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ISOLATION AND CHARACTERIZATION OF THE BIOACTIVE PHENOLIC COMPOUNDS FROM *MORUS ALBA* LEAVES GROWING IN EGYPT

Ghareeb, M.A.[1*]; Ahmed, W.S.[1,2]; Refahy, L.A.[1]; Abdou, A.M.[1]; Hamed M.M.[1]; Abdel-Aziz, M.S.[3]

[1]Medicinal Chemistry Department, Theodor Bilharz Research Institute, Giza, 12411, Egypt.
[2]Department of Chemistry, College of Science and Arts, Sajir, Shaqra University, Kingdom of Saudi Arabia
[3]Microbial Chemistry Department, National Research Center, Dokki, Giza, Egypt.

[*]m.ghareeb@tbri.gov.eg

Abstract

Morus alba Linn. (Whit Mulberry) is known for its medicinal and nutritive benefits. In the current study, different extracts of *Morus alba* leaves were investigated for their antioxidant activities which was evaluated via phosphomolybdenum & reducing power, and dot-blot-DPPH staining assays, as well as their total phenolic contents (TPCs). Also, their antimicrobial activity was evaluated via disc diffusion technique toward four human pathogenic strains including; *Staphylococcus aureus*, *Pseudomonas aeruginosa, Candida albicans*, and *Aspergillus niger*. The results revealed that TPCs were ranged from 26.53 to 387.50 (mg gallic acid equivalent GAE/ g dry extract), the total antioxidant capacity values were ranged from 47.06 to 430.50 (mg AAE /g dry extract), whereas the reducing power activities (OD values) were ranged from 0.102 to 0.659, compared to ascorbic acid of OD= 0.915. Moreover, the antimicrobial results revealed that the EtOAc extract was the strongest one against three strains with inhibition zones between 16-21 mm, followed by *n*-BuOH with inhibition zones between 10-18 mm. Furthermore, the chromatographic isolation of the *n*-BuOH upon polyamide column resulted in the isolation of six compounds, their structures were determined on the basis of 1D (^{1}H,^{13}C-NMR), and IR spectroscopy as; sinapic acid-*O*-glucoside (1), 3,4,5-trimethyoxyphenol-1-*O*-β-D-rhamnopyranoside (2), chlorogenic acid (3), gallic acid (4), rutin (5), and quercetin. Furthermore, the free radical antioxidant activities (DPPH) SC_{50} values of the compounds 1-6, were found to be 7.45, 11.65, 6.55, 5.20, 9.0, and 3.45 µg/ml respectively, relative to ascorbic acid as positive standard (7.80 µg/ml), whereas that of the total antioxidant capacity values were 560.40, 410.25, 570.15, 600, 535.50, and 630.23 (mg AAE/g compound) respectively. This is the first report of the compounds 1, 2 being isolated from the leaves of *Morus alba* growing in Egypt.

Key words: *Morus alba* L., antioxidant, antimicrobial, phenolics, sinapic acid-*O*-glucoside.

Introduction

The mulberry belongs to the genus Morus (Family Moraceae), including approximately 24 Morus species and one subspecies, with at least 100 known varieties [1]. It is distributed in different areas including North America, South America, Africa, Europe, and Asia [2]. In folk medicine, different parts of *Morus alba* L. are traditionally used for the treatment of several kinds of diseases [3], and to treat fever, hypertension, arthritis, liver disorders and urinary system problems [4]. Furthermore, several studies have been reported on the bioactivity, and health benefits of mulberry [5-11]. In addition, previous reports documented that the different parts of *M. alba* possess remarkable biological activities *i.e.*, antioxidant [12-14], antimicrobial [15], anticancer [16, 17], hypolipidemic [18, 19], macrophage activating [20], neuroprotective [21], antidiabetic [22], and antihypertensive [23]. Moreover, numerous bioactive compounds have been isolated from different parts of *M. alba* such as; kuwanon-G, and 1-deoxynojirimycin with antibacterial activity against *Streptococcus mutans* [24, 25], quercetin 3-(6-Malonylglucoside) with antioxidant & antiatherogenic activities [26], deoxynojirimycin & fagomine with hypoglycemic activity [27], albanol A with anticancer activity [27], (2R,3R,4R)-2-hydroxymethyl-3,4-dihydroxypyrrolidine-N propionamide, 4-*O*-R-D-galactopyranosyl-calystegine B2 & 3β,6β- dihydroxynortropane with glycosidase inhibition effect [28], leachianone G with antiviral activity [29], and moralbosteroid with anxiolytic activity [30]. On the other hand, the Egyptian researchers reported on the phytochemical and biological investigations of the Egyptian *Morus* species *i.e.*, Singab et al. (2005) reported the isolation of morusin, cyclomorusin, neocyclomorusin, kuwanon E, 2-arylbenzofuran, moracin M, betulinic acid, and methyl ursolate from the root bark [31], also the antioxidant activity was reported [32, 33]. Therefore, the aim of the current study is to isolate and characterize the bioactive secondary metabolites from the Egyptian *Morus alba* leaves together with the evaluation their antioxidant, and antimicrobial activities.

Methods

Plant material

The leaves of *Morus alba* L. (Family Moraceae), were collected during May-June 2014, El-Kaluibia Governorate, Egypt. The plant was kindly identified by Prof. Dr. Wafaa Amer, Professor of Plant Taxonomy, Faculty of Science, Cairo University, Giza, Egypt.

Extraction and fractionation

The air-dried powdered leaves of *Morus alba* (2 Kg) were soaked on aqueous 70% methanol (4 X 3L) at room temperature (25±2°C) on cold. The crude methanolic extract (ME) was concentrated via rotatory evaporator to afford 360 g, and then 350 g from ME was defatted with petroleum ether (60-80°C) (3 X 2 L) to afford petroleum ether extract (40 g). The defatted 70% ME (300 g) was suspended in distilled water, and extracted successively with dichloromethane DCM (2 X 3L), ethyl acetate (3 X 2 L), and *n*-butanol (3 X 2.5 L), to afford methylene chloride (45 g), ethyl acetate (17 g), *n*-butanol (47 g), and water (65 g) extracts.

Chemicals and experimental procedures

All solvents and reagents used were of analytical grade. The ^{1}H NMR & ^{13}C NMR spectra were recorded on Varian Mecauy 300 MHz spectrometer, (^{1}H 300 & ^{13}C 75 MHz, DMSO-d_6). The chemical shifts are expressed in δ (ppm) with reference TMS and coupling constant (*J*) in Hertz. The absorbance measurements were recorded using the UV-Vis spectrophotometer Spectronic 601 (Milton Roy, USA). Melting points were determined on an electrothermal apparatus. Sephadex LH-20 (25-100 µm, Pharmacia Fine Chemicals Inc., Uppsala, Sweden), paper chromatography (PC) was carried out on Whatman No. 1 and No. 3 paper sheets (57 x 46 cm) (Maidstone, England) in (S_1, *n*-BuOH: AcOH: H_2O; 4:1:5; v/v/v; upper layer & S_2, H_2O: AcOH; 85:15 v/v), while thin layer chromatography (TLC) was performed over pre-coated silica plates (GF254, Merck) for dot-blot-DPPH staining assay. Spots were visualized under Vilber Lourmat UV lamp (VL-6LC, France) at 254 and 365 nm, then spraying with methanolic 1% $FeCl_3$ and/or 5% $AlCl_3$ for PC.

Determination of total phenolic contents (TPCs)

The total phenolic contents of plant extracts was determined using Folin-Ciocalteu's reagent according to the reported method [34].

Antioxidant assays

Rapid screening of antioxidant by dot-blot and DPPH staining

The antioxidant dot-blot and DPPH staining was carried out according to the detailed reported method [35].

Determination of total antioxidant capacity (TAC)
The antioxidant activity of plant extracts was determined according to phosphomolybdenum method, using ascorbic acid as standard [36].

Reducing power antioxidant assay (RPAA)
The reducing power activity was evaluated according to the reported procedure [37].

Antimicrobial activity
Disc agar plate method was used to evaluate the antimicrobial activity of ME as well as its derived sub-fractions according to the reported method [38].

Statistical analysis
All data were presented as mean **±** S.D. using SPSS 13.0 program [39].

Chromatographic isolation of n-BuOH fraction
The *n*-butanol fraction (dark brown mass; 40 g) was subjected to polyamide column chromatography (100 X 6 cm, 250 gm). A gradient elution was started with 5% MeOH and the polarity was gradually increased by methanol to pure MeOH at the end. Fractions (250 ml each) were collected, concentrated and examined (PC, S_1&S_2, 5% $AlCl_3$ and 1% $FeCl_3$, UV light for detection). A total of 165 fractions were collected, all the fractions were monitored via PC (S_1&S_2), and also TLC. Similar fractions were pooled together, concentrated to yield four major promising fractions (*Fr.1*, *Fr.2, Fr.3* and *Fr.4*). These fractions (1-4) were undergoing further purification over Sephadex LH-20 sub-column using a methanol/water gradient system. *Fr.1* (1 g eluted via 10% MeOH) was subjected to further purification upon Sephadex LH-20 column chromatography to yield compounds **1** (10 mg) & **2** (30 mg) eluted from sub-column via H_2O: MeOH (40:60 & 35:65; v/v) respectively. *Fr.2* (0.9 g eluted via 20% MeOH) was subjected as mentioned to yield compounds **3** (25 mg) & **4** (15 mg) eluted from sub-column via H_2O: MeOH (20:80 & 10:90; v/v) respectively. *Fr.3* (0.5 mg eluted via 30% MeOH) was subjected to yield compound **5** (20 mg) eluted from sub-column via H_2O: MeOH (85:15; v/v). Finally, *Fr.4* (0.6 mg eluted via 50% MeOH) was subjected to yield compound **6** (18 mg) eluted from sub-column via H_2O: MeOH (5:95; v/v).

Results

Total phenolic content and antioxidant activities
The total phenolic contents of the defatted 70% ME of *M. alba* as well as its successive sub-fractions were arranged in the descending order as; *n*-BuOH (387.50) > EtOAc (255.0) > defatted 70% ME (186.66) > H_2O (95.0) > CH_2Cl_2 (79.47) > pet. ether (26.53) (mg gallic acid equivalent/g dry extract) (Table 1). Furthermore, the total antioxidant capacity was arranged in the descending order as; *n*-BuOH (430.50) > EtOAc (314.81) > defatted 70% (207.55) > H_2O (136.30) > CH_2Cl_2 (57.0) > pet. ether (47.06) (mg AAE /g dry extract) (Table 1). Therefore, there are a highly positive correlation between the total phenolic content and the total antioxidant capacity with correlation coefficient (R^2 = 0.9925), indicating that the presence of phenolic compounds are mainly responsible for the antioxidant activity (Figure 1). Furthermore, most tested extracts showed a white zone upon the purple background indicating their potent antioxidant activity respect to ascorbic acid as standard (Figure 2). Moreover, the results in (Figure 3) revealed that the reducing power (optical density OD) were ranged from 0.659 to 0.102, in which the *n*-BuOH fraction has the highest activity (OD value = 0.659) followed by the EtOAc (OD value= 0.584), and defatted 70%ME (OD value= 0.452) respectively, comparated ascorbic acid as standard (OD value= 0.915) at concentration 200 µg/ml.

Antimicrobial activity
The results in Table 2 represent the *in vitro* antimicrobial activity of the defatted 70% ME of *M. alba* as well as its derived sub-fractions against four strains of the clinical antibiotic resistant pathogens including; *Staphylococcus aureus* (G+ve bacteria), *Pseudomonas aeruginosa* (G-ve bacteria), *Candida albicans* (yeast), and *Aspergillus niger* (fungus). The inhibition zones against three strains of the tested fractions were ranged from 10-21 mm, and there is no any effect against *A. niger*. Our results revealed that the EtOAc fraction was the most active fraction with inhibition zones; *C. albicans* (16/18 mm), *P. aeruginosa* (19/21 mm), & *S. aureus* (18/20 mm), followed by *n*-BuOH fraction with inhibition zones; *C. albicans* (15/17 mm), *P. aeruginosa* (16/18 mm), & *S. aureus* (14/15 mm), and the defatted 70% ME with inhibition zones; *C. albicans* (14/15 mm), *P. aeruginosa* (10/11 mm) & *S. aureus* (12/13 mm), in comparison with Penicillin G, as positive control, and there is no any activity was detected for the remaining fractions.

The isolated phenolic compounds from n-BuOH
The phytochemical investigation of *n*-butanol extract led to isolation of six compounds identified on the basis of certain spectrosocpic techniques as; sinapic acid-glucoside (1), 3,4,5-

trimethyoxyphenol-1-*O*-β-D-rhamnopyranoside (2), chlorogenic acid (3), gallic acid (4), rutin (5), and quercetin (6) (Table 3, Figure 4).

Antioxidant activity of the isolated phenolics

The antioxidant activities of the isolated phenolic compounds were evaluated via DPPH and phosphomolybdenum antioxidant assays. The results revealed that SC_{50} ± S.D. values of the sinapic acid-glucoside (1), 3,4,5-trimethyoxyphenol-1-*O*-β-D-rhamnopyranoside (2), chlorogenic acid (3), gallic acid (4), rutin (5), and quercetin (6), were found to be 7.45 ± 1.18, 11.65 ± 3.25, 6.55 ± 1.24, 5.20 ± 1.33, 9.0 ± 1.35 and 3.45 ± 1.25 µg/ml, respectively, for the DPPH assay relative to 7.50 ± 1.10 of the positive standard ascorbic acid, whereas that of the total antioxidant capacity values were 560.40 ± 1.22, 410.25 ± 2.20, 570.15 ± 1.85, 600.0 ± 1.65, 535.50 ± 2.10, and 630.23 ± 2.33 (mg AAE/g compound), respectively (Table 4).

Discussion

Total phenolic contents and antioxidant activities

Memon et al. (2010) reported on the antioxidant activity, and total phenolic contents of mulberry leaves, which were attributed to the presence of major phenolic acids *i.e.*, gallic, protocatechuic, *p*-hydroxybenzoic, vanillic, chlorogenic, syringic, *p*-coumaric, ferulic, and *m*-coumaric acids [40]. Also, Yi et al. (2002) reported that twigs of *M. alba* showed a potent antioxidant activity against lipid peroxidation, which may be attributed to the presence of phenolic compounds like 6-geranylapigenin, 6-geranylnorartocarpetin, resveratrol, oxyresveratrol, and quercetin [41].

Antimicrobial activity

Sheikhar et al. (2013) reported that, the aqueous and methanol extracts of *M. alba* foliage were tested against two Gram-negative bacteria, *Aeromonas hydrophila* & *Escherichia coli*, and two Gram-positive bacteria, *Streptococcus agalactiae* & *Streptococcus aureus* which exhibit a strong antimicrobial activity with inhibition zones; *A. hydrophila* (21.6 mm), *E. coli* (14.1 mm), *S. agalactiae* (20.1 mm), and *S. aureus* (13.1 mm) at 100 mg/ml [42]. Also, the antibacterial activity of crude extract from *M. alba* leaves was evaluated against oral pathogens *S. mutans* as well as the isolated compound 1-deoxynoijirimycin which strongly inhibited biofilm formation of *S. mutans* [24]. In addition, Omidiran *et al.* (2012) reported the potent antimicrobial activity of the ethanolic and aqueous extracts of *M. alba* leaves against certain human pathogenic microbes like; *S. aureus, P. aeruginosa, S. faecium, E. coli, N. gonorrheae, P. vulgaricus, A. niger, A. tamari, F. oxysporum* and *P. oxalicum* [43]. Our results were also supported via many prior studies reported on antimicrobial activity of *M. alba* against Gram-positive and Gram-negative bacteria [44, 45].

Identification and characterization of phenolic compounds

Six cmpounds were isolated and identified from the *n*-butanol fraction of *M. alba* leaves. Moreover, compounds 3-6 were previously isolated and identified [41, 46]; while compounds 1-2 were isolated for the first time from the leaves of *M. alba* growing in Egypt.

Compound **1** was isolated as pale yellow powder, m.p. 222-224°C. ^{1}H NMR (300 MHz, DMSO-d_6) spectrum showed presence of two signals at δ 6.83 ppm (1H, d, *J* = 15.9 Hz, H-7) and 7.69 ppm (1H, d, *J* = 15.9 Hz, H-8), corresponding to two coupled olefinic protons characteristic to cinnamic acid moiety, as well as an aromatic singlet at 6.85 ppm (2H, s, H-2/6), which suggested a symmetrically substituted ring system with an olefinic side chain. The geometry of the olefinic double bond was determined to be *trans* on the basis of the coupling constants. The ^{1}H NMR spectrum was consistent with trioxy-substituted cinnamic acid fragments, as well as two methoxy-resonances at 3.57 ppm (6H, s, H-3/5-OMe). This unite of the compound was identified as sinapine. In addition the compound includes other signals; the anomeric signal of glucose moiety appears at δ 4.08 (1H, d, *J* = 7.2 Hz, 1') and the rest of glucose protons appear at the range δ 3.10-3.31 ppm as multiplets (Table 3). The ^{1}H-NMR data for this compound compared with that shown for sinapic acid glucoside isolated from *Vaccinium Corymbosum* [47]. The above data suggested that compound 1 identified as sinapic acid-*O*-glucoside.

Compound **2** was obtained as white fine crystals, m.p. 205-207°C. ^{13}C NMR spectroscopic data (Table 3) showed presence of twenty carbons; three methyl, two methylene, nine methine, two sp2 CH, and four sp2 quaternary carbons. The ^{1}H NMR spectrum showed the presence of three methoxy groups signal at δ 3.85, 3.68 and 3.76 ppm (9H, s, 3-OMe) and two aromatic protons at δ 6.77 (2H, s), suggesting that the molecule contained an aromatic ring with three methoxyl substitution. The presence of the 3,4,5-trimethoxyphenol nucleus was supported by the ^{13}C NMR, it showed three signals at δ 157.42 (C-1), 156.36 (C-3 and 5) & 133.46 (C-4), and two methine carbons at δ 99.9 ppm (C-2 and 6).

These spectral data confirmed that a 3,4,5-trimethoxyphenol moiety was present in the molecule [48]. The presence of a rhamnose moiety was also confirmed from the presence of one anomeric proton signal at δ 4.08 ppm and confirmed by ^{13}C-NMR signals at δ 99.9 ppm (anomeric carbon), 70.96, 73.11, 73.93, 68.35 and 19.84 (methyl groups) (Table 3). The complete ^{1}H and ^{13}C-NMR spectral assignments for compound 2 in comparison with published data suggested that compound 2 was identified as 3,4,5-trimethyoxyphenol-1-*O*-β-D-rhamnopyranoside.

Compound **3** white powder, m.p. 201-203°C, it gave rosy red colour with phenol and effervescence with $NaHCO_3$ reagents indicating the presence of hydroxyl and carboxylic groups as well as deep blue with $FeCl_3$ confirming presence of phenolic OH group [49]. IR ν_{max} (KBr) cm^{-1} spectrum showed the absorption bands at: 3353 (–OH aromatic alcohol stretching); 2945 (-CH=CH- stretching); 1230, (-C–O- stretching); 1682, (>C=O stretching); 1520 and (–C=C– aromatic ring). Based on chromatographic properties, color reaction with different spraying reagents, and IR spectra, compound 3 was expected to be phenolic compound. ^{1}H-NMR of the compound indicate the characteristic resonance in the aromatic region for a caffeoyl moiety, represented by presence of an AX spin coupling system as olefinic doublets at 7.45 and 6.19 ppm with large *J*-values (2H, d, *J*= 15.0 Hz) for H-7' and H-8', respectively to confirm a geometrical isomerism as *E*-form for the caffeic acid moiety. Moreover an ABM system at 7.03 (1H, d, *J*= 2.1 Hz), 6.96 (1H, d, *J*= 8.4 Hz) and 6.75 (1H, d, *J*= 8.4 Hz) ppm, for H-2', H-5' and H-6' respectively. The quinic acid moiety of the compound was confirmed via the presence of four resonances in aliphatic region 1.98, 2.03 ppm for the two methylene protons at C-2, 3.93 ppm for H-3, 3.55 for H-4, the most downfield signal at 5.13 for H-5 due to the location of the caffeoyl moiety and 1.99, 2.02 for the two methylene protons at C-6 (Table 3). ^{13}C NMR spectra showed two carbonyl carbons at 175.23 and 165.81 ppm respectively for quinic and caffeoyl moieties, six signals at 125.54, 114.34, 144.79, 148.31, 115.73, 121.23 ppm for the aromatic carbons C-1', C-2', C-3', C-4', C-5' and C-6' respectively, also two characteristic signals at 145.55 and 114.13 ppm corresponding to two olefinic carbons C-7' and C-8'. The six carbons from the cyclohexane ring appeared at 81.35, 37.39, 71.87, 71.0, 68.85 and 37.02 ppm (Table 3). The spectral data showed basically agreement with the literature [50, 51]. Finally, based on Co-PC with the authentic sample, compound 3 was identified as 5'-*O*-E-caffeoyl- quinic acid (chlorogenic acid).

Compound **4** off-white powder; m.p. 249-251°C, Rf : 0.78 (S_1) and 0.52 (S_2). It gave a positive test with $FeCl_3$ and effervescence with $NaHCO_3$ reagents indicating the presence of phenolic and carboxylic groups respectively [49]. UV λ_{max} (nm): showed two characteristic bands at; 220 nm (due to conjugation of hydroxyl groups with benzene ring) and 271 nm (due conjugation of carbonyl group with benzene ring [51]. IR ν_{max} (KBr) cm^{-1} spectrum showed the absorption bands at: 3510 (Ar-OH), 3280 (carboxylic-OH), 3055 (Ar-CH-), 1730 (-C=O) and 1610 (Ar-C=C-). ^{1}H NMR (300 MHz, DMSO-*d*6) spectra showed a characteristic signal in the aromatic region at 6.92 (2H, s, H-3,7), and ^{13}C NMR (75 MHz, DMSO-*d*6) spectra showed peaks at: 121.07 (C-2), 108.85 (C-3 & C-7), 145.49 (C-4 & C-6), 138.08 (C-5), and 167.56 ppm (C-1). Therefore, compound 4 was identified as 3,4,5-trihydroxybenzoic (gallic acid) through comparison with the mentioned spectral data and (Co-PC) [50, 52].

Compound **5** was obtained as yellow powder, m.p. 209-211°C. IR (KBr cm^{-1}): 3423 cm^{-1} (OH stretch) 2938 cm^{-1}, 2909 cm^{-1} (C-H stretch), 1457 cm^{-1} (C-H bend), 1656 cm^{-1} (C=O) and 1505 cm^{-1} (C=C). ^{1}H NMR (300 MHZ, DMSO-d_6): 12.58 (1H, s, OH-5), ABX system including; 7.54 (1H, H-2', B-ring), 7.52 (1H, H-6', B-ring), and 6.85 (1H, d, *J* =8.7 Hz, H-5', B-ring), as well as AM system of two meta coupled protons at 6.38 (1H, d, *J* =1.5 Hz, H-8 A-ring), and 6.19 (1H, d, *J* =1.5 Hz, H-6 A-ring), beside a characteristic signals for the anomeric protons at 5.33 (1H, d, *J* =6.9 Hz, Glu-1''), 5.33 (1H, s, Rha-1'''), and one signal in the aliphatic region at 0.99 (3H, d, J= 6 Hz, CH_3-Rha). ^{13}C NMR (75 MHZ, DMSO-d_6): 144.77 (C-2), 133.33 (C-3), 177.39 (C-4), 161.24 (C-5), 98.73 (C-6), 156.63 (C-7), 93.62 (C-8), 100.76 (C-9), 156.45 (C-10), 121.62 (C-1'), 116.29 (C-2'), 144.77 (C-3'), 148.43 (C-4'), 115.25 (C-5'), 116.29 (C-6'), 101.22 (C-1''), 74.11 (C-2''), 76.49 (C-3''), 68.26 (C-4''), 75.93 (C-5''), 67.03 (C-6''), 100.76 (C-1'''), 70.40 (C-2'''), 70.04 (C-3'''), 70.60 C-5'''), 68.26 (C-5'''), 17.73 ppm (-CH_3-Rha). Thus compound 5 was established as quercetin-3-O-rutinoside (rutin) [53].

Compound **6** was obtained as dark yellow amorphous powder, m.p. 300-301°C. Rf: 0.52 (S_1) and 0.05 (S_2); it is gave a yellow fluorescence under UV, turned to bright yellow with ammonia, and green with $FeCl_3$. IR ν_{max} (KBr) cm^{-1} spectrum showed the absorption bands at: 3290 (-OH), 3045 (-CH-Ar), 1655 (-C=O), 1595 (Ar-C=C-), and 1140 (-C-O-). ^{1}H NMR specral data (300 MHz, DMSO-d_6) showed two characteristic spin coupling systems, the first one is

an ABX of three types of proton signals at δ ppm 7.68 (1H, d, *J* = 1.8 Hz, *meta* doublet), 7.55 (1H, dd, *J* = 1.8, 8.4 Hz, *ortho-meta* doublet), 6.90 (1H, d, *J* = 8.4 Hz, *ortho* doublet), assignable to H-2′, 6′ and 5′, respectively of 3′, 4′-dihydroxy B-ring. The second one is an AM of two *meta* coupled proton signals at 6.43 (1H, d, *J* = 1.8 Hz), 6.18 ppm (1H, d, *J* = 1.8 Hz) assignable to H-8 and H-6 respectively of 5, 7-dihydroxy A- ring and characteristic signal at 12.47 assignable to 5-OH A- ring. ^{13}C NMR spectral data (75 MHz, DMSO-d_6); exhibited 15 carbon resonances at 146.80 (C-2), 135.71 (C-3), 175.82 (C-4), 160.71 (C-5), 98.17 (C-6), 163.88 (C-7), 93.34 (C-8), 156.13 (C-9), 103.0 (C-10), 121.96 (C-1'), 115.07 (C-2'), 145.04 (C-3'), 147.69 (C-4'), 115.60 (C-5'), and 119.97 ppm (C-6'). Accordingly, compound 6 was identified as quercetin via comparative study with published data and authentic quercetin sample (Co-PC) [54]. Furthermore, the isolated compounds showed a promissing *in vitro* antioxidant activity. The results revaeld that there are remarked variations in the antioxidant potential of the tested compounds (1-6), and this may be return to the variation in their chemical structures and structural requirements for their hydrogen donating activity. Also, previous reports revealed that the variation in the antioxidant activities of different subclasses of flavonoid and phenolic compounds may be due to the presence or absence of the some structural criteria [50, 53, 54].

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Table 1. Total extractable content,s total phenolic content, and total antioxidant capacity of the defatted 70% methanolic extract of *M. alba* as well as its sub-fractions.

Sample	TEC (Yield %)[1]	Total phenolic contents (mg GAE/ g dry extract)[2]	Total antioxidant capacity (mg AAE /g dry extract)[3]
Defatted 70 % MeOH	16.25	186.66± 2.08	207.55 ± 3.53
Pet. ether	2.0	26.53± 1.32	47.06 ± 1.30
CH_2Cl_2	2.25	57.0± 1.64	79.47 ± 1.33
Ethyl acetate	0.85	255.0 ± 1.35	314.81 ± 2.31
n-butanol	2.35	387.50 ± 1.15	430.50 ± 1.20
Water	3.25	95.0 ± 1.20	136.30 ± 1.40

** Results are expressed as mean values ± standard deviation (n = 3).*
[1]TEC (total extractable contents).
[2]TPC (total phenolic contents) values are expressed as mg gallic acid equivalent/g extract (mg GAE/g ext.).
[3]Total antioxidant capacity values are expressed as mg ascorbic acid equivalent/g extract (mg AAE/g ext.).

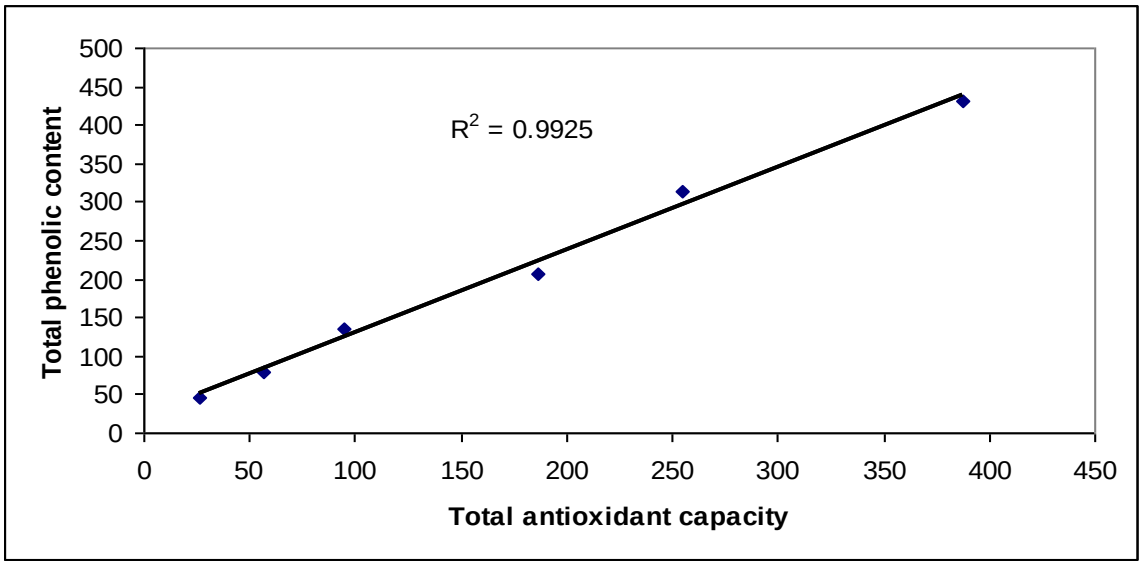

Figure 1. Correlation between the total antioxidant capacity and total phenolic contents (TPCs) of the defatted 70% methanolic extract of *M. alba* as well as its derived sub-fractions.

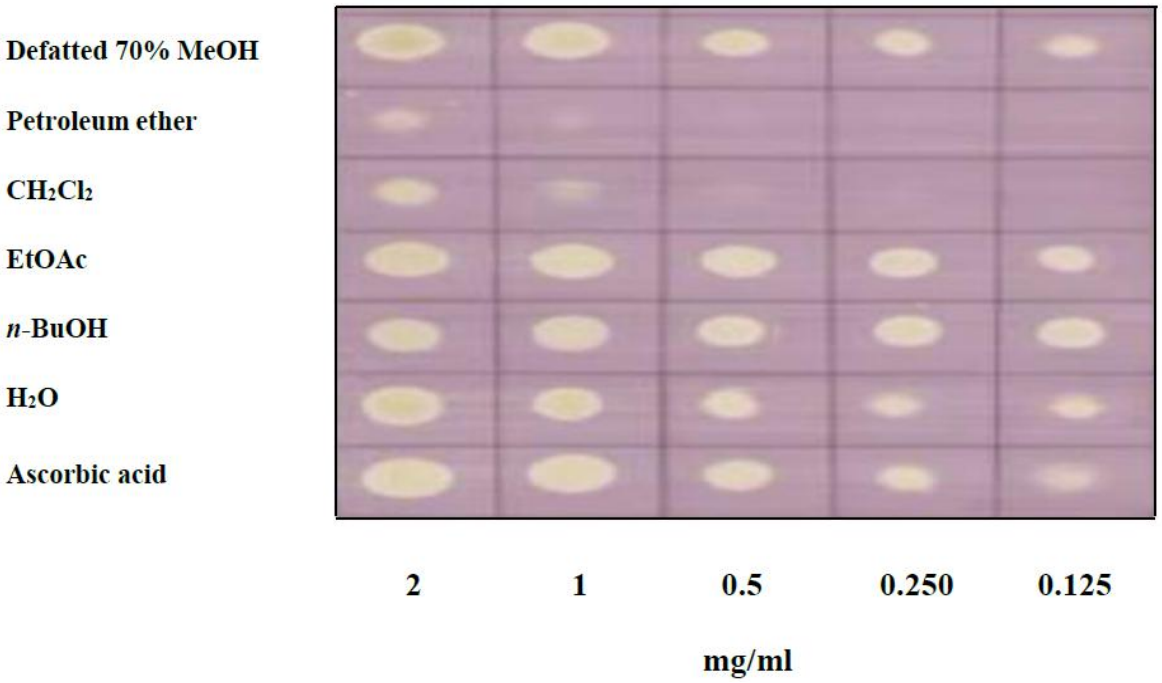

Figure 2. Dot-blot qualitative antioxidant assay of different fractions of *M. alba* on silica sheet stained with DPPH· solution in methanol.

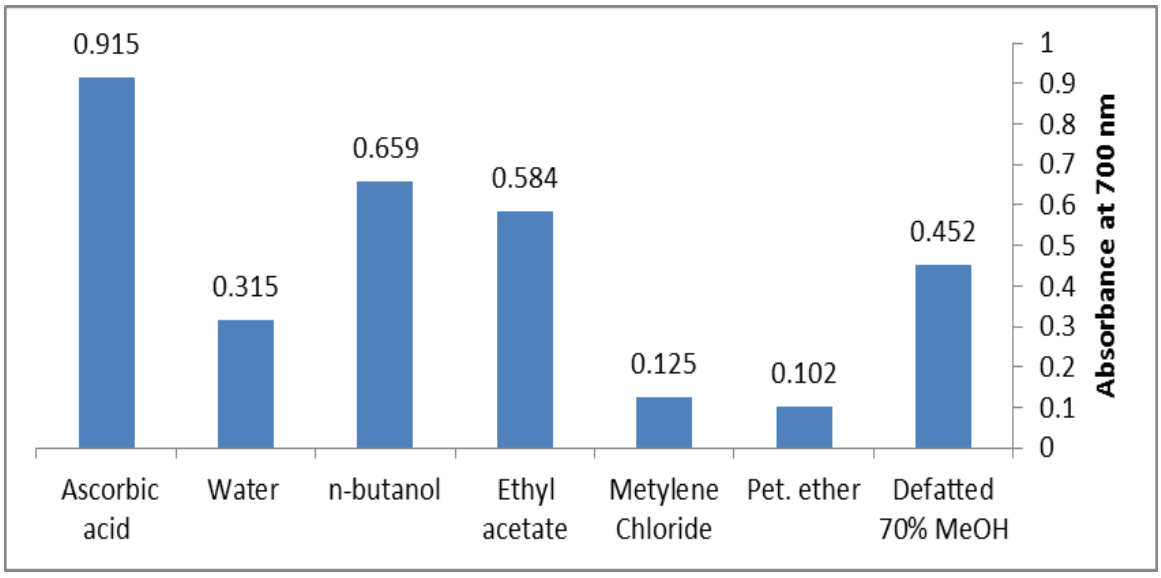

Figure 3. Reducing power activity of the defatted 70% methanolic extract of *M. alba* as well as its derived sub-fractions at concentration 200 µg/ml in comparison with ascorbic acid.

Table 2. Antimicrobial activity of the defatted 70% methanolic extract of *M. alba* as well as its derived sub-fractions.

Sample	Clear Inhibition zone (ϕmm)			
	Staphylococcus aureus	*Pseudomonas aeruginosa*	*Candida albicans*	*Aspergillus niger*
Defatted 70 %	12.50 ± 0.70	10.50 ± 0.84	14.50 ± 0.63	-
MeOH	-	-	-	-
Pet. ether	-	-	-	-
CH_2Cl_2	19.0 ± 1.40	20.0 ± 1.23	17.0 ± 1.34	-
EtOAc	14.50 ± 0.85	17.0 ± 0.76	16.0 ± 1.15	-
n-butanol	-	-	-	-
H_2O	28.35 ± 0.75	23.45 ± 0.68	26.0 ± 0.84	-
Penicillin G				

* The results of samples against *Staphylococcus aureus* (G+ve bacteria); *Pseudomonas aeruginosa* (G-ve bacteria); *Candida albicans* (yeast); *Aspergillus niger* (fungus); (-); inactive; Penicillin G, as positive control.

Table 3: ^{1}H& ^{13}C-NMR spectral data of the compounds **1-3** (300/75 MHz, DMSO-$d_{6;}$ TMS as internal standard, δ in ppm, *J* in Hz).

Compound 1		**Compound 2**			**Compound 3**		
Carbon No.	**^{1}H-NMR**	**Carbon No.**	**^{1}H-NMR**	**^{13}C-NMR**	**Carbon No.**	**^{1}H-NMR**	**^{13}C-NMR**
1	--	**1**	--	157.42	**1**	--	81.35
2	6.85	**2**	6.77	99.9	**2**	1.98, 2.03 (2H, -CH2)	37.39
3	-	**3**	--	156.36	**3**	3.93	71.87
4	8.43 (4-OH)	**4**	--	133.46	**4**	3.55	71.0
5	-	**5**	--	156.36	**5**	5.13	68.85
6	6.85	**6**	-	99.9	**6**	1.99, 2.02 (2H, -CH2)	37.02
7	7.69	**(3-*O*-Me)**	3.85	57.88	**7**	--	175.23
8	6.83	**(4-*O*-Me)**	3.68	62.83	**1'**	--	125.54
9	-	**(5-*O*-Me)**	3.76	59.69	**2'**	7.03 d, *J*= 2.1	114.34
-CH_3		**Glu**	--	--	**3'**	--	144.79
-OCH_3	3.57	**1'**	4.08	99.9	**4'**	--	148.31
Glu	--	**2'**	3.10	70.96	**5'**	6.96 d, *J*= 8.4 Hz	115.73
1'	4.08	**3'**	3.31	73.11	**6'**	6.75 d, *J*= 8.4 Hz	121.23
2'	3.10	**4'**	3.18	73.93	**7'**	7.45 d, *J*= 15.0	145.55
3'	3.31	**5'**	3.35	68.35	**8'**	6.19 d, *J*= 15.0	114.13
4'	3.18	**6'**	4.12	19.84	**9'**	--	165.81
5'	3.35	**--**	--	--	**--**	--	--
6'	4.12	**--**	--	--	**--**	--	--

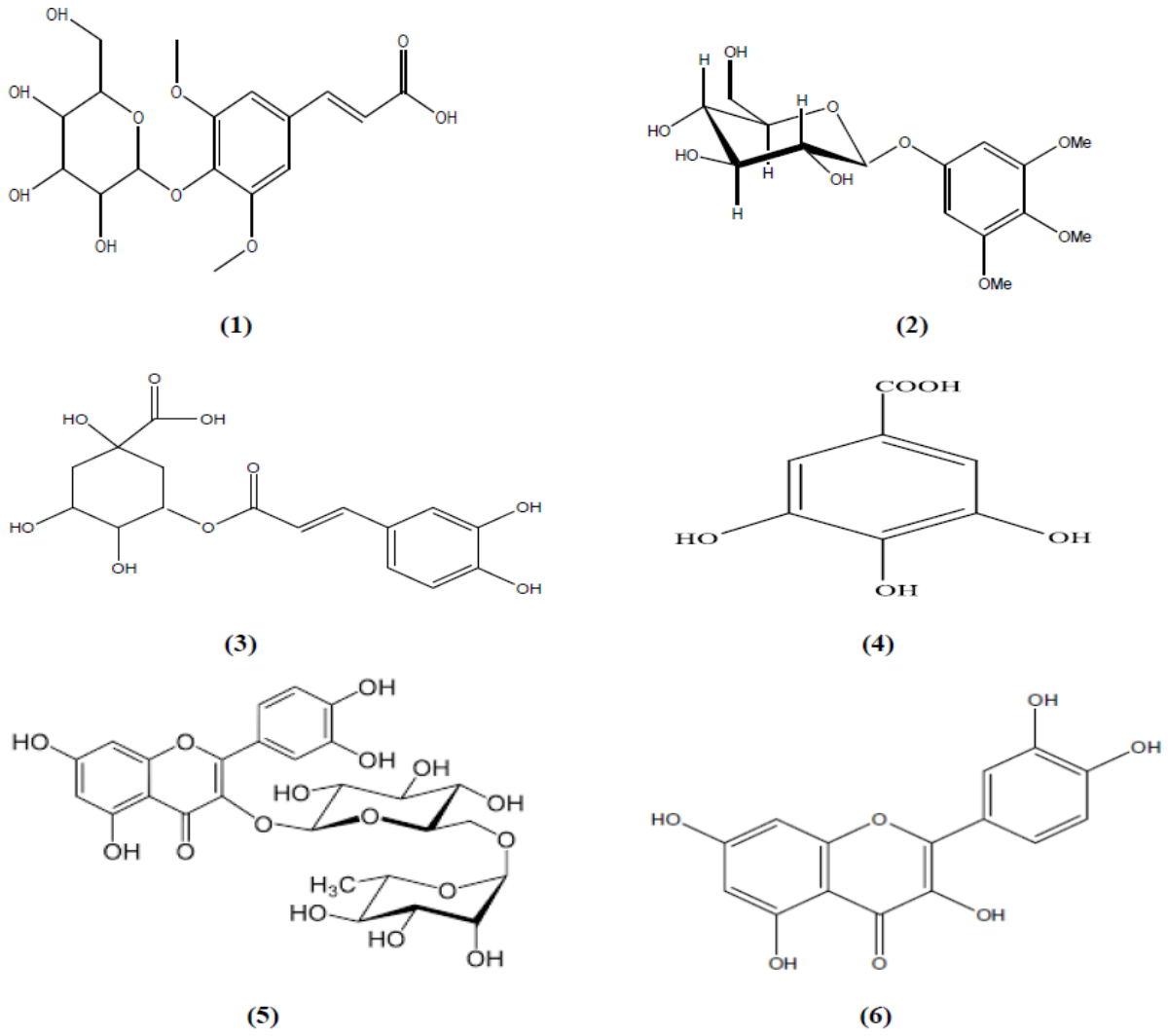

Figure 4. The chemical structures of the isolated compounds from *M. alba* leaves.

Table 4. Free radical scavenging antioxidant activity (DPPH), and total antioxidant capacity of the isolated compounds **(1-6)** from *M .alba*.

Compound	DPPH SC_{50} [µg/ml]	Total antioxidant capacity (mg AAE/g compound)
1	7.45 ± 1.18	560.40 ± 1.22
2	11.65 ± 3.25	410.25 ± 2.20
3	6.55 ± 1.24	570.15 ± 1.85
4	5.20 ± 1.33	600.0 ± 1.65
5	9.0 ± 1.35	535.50 ± 2.10
6	3.45 ± 1.25	630.23 ± 2.33
Ascorbic acid	7.5 ± 1.10	---