= H

Fig. 1.

The pure yellow amorphous material of (A_6) showed chromatographic and UV spectral properties similar to those of myricetin 3-*O*-glycoside. Complete acid hydrolysis of (A_6) yielded myricetin and rhamnose, identified by CoPC with authentic samples in different solvents. Compound (A_6) exhibited a Molecular weight of 464 in Negative ESI-MS spectrum analysis which showed $[M-H]^-$ at m/z: 463.1. In order to determine the final structure of (A_6), it was subjected to ^{1}H NMR spectroscopic analysis. The proton resonances in the recorded spectrum (DMSO-d_6) revealed an anomeric proton resonance, appearing as doublet signal of coupling constant (J = 1.44 Hz), at δ 5.21 ppm assignable to the rhamnoside proton H-1". While, the methyl rhamnose proton resonance revealed at δ 0.85 ppm (d, J = 6.15 Hz). Finally, the confirmation of the identity was achieved

through ^{13}C NMR spectroscopic analysis which revealed the presence of a rhamnose moiety followed from the signal of the methyl carbon at δ 17.52 ppm. The signal of the C-3 carbon of the flavonol moiety at δ 134.29 ppm showed the direct bonding between aglycone (myricetin) and sugar (rhamnose) moieties at the flavonol C-3 position. Thus compound (A_6) was identified to be Myricetin-3-*O*-α-L-rhamnopyranoside[15]. Also, compound (A_7) exhibited R_f-values and UV spectral data similar to those of myricetin 3-*O*-glycoside where complete acid hydrolysis of (A_7) yielded myricetin and arabinose which were identified by CoPC using authentic markers. This was supported by Negative ESI-MS analysis which revealed $[M-H]^-$ peak at m/z: 449.1 as well as ^1H NMR spectrum which gave data identical to those reported for myricetin 3-arabinose[15]. The pyranose form of sugar arabinose showed a doublet signal of δ 5.26 ppm of the anomeric sugar proton of coupling constant 5.13 Hz. This analysis confirmed the characteristic pattern of myricetin, including δ 6.38 ppm (*d*, *J* =1.8Hz) and δ 6.19 ppm (*d*, *J* =1.8Hz) which correspond to H-8 and H-6 respectively. Finally compound (A_7) was identified to be Myricetin-3-*O*-α-L-arabinopyranoside[15] .

Compound (A_9) appeared on PC as dark brown spot under UV light, turning yellow when fumed with ammonia vapor and bright yellow with $AlCl_3$ reagent. (A_9) showed R_f-values and UV spectral data similar to those of quercetin 3-glycoside[14]. Complete acid hydrolysis of (A_9) yield quercetin and arabinose which were identified by CoPC using authentic markers. This was supported by Negative FAB-MS analysis which revealed $[M-H]^-$ ion peak at m/z: 433. The structure was further confirmed through ^1H NMR spectrum which gave data identical to those reported for quercetin 3-arabinoside[16]. The pyranose form of sugar arabinose showed a doublet signal of δ 5.26 ppm of the anomeric sugar proton of coupling constant 5.13 Hz. This analysis confirmed the characteristic pattern of quercetin, including δ 6.39 ppm (*d*, *J* =1.86Hz) and δ 6.18 ppm (*d*, *J* =1.83Hz) which correspond to H-8 and H-6 respectively. Final confirmation of compound (A_9) achieved by ^{13}C NMR, spectrum where the anomeric carbon of sugar appeared at δ 101.76 ppm and the other sugar carbon signals appeared at their proper positions as reported for Quercetin-3-*O*-α-L-arabinopyranoside.

The yellow amorphous powder of compound (A_{10}) was preliminary identified as a flavonol 3-glycoside; whose R_f-values and UV spectral data was similar to those reported for myricetin 3-*O*-glycoside[17]. Complete acid hydrolysis of (A_{10}) gave myricetin and glucose which were identified by CoPC using authentic markers. Both the ^1H NMR and ^{13}C NMR spectra confirmed the structure of (A_{10}) to be myricetin 3-*O*-glucoside, where the presence of β-glucopyranoside moiety in the compound of (A_{10}) followed from δ ppm of sugar at 5.40 (*d*, *J* = 7.77 Hz, H-1" of glucose), 3.36-3.73 (m, rest of glucose protons) and the anomeric carbon resonance at δ 102.30 ppm. Resonances of the protons and carbons of the flavonoid moiety were assigned by comparison with the corresponding signals in the published spectrum of Myricetin-3-*O*-β-D-glucopyranoside.

We conclude from this study that, the chemical constituents of plant especially phenolic compounds, flavonoids and other compounds present in the plant may be involved in the observed antimicrobial effect of the plant's extract[18,19].

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دراسات فيتوكيميائية وميكروبية على أوراق نبات أكاسيا سالجينا

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تهدف هذه الدراسة الى التعرف على المكونات الكيميائية وخاصة عديدة الفينول من نبات مصرى ينمو محليا وهو نبات اكاسيا سالجنا وينتمى الى العائلة البقولية، ودراسة تطبيقاته خاصة كمضادات للميكروبات. وقد تم اختيار هذا النبات لما له من اهمية اقتصادية و لقلة ماورد عنه فى المراجع العلمية المتداولة.

تم تجميع الاوراق وتجفيفها وطحنها حيث تم استخلاص المكونات الفينولية باستخدام ٧٠٪ كحول ميثلى ، وبعد تجفيف هذا المستخلص تم استخلاص المادة المتبقية جزئياً بأستخدام المذيبات المختلفة كالأثيل أستيات والميثنول والماء على التوالي. أظهرت خلاصة الأثيل أستيات الناتجة انها اكثر خلاصة مثبطة للميكروبات ولذلك تم تجزئتها باستعمال العمود الكروماتوجرافى المحتوى على البولى اميد ٦ اس كمادة ادمصاص واستعمال الماء ثم خليط من الكحول الايثلى والماء حيث امكن الحصول على ثمانى اجزاء من خلاصة النبات حيث اظهرت النتائج احتواء الجزء الاول من خلاصة النبات على سكريات حرة مثل جلوكوزو اربينوزو ورامنوز. ولقد تم التعرف على مركبين فينول وهم حمض الجاليك ، استرجالات الميثيل من الجزء الثانى من الخلاصة. اما فى الجزء الثالث من خلاصة النبات ، تم التعرف على كاتشين ، كاتشين-٧-جالات. وتم فصل وتعريف كويرستين-٣-رامنوزايد، ميريسيتين- ٣-رامنوزايد وميريسيتين-٣-اربينوزايدو من الجزء الرابع من خلاصة النبات وهناك ثلاثة مركبات فلافونية وهم كويرستين-٣-جلوكوزايد، كويرستين - ٣- اربينوزايد وميريسيتين -٣-جلوكوزايد تم فصلهم من الجزء الخامس. ولقد تم التعرف على مركبين فينولين من الجزء السادس وهم الابيجنيين-٧-جلوكوزايد والاليتيولين -٧- جلوكوزايد وفى النهاية ، تم تعريف وفصل الابيجنيين ، الاليتيولين ، كويرستين وميريسيتين من الجزئين السابع والثامن من الخلاصة.

وتم التعرف على المركبات المفصولة بعد تنقيتها واثبات تركيبها الكيميائى بالطرق الكيميائية المختلفة مثل طريقة التميوء فى الوسط الحمضى ، وباستخدام القياسات الطيفية المتعددة منها UV، MS ، ^{1}H-NMR، ^{13}C-NMR بجانب الخواص الكروماتوجرافية.

وأجريت دراسات للنشاط البيولوجى على الخلاصات الناتجة المختلفة مثل الأيثيلى أستيات ، الميثنول والماء حيث أظهرت خلاصة الأثيل أستيات الناتجة انها اكثر خلاصة مثبطة للميكروبات.