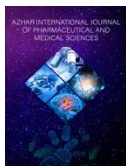

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Gastroprotective effect of abscisic acid O-β-D- glucopyranoside isolated from *Malpighia glabra* L. cultivated in Egypt

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Abstract: The secondary metabolites profile of *Malpighia glabra* L. leaves methanol extract using UPLC-Quadra- TOF-MS/MS analysis detected fifty-one compounds, including eleven phenolic acids, eighteen flavonoids of different classes flavonoid glycosides, flavanone, flavone, flavanol. Moreover, one stilbene glycoside, two triterpenes; one iridoid, three growth regulators in addition to primary metabolites. All metabolites were tentatively identified by comparing retention periods and fragmentation patterns of their mass spectra and MS/MS spectra to previously reported data. Six compounds; tithoniaquinone A glucoside, epicatechin, epiafzelechin, epicatechin 8-C-β-D-galactoside, physalin B, abscisic acid O-β-D-glucopyranoside (ABAGE) underwent isolation and innovatively identified from butanol extract of M. glabra leaves. Measurement of the antioxidant activity of ABAGE pure compound was done using Diphenyl-1-picrylhydrazyl (DPPH) assay and Trolox as standard. Gastroprotective activity of ABAGE (single oral dosages between 200 and 400 mg/kg body weight) one hour before ulcer induction by ethanol in Albino rats was examined using Omeprazole as the standard drug. ABAGE exhibited no liver or renal toxicity. ABAGE greatly improved the action of superoxide dismutase (SOD) and glutathione (GSH); the antioxidant enzymes, and enhanced GSH together with decreasing MDA which are suggested to be through avoiding oxidative damage caused by free radicals. ABAGE had the effect of decreasing nitric oxide (NO) activity which is suggested to be by inhibiting signal-induced tumor necrosis factor (TNF) transcription in addition to control of cytokine induction during inflammation. ABAGE exhibited a great gastroprotective impact on acute gastric ulcers caused by ethanol in rats.

Keywords: *Malpighia glabra L.,* Abscisic acid, Gastroptective effect.

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1. INTRODUCTION

Genus *Malpighia* contains thirty species of small trees and shrubs, that produce fruits and flowers at various ages, and are known to own lengthy fruiting seasons all year round. Acerola (*Malpighia punicifolia* L.; *Malpighia glabra* L.; *Malpighia emarginata* DC.), commonly identified as West-Indian cherry or Barbados cherry [1], is an essential part of the human diet and a viable alternate supply of revenue for agricultural companies. Acerola extracts from the fruit showed strong anti-oxidant, anti-mutagenic, and skin-protective properties. The highly significant natural supply of vitamin C is acerola [(1000 to 4500) mg100-1 g of pulp, which is better absorbed by humans than manufactured ascorbic acid] [2, 3]. Pectolytic enzymes and pectin, carotenoids, vitamin B, niacin, riboflavin, thiamin, plant fiber, mineral salts, and proteins are abundant in it. Acerola is used to manufacture ice cream, gelatin, nectar, jelly, gum, fruit preserves, juice, soft drinks, yogurts, and as well as nutraceuticals. *Malpighia* is known to have antigenotoxic, antipyretic, cytotoxic against tumor

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cell lines, antimicrobial, antihyperglycemic, antifungal, antioxidant, anti-inflammatory, free radical scavenging, immune-stimulant, and hepatoprotective activity. Additionally, it is utilized to cure diarrhea, fever, coughs, colds, and liver problems[4]. This work was done to examine the phytochemical profile of *Malpighia glabra* leaf extracts; identify separated pure chemicals using chromatographic characteristics, chemical, spectroscopic (UV, ^{1}H- NMR and ^{13}C-NMR), in addition to ESI-MS studies. Additionally, using ethanol to cause an acute gastric ulcer in rats, then we estimated the pure compound's antioxidant and gastro-protecting effects.

2. METHODS

2.1. Plant Material

Malpighia glabra L. leaves were gathered from cultivated plants in El-Orman Garden, Cairo, Egypt, in July 2018. The plant was kindly confirmed by Professor Dr. Abduo Marie Hamed, the Professor of Plant Ecology at the Faculty of Science, Al Azhar University, Nasr City Voucher specimens (code: M. g. Giza 2018) were kept in the Pharmacognosy Department, Faculty of Pharmacy (Girls), Al Azhar University.

2.2. Extraction and isolation

Malpighia glabra L. air-dried-powdered leaves (1.8 Kg) were extracted in 70% aqueous methanol (25±2°C), several times for two weeks (6-liter x 9 times). The concentration of crude extracts was done *in vacuo* at 45C°. The concentrated methanol extract (260 g) was defatted with the aid of pet-ether (60-80°C) (4 L); to give a dark brown concentrated defatted crude extract (200 g), which was separated with methylene chloride and ethyl acetate (6 L X 4 times). Then overstrained with *n*-butanol: water in a separating funnel to get a brown extract (butanol layer). The fractions of methylene chloride, ethyl acetate, and n- butanol were condensed under vacuum at temperatures below 40 °C to yield 30 grams, 45 grams, and 35 grams of methylene chloride, ethyl acetate, and n- butanol, respectively. The remaining aqueous fraction (70 g) was partitioned with methanol to give a 40 g methanol fraction.

The obtained plant fractions were subjected to chromatographic comparison using 2D-PC (2-dimension paper chromatography) using the solvent systems S1 & S2 (S1:15% acetic acid, S2: BAW). The promising n-butanol fraction (35 g) was placed onto a polyamide column chromatography for separation (5 x 120 cm, 250 g), eluted with 5% methanol followed by a gradual increase in polarity till 100% methanol. Similar fractions were pooled and concentrated then compared based on their paper chromatogram (PC) characteristic as well as thin layer chromatogram (TLC) to give five (i-v) fractions which were subjected separately for further purification to obtain a pure compound [5].

Fraction i (760 mg) was obtained from elution with 15% methanol then it was additionally purified using a column of Sephadex LH-20 with 20% methanol affording compound **(1)** (40 mg). Moreover, 450 mg of fraction ii was eluted with 35% methanol from a polyamide column and purified over a Sephadex column LH-20 twice with 30% methanol to acquire the chemical **(2)** (28 mg). However, fraction iii (490 mg) was eluted with 50 percent methanol from the polyamide column and chromatographed for further purification over the Sephadex column LH-20, followed by elution with 55 percent methanol to produce the chemical **(3)** (70 mg), and **(4)** (55 mg) which were crystallized with chloroform-methanol. While fraction iv (545 mg) eluted with 85% methanol from the polyamide column was recovered and purified on a Sephadex column eluted with 60% methanol. The compound (5) (75 mg) was isolated from fraction iv on the polyamide column by purification on a Sephadex LH-20 column and elution with 70% methanol. Fraction v (2g) was obtained by elution with 80% methanol and further cleansed with a silica gel column utilizing an eluent system with a chloroform mobile phase: Methanol (6:4) to yield a pure compound **(6)** (750 mg).

2.3. High-resolution UPLC-Quadra- TOF-MS/MS analysis

Tentative Determining the chemical constituents of the methanolic extract was done by LC-MS. A binary pump and Mariner Bio spectrometry were used for the LC-MS analysis. Connected to the UPLC was a Q-TOF mass spectrometer equipped with an ESI source. A 140°C source temperature was used during full-scan mode from m/z 100 to 1200. The analysis was conducted using the (150 × 2 mm i.d.) Phenomenex 5µ C18 HPLC column. The solvent used was formic acid0.3% in methanol. The solvent was eluted using isocratic elution with a total flow rate of 0.1 mL/min. Utilized N2 gas at a flow rate of 6 mL/min, a nebulizing pressure of 25 psi, and a drying gas temperature of 350o C. Prior to analysis, extracts of 0.5 g were diluted with methanol and filtered through a 0.22 m nylon filter. The excerpts were injected onto the analytical column in a 5 l volume for analysis. The mass fragmentations were discovered utilizing a spectrum database for organic chemicals and the negative ion mode in the SDBS tool.

2.4. Biological evaluation of Abscisic acid O-β-D-glucopyranoside (ABAGE)

2.4.1. Evaluation of antioxidant activity by Diphenyl-1-picrylhydrazyl (DPPH)

ABAGE's antioxidant action was evaluated utilizing the DPPH technique[6]**;** in terms of a free radical assay and compared with Trolox as a positive control.

2.4.2. Gastroprotective activity evaluation of ABAGE

2.4.2.1. Animals and housing conditions

Female albino rats that weighed between 120 and 150 grams each were kept in a controlled environment at the National Center for Radiation Research and Technology in Cairo (at 25˚C and humidity of 60 ± 5%; standard diet and tap water *ad libitum).* The research was achieved by international guidelines for animal experiments and approved by the Ethics Committee of the Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt (No: 350-2022).

2.4.2.2. Drugs and chemicals for treatment

- ABAGE extracted from *Malpighia glabra* L. leaves.

ABAGE; a naturally occurring conjugate of abscisic acid, was used to prepare a solution of 200 and 400 mg/kg body weight knowing that the lowest therapeutic dosage of dietary abscisic is 100 mg/kg body weight [7] while the LD50 for acute oral toxicity is more than 5,000 mg/kg body weight [8].

- Omeprazole as standard drug

Suspension of 20 mg/kg Omeprazole was prepared from Pepzol® capsules (Hikma Pharma SAE - Egypt) by addition of water [9].

- Utilized kits and other chemicals

GOT (AST) / GPT (ALT) (Biodiagnostic, Egypt); Urea kit (Biodiagnostic, Egypt); Creatinine kit (Biodiagnostic, Egypt); Reduced Glutathione (GSH) Assay Kit (Colorimetric) (Biovision, Egypt); Superoxide Dismutase (SOD) Activity Assay Kit (Biovision, Egypt); Lipid Peroxidation (MDA) Colorimetric Assay Kit (Biovision, Egypt); nitric oxide (NO) Colorimetric Assay Kit (Biovision, Egypt).

2.4.3. Experimental design

Thirty overnight fasted female albino rats were equally categorized into five groups. **Group 1**: normal control group; in this group rats were administered with distilled water one hour before decapitation. **Group 2:** ulcer group; received a single oral ethanol dose (5 ml/kg b. wt) one hour before decapitation[10]. **Group 3:** low concentration ABAGE group; received an oral single dose of ABAGE (200mg/ kg b. wt) one hour before ulcer induction. **Group 4:** high concentration ABAGE group; administrated a single oral dose of ABAGE (400mg/kg b. wt) one hour before ulcer induction. **Group 5:** standard group; administrated a single dose of Omeprazole (20 mg/kg b. wt.) one hour before ulcer induction. At the conclusion of the trial, all groups of rats were euthanized with urethane (at a dosage of 1.2 g/kg b. wt [11]. Blood samples were rapidly obtained by decapitation and rats were rapidly dissected for excision of the stomach[12].

2.4.4. Blood sampling:

After blood collection, blood was allowed to remain at room temperature for one hour before being centrifuged at 3000 rpm for fifteen minutes to extract the serum, which was then frozen at -20°C until used for biochemical measurement of hepatic and renal functions.

2.4.5. Tissue sampling:

Stomach was excised from rats immediately after the rats were sacrificed, washed with normal saline and divided into 2 portions. The first portion was taken for histological examination. The last portion of the stomach was taken for biological examination of oxidative stress and antioxidant parameters

2.4.6. Assay of some various biochemical parameters in serum

2.4.6.1. Determination of ALT and AST activities

ALT and AST activity was determined using the Reitman and Frankel technique (1957) [13] by the kits.

2.4.6.2. Determination of urea level

Urea was estimated by Fawcett and Soctt (1960)[14] by the kit.

2.4.6.3. Determination of creatinine concentration

Estimation of the creatinine level in the samples *via* the kit by the method of Bartles et al. (1972) [15].

2.4.7. Assay of antioxidants in tissue homogenates:

Ice was used to properly cleanse and sanitize stomach tissue. They were weighed after being gently wiped between filter paper folds. Using a Polytron homogenizer at 40°C, a 10 percent homogenate was produced in a 0.05 M phosphate buffer (pH 7) at a pH of 7. 20 minutes were spent centrifuging the homogenate at 10,000 rpm to remove debris, intact cells, nuclei, red blood cells, and mitochondria. According to the manual's recommendations, the supernatant (cytoplasmic extract) was utilized to measure MDA, SOD, GSH, and NO.

2.4.7.1. Determination of reduced GSH content:

Using an enzymatic cycling technique in GSH presence and a chromophore, the reduced GSH concentration of stomach homogenate is determined. The chromophore reduction produces a stable product whose kinetics may be seen at 450 nm [16].

2.4.7.2. Estimation of SOD:

SOD is among the most essential antioxidant enzymes. It catalyzes the breakdown of SOD anions into hydrogen peroxide and molecular oxygen. The Sensitive SOD Assay Kit employs WST-1, which, upon reduction with superoxide anions, generates a water-soluble formazan dye. The rate of SOD anion reduction is proportional to Xanthine oxidase (XO) activity and is decreased by SOD [17].

2.4.7.3. Estimation of Lipid Peroxidation (MDA):

MDA in samples interacts with thiobarbituric acid (TBA) to create colorimetrically quantifiable MDA-TBA adducts (OD: 532 nanometers). This test identifies MDA concentrations as lowest 1 nmol/well using a colorimetric method [18].

2.4.7.4. Estimation of NO content:

NO is quickly reduced to nitrite and nitrate and employed in a straightforward two-step technique to measure NO generation. Initially, nitrate reductase turns nitrate to nitrite. In the second stage, Griess reagents are used to transform nitrite into a dark purple azo molecule. The quantity of azo chromophore properly represents the amount of NO in the sample [19].

2.5. Statistical Analysis

The software GraphPad Prism (Version 9.3.1, California, United States) was used. One-way analysis of variance (ANOVA) and the Tukey-Kramer multiple comparison test were used to examine the differences between the groups. Considered significant were p values less than 0.05.

3. RESULTS

3.1. Experimental data of purified isolated compounds

Compound (1)

Yellow needles, m. p. 206 ◦C, TLC; Rf: 0.52 [Acetic acid: ethyl acetate: hexane (2:5: 10)], showed a positive anthraquinone glycoside test; UV spectrum of 1 exhibited absorption bands at 226, 272, 290, and 401 nm.^{13}C-NMR (100 MHz, DMSO-d_6): δ = 65.35(OMe), 113.1 (C-4), 117.7 (C-1), 118.0 (C-2), 127.1 (C-5), 127.4 (C-8), 133.6 (C-7), 135.1 (C-6), 149.55(C-4), 166.7 (C-3), 180.1 (C-9), 181.9 (C-10), 195.4 (CHO).

Compound (2)

Brownish-yellow amorphous solid with melting point 240°C, PC; R_f: 0.50 (15% AcOH), 0.65 (BAW); it gave yellow color with $AlCl_3$, greenish-brown with $FeCl_3$. ^{1}H NMR (400 MHz, CD_3OD-d_4): δ 2.49 (dd, *J* 15.9 and 8.4 Hz, Hβ -4), 2.84 (dd, *J* 15.9 and 5.4 Hz, Hα-4), 4.162 (m, H-3), 4.802 (d, *J* 7.5 Hz H-2), 5.93 (d, *J* 1.8 Hz, H-6), 5.90 (d, *J* 1.8 Hz, H-8), 6.77 (d, *J* 8.2 Hz, H-5’), 6.70 (dd, *J* 8.2 and 1.5 Hz, H-6’), 6.966 (d, *J* 1.5 Hz, H-2’).

Compound (3)

Under UV light, white crystals appear violet-black, melting point 249 °C, PC; R_f: 0.47 (15% AcOH), 0.59 (BAW); it gave yellow color with $AlCl_3$, green with $FeCl_3$. ^{1}H-NMR (CD_3OD) = δ 2.25 (1H, dd, *J* = 2.9 and 16.9 Hz, H-4a), 2.5 (1H, dd, *J* = 4.5 and 16.9 Hz, H-4b), 4.25 (1H, m, H-3), 5.0 (1H, br s, H-2), 6.60 (IH, d, *J* = 2.4 Hz, H-8), 6.98 (1H, d, *J*= 2.4 Hz, H-6), 6.75 (2H, d, *J*= 8.6 Hz, H-3',5'), and 7.45 (2H, d, *J* = 8.6Hz, H-2',6'). ^{13}C-NMR (CD_3OD): 30.0 (C-4), 61.46 (C-3), 76.91 (C-2), 87.29 (C-8), 98.35 (C-6), 104.09 (C-9), 115.52 (C-3',5'), 128.0 (C-2',6'), 157.7 (C-5), 159.03 (C-7), and 158.9 (C-4')

Compound (4)

^{1}H NMR spectrum of 4 showed three aromatic proton signals corresponding to an ABX system at δ 7.4 (1H, d, *J*=2 Hz, H-2'), 6.78 (1H, d, *J*=8 Hz, H-5') and 6.99 (1H, dd, *J*=2, 8 Hz, H-6') and an aromatic A-ring singlet signal at δ 6.66. The spectrum exhibited four aliphatic proton signals at δ 4.89 (1H, br s, H-2), 4.28 (1H, br m, H-3), 2.51 (1H, dd, *J*=4, 17.5 Hz, H-4) and 2.07 (1H, dd, *J*=2, 17.5 Hz, H-4). Seven aliphatic proton signals of a sugar moiety are also observed in the spectra; the anomeric proton emerged at δ 4.56 (1H, d, *J*=9.5 Hz, H-1''). The remaining proton signals are at δ 4.13 (1H, t, *J*=9.5 Hz, H-2''), 3.36 (1H, dd, *J*=3, 9.5 Hz, H-3''), 3.74 (1H, br d, *J*=3 Hz, H-4''), 3.07 (1H, dd, *J*=5.5, 6.5 Hz, H-5''), 3.59 (1H, dd, *J*=6.5, 11.5 Hz, H-6'') and 3.76 (1H, dd, *J*=5.5, 11.5 Hz, H-6'').

Compound (5)

White amorphous powder with melting point 248-250 °C, TLC; R_f: 0.50 (Chloroform: Methanol: H_2O; 8:6:0.9). ^{13}C-NMR &^{1}H-NMR spectral data of compound 5 (100&300 MHz, DMSO-d_6) **(Table 1)**.

Compound (6)

Colorless crystal with melting point 165-167 ◦C, TLC; R_f: 0.50 (Chloroform: Methanol: H_2O; 9:9:1.2). It revealed a positive Salkowski test for terpenoids. ^{1}H-NMR spectral data of compound 6

(300 MHz, DMSO-d_6) **(Table 1)**. The structures of isolated compounds **1-6 are** presented in **Fig.1**

Table 1: ^{13}C-NMR &^{1}H-NMR spectral data of compound 5&6 (100&300 MHz, DMSO-d_6; TMS as internal standard, δ in ppm, J in Hz).

Compound 1			Compound 2	
Position	**δ_C (ppm)**	**δ_H (ppm)**	**Position**	**δ_H (ppm)**
1	193.4	-	H-1	-
2	116.7	-	H-2	1H, 4.69, *s*
3	147.09	1H, 7.48, ddd, J = 10, 4.8, 2.2 Hz	H-3	-
4α **4β**	30.45	1H, 2.51, dd, J = 20, 5 Hz 1H, 3.35, br d, J = 20 Hz	H-4	1H, 7.95, d
5	135.81	-	H-5	1H, 7.21, d
6	123.7	1H, 5.43, br d, J = 6.2 Hz	H-6	3H, 2.02, d
7	21.25	-	H-1'	-
8	39.91	-	H-2'	-
9	30.45	-	H-3'	1H, 6.69, s
10	10.53	-	H-4'	-
11	21.25	-	H-5a'	1H, 2.18, d
12	25.09	-	H-5b'	1H, 2.60, d
13	78.08	-	H-6'	-
14	106.1	-	H-7'	3H, 1.48, d
15	207.10	-	H-8'	3H, 1.35, s
16	53.98	-	H-9'	3H, 1.18, s
17	79.74	-	H-1''	4.46
18	176.15	-	H-2''	1H, 3.41, d, *J*=8 Hz
19	18.04	3H, 1.03, s	H-3''	1H, 3.49, t, *J*=8 Hz
20	79.74	-	H-4''	1H, 3.01, d, *J*=8 Hz
21	21.25	3H, 1.06, s	H-5''	1H, 3.38, m
22	78.08	-	H-6a''	1H, 3.51, dd, *J*=3, 12 Hz
23	30.42	-	H-6b''	1H, 3.75, dd
24	30.45	-	OH	8.20
25	56.07	-	Position	δ_H (ppm)
26	167.73	-		
27	63.01	-		
28	27.43	3H, 1.24, s		

3.2. Results of biological evaluation:

The antioxidant potential of ABAGE isolated from Malpighia glabra L. leaves was evaluated using DPPH, and its IC50 value was determined to be 294.4 ± 13.9%.

3.2.1. Liver enzymes

The ulcer (ethanol) group showed neither elevation of ALT nor AST activities. On the same line, the effect of ABAGE (200mg/kg and 400mg/kg) on acute ulcers recorded non-significant changes in serum ALT and AST activity compared to control in rats orally administered with ethanol **(Fig. 2)**.

3.2.2. Antioxidants parameters

3.2.3. The ulcer (ethanol) group showed a significant GSH concentration and SOD activity decrease compared to the control recording -72% for both. ABAGE 200 group reflected an improvement in GSH concentration and SOD activity than Ulcer group but still less than the normal one. While ABAGE 400 and Omeprazole showed restoration of GSH concentration and SOD activity levels **(Table 2)**. In comparison with the control, the ulcer group exhibited a significant change in MDA and NO levels. Administration of ABAGE 200, and ABAGE 400 exhibited a significant reduction in MDA and NO levels. Omeprazole Administration also revealed

a decline in MDA and NO levels (**Table 3**). On the other hand, in the normal group, the rat stomach mucosa was smooth and had no bleeding, however in the ulcer group, the rat gastric mucosa revealed severe bleeding injuries. Compared to ulcer group, the ABAGE high and medium dosage groups and the Omeprazole group displayed substantial inhibitory impacts on the bleeding damage of gastric mucosal **(Fig. 3)** *Histopathological results.*

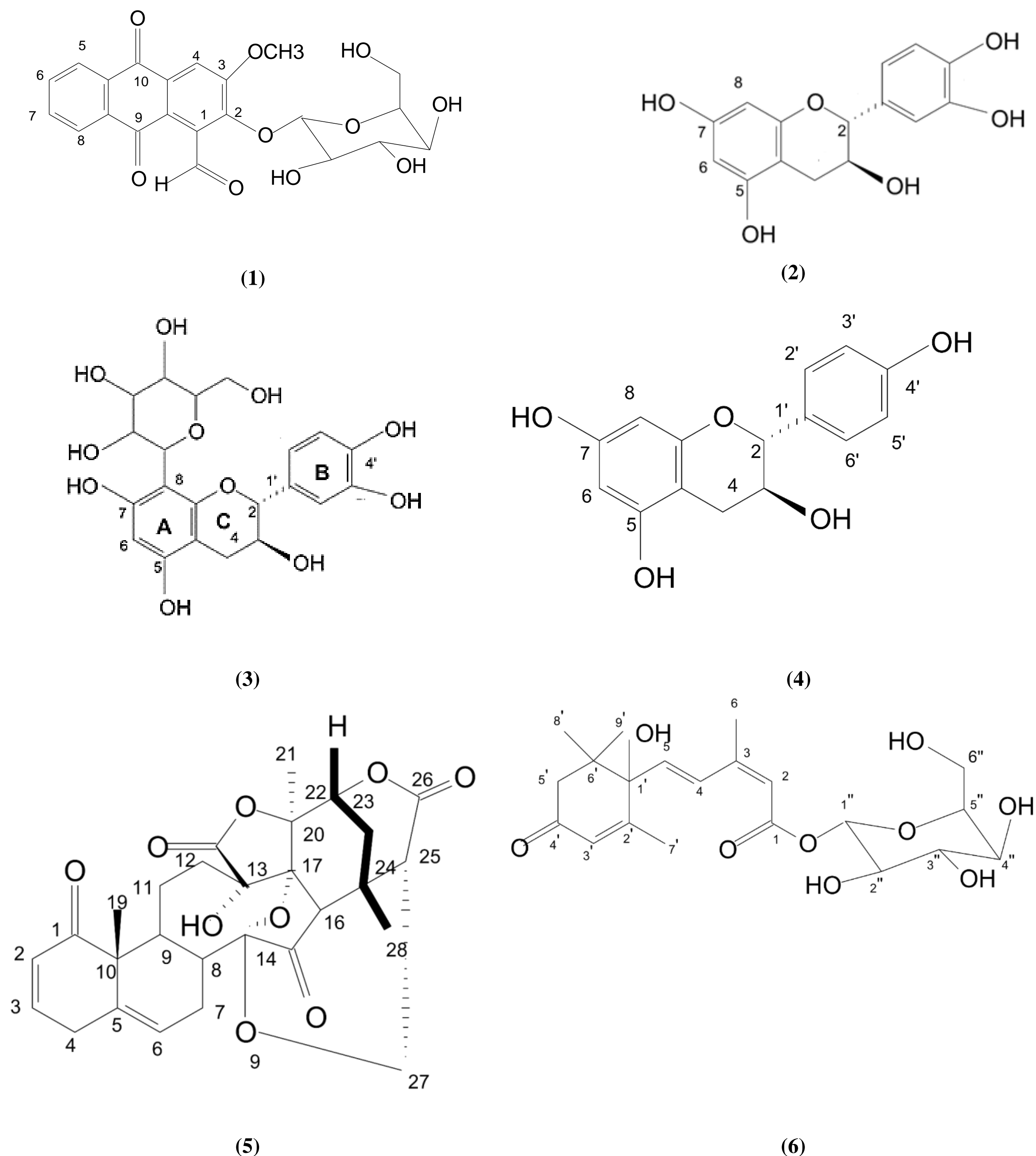


Figure 1 : The purified identified compounds 1-6

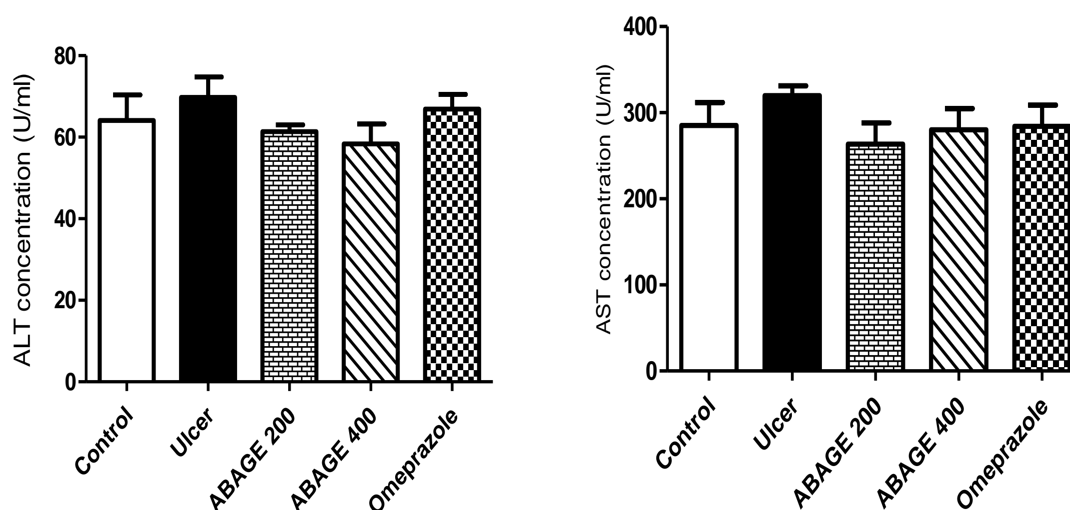


Figure 2: Effect of oral administration of ABAGE on serum ALT and AST activities in rats orally administered Ethanol.

For ABAGE groups; Rats were administered ABAGE in a dose of 200mg/kg and 400mg/kg orally 1 hour before the ethanol ulcer-induced effect. For Omeprazole groups; Rats were administered Omeprazole in a dose of (20 mg/kg) orally 1 hour before the ethanol ulcer-induced effect.

Photomicrograph of gastric mucosa showing that: the normal control rats group showed normal architecture with intact mucosa, submucosa, muscularis, and serosa. Gastric mucosa appeared with an intact wall and also no exfoliation in the mucosal epithelium. The gastric glands and sub-mucosal layer showed a normal histological structure **score 0 (Fig. 4A, 5)**. The gastric mucosa of the model rats group showed ulceration of the surface mucosa extended to the lamina muscularis mucosa. Severe exfoliations in the mucosal cells and exhibited massive necrotic debris were seen at the base of ulcer. The lower lamina propria desquamation or gastric glands decrease by >2/3, as well as submucosal exposure, was seen (4). This was accompanied by necrosis of gastric glands, atrophy of gastric folds, sub-mucosal edema, distortion of the gastric glands, inflammatory cells infiltration **score (2)** in the mucosa, and submucosa. Gastric mucosal hemorrhage was seen in amount and mixed with exfoliated surface epithelial lining **score (1) Fig (5b-c)**.

Table 2: Effect of oral administration of ABAGE on Glutathione (GSH) and superoxide dismutase (SOD) activities in stomach tissue of rats on various experimental groups:

Parameters Groups	GSH (mmol/mg protein)	SOD (U/mg protein)
Control	1.68 ± 0.06	4.66 ± 0.16
Ulcer	0.47 ± 0.04 *	1.30 ± 0.07 *
% Change from control	-72%	-72%
ABAGE 200	0.85 ± 0.04 *#	2.12 ± 0.18 *#
% Change from control	-49%	-55%
ABAGE 400	1.50 ± 0.07 #	4.12 ± 0.27 #
% Change from control	-11%	-12%
Omeprazole	1.52 ± 0.09 #	3.94 ± 0.16 #
% Change from control	-10%	-15%

Data were presented as a mean ± standard error (S.E), n=6.

* Significantly change from control at p<0.05. # Significantly change from ulcer at p<0.05.

Table 3: Effect of oral administration of ABAGE on Malondialdehyde (MDA) and nitric oxide (NO) activities in stomach tissue of rats on various experimental groups:

Parameters Groups	MDA (nmol/mg protein)	NO (nmol/mg protein)
Control	0.54 ± 0.05	1.28 ± 0.07
Ulcer	2.58 ± 0.07 *	3.68 ± 0.20 *
% Change from control	374%	188%
ABAGE 200	1.82 ± 0.09 *#	2.68 ± 0.14 *#
% Change from control	234%	109%
ABAGE 400	0.93 ± 0.08 *#	1.42 ± 0.09 #
% Change from control	71%	11%
Omeprazole	0.95 ± 0.10 *#	1.38 ± 0.07 #
% Change from control	75%	8%

Data were presented as a mean ± standard error (S.E), n=6.

* Significantly change from control at p<0.05. # significantly change from ulcer at p<0.05

The gastric mucosa of the animal model and ABAGE 200 revealed superficial ulceration of the surface mucosa with intact lamina muscularis mucosa. the middle lamina propria desquamations and stomach glands decrease was observed (3). Atrophy of gastric folds and necrosis of the gastric gland were also seen. Inflammatory cell infiltration was detected in the lamina propria score (1). Gastric mucosal hemorrhage was not detected score (0) **Fig (5 d-e)**. On the other side, the animals' model and ABAGE 400 showed sloughing of the surface epithelial lining of the gastric mucosa. Organizations of gastric folds with few numbers of necrotic mucosal glands were observed. Superficial lamina propria desquamation or gastric gland decrease by a one-third score (2). Inflammatory cells infiltration score (1) and submucosal edema were noticed. Gastric mucosal hemorrhage was not detected score (0) **Fig (5 f-g)**.

Animal's model and the standard drug showed desquamation of the surface epithelial lining of gastric folds with intact subepithelial glands score (1). Submucosal edema and leukocytic infiltration score (1), and the restoration of the normal thickness of the mucosal, submucosal, and muscular layers gastric mucosal hemorrhage was not detected score (0) **Fig (5 h-i)**.

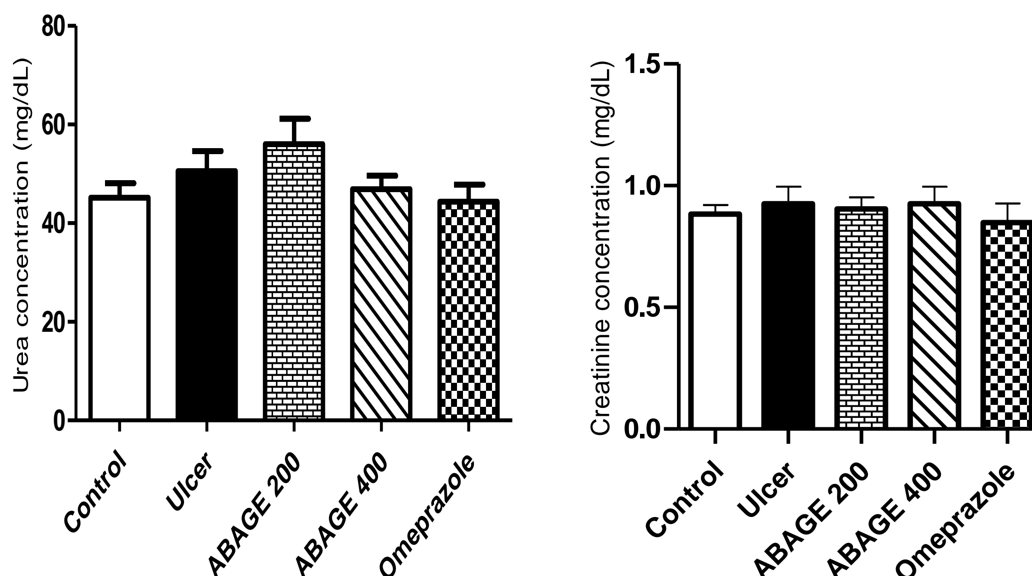


Figure 3: Effect of oral administration of ABAGE on serum Urea and Creatinine activities in rats of various experimental groups.

For ABAGE groups; Rats were administered ABAGE in a dose of 200mg/kg and 400mg/kg orally 1 hour before the ethanol ulcer-induced effect. For Omeprazole groups; Rats were administered Omeprazole in a dose of (20 mg/kg) orally 1 hour before the ethanol ulcer-induced effect.

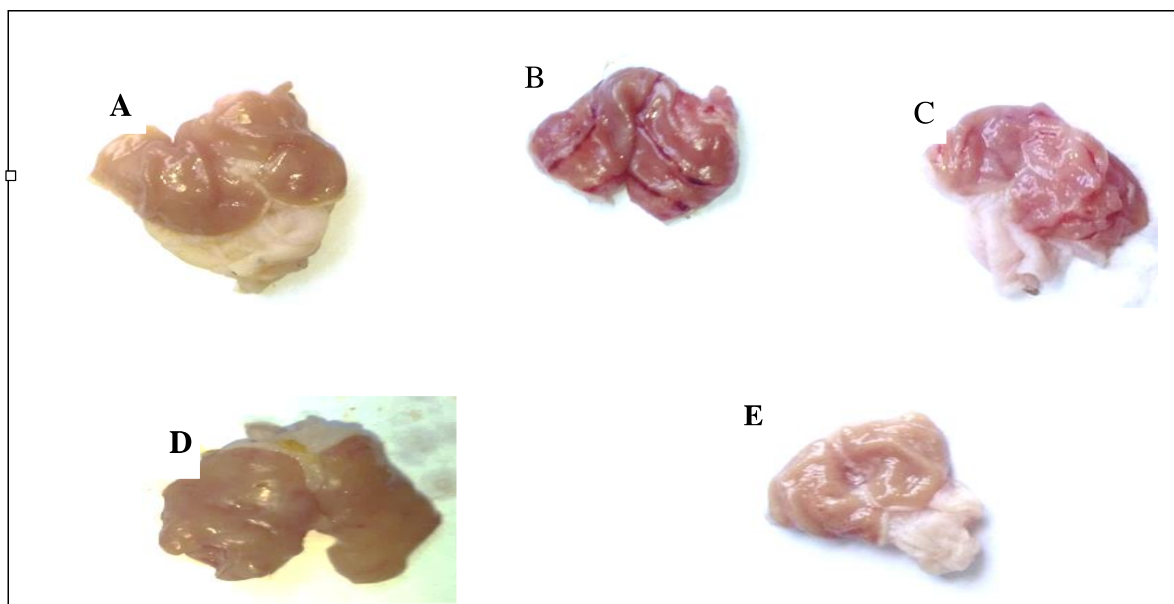


Figure 4: Morphological appearance of different treatments against ethanol-induced gastric lesions. (A) Normal; (B) Ulcer (Ethanol); (C) ABAGE 200 mg/kg + Ethanol; (D) ABAGE 400 mg/kg + Ethanol and (E) Omeprazole 200 mg/kg + Ethanol.

4. DISCUSSION

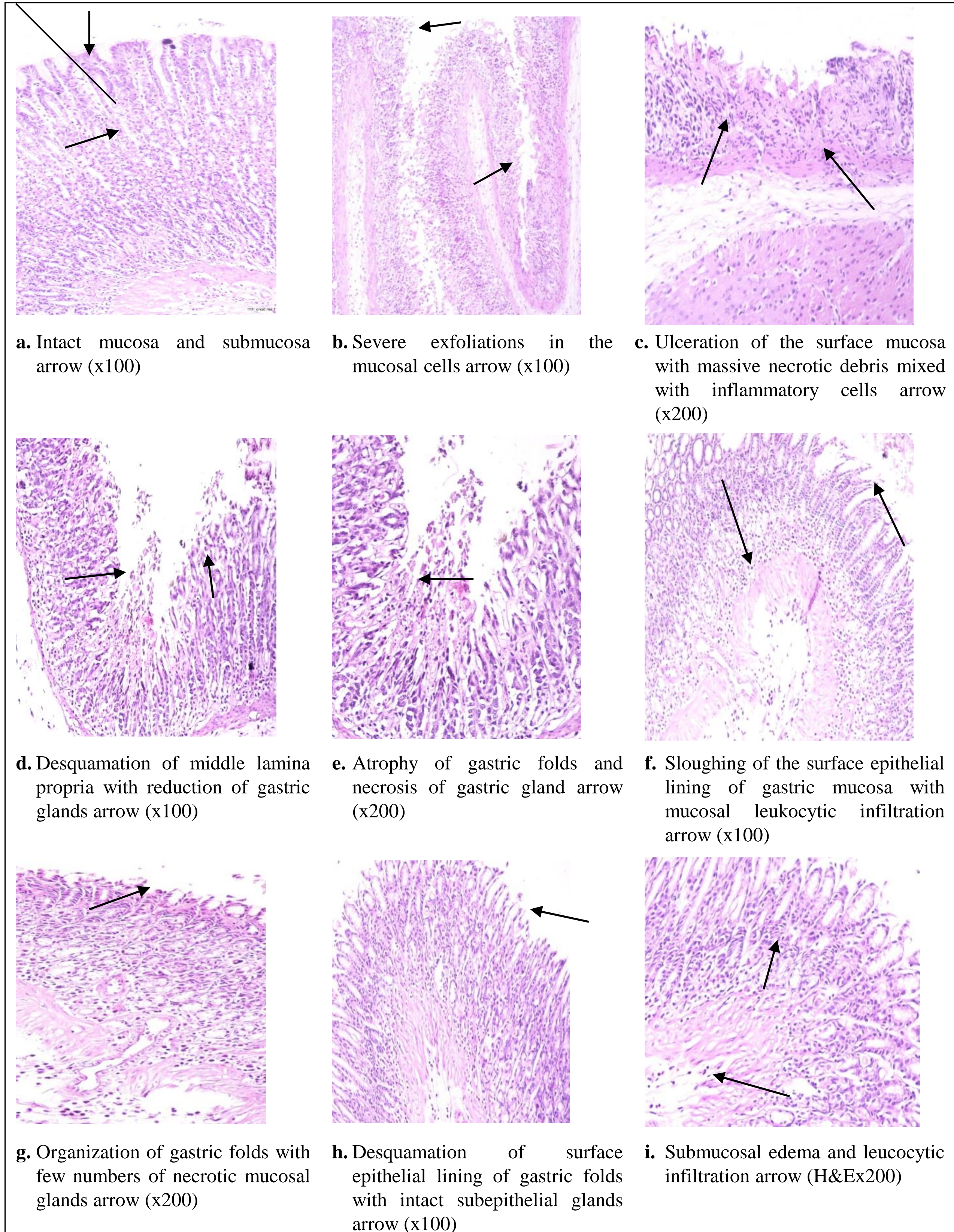
HPLC–ESI–MS analysis of Malpighia glabra methanol extracts had detected tentatively identified fifty-one compounds. Each peak was fragmented; producing fragmentation spectra that included the suggested compounds' candidates' masses (m/z). The results of spectrum interpretation of Malpighia glabra methanol extract showed eleven phenolic acids mainly as: [Galloyl glucose 20; Syringic acid 21; Ferulic acid 4-O-glucuronide 22; Feruloylquinic acid 23; 3-Cafeoylquinic acid 24; Gallocatechin 25; 4- hydroxycinammic acid 26]. Moreover, eighteen flavonoids of different classes were recognized, flavonoid glycosides considerably as: [eriocitrin 27; luteolin-7-O-rutinoside 28; miquelianin 29; flavanol (isorhamnetin 30; Fisetin 31; Quercetin 32; flavone (Chrysin 33 and one iridoid (Acetylaucubin) 34] were detected. The identification was depend on the retention durations and fragmentation characteristics of the mass spectra and MS/MS spectra, which were compared to previously reported data.

From the butanol fraction; six purified isolated compounds were identified as Tithoniaquinone A glucoside (**1**) [35]; Epicatechin (**2**) [36]; Epiafzelechin (**3**) [37]; Epicatechin 8-C-β-D-galactoside (**4**) [38]; Physalin B (**5**) [39]; ABAGE (**6**) [40]. On the basis of their chromatographic, physiochemical, and spectroscopic characteristics (UV, ESI-MS, 1H-NMR, and 13C-NMR), and on comparisons to authentic samples and previous reports in the literature, their structures were clarified.

Antioxidant effect: IC_{50} value of ABAGE isolated from *Malpighia glabra* L. leaves was determined by DPPH assay and the findings exhibited the IC_{50}=294.4 ± 13.9, which compared with total butanol extract IC_{50}=425.0 ± 16.8 and ethyl acetate fraction of *Malpighia glabra* leaves $IC5_0$ = 4.98(mg/ml) [41]previously reported by Boly et al.; 2016 [6].

There was neither significant rise in hepatic parameters (ALT and AST) nor renal parameters (Urea and Creatinine) in all groups in this study; showing that ABAGE concentrations used (200mg/kg and 400mg/kg) has no hepatic toxicity or renal toxicity [42]. Adel *et al*.; 2021[43] further validated this and elucidated the function of Abscisic acid in enhancing the damage of the kidney after Renal ischemia/reperfusion injury, as it reduces epithelial sloughing, tubular necrosis, and cast formation**.** In contrast to studies [44, 45], which are different from our study in that they reported an increase in ALT and AST in the ethanol group**.**

In the present work, it was noticed that rat with induced ulcer effect without previous treatment with ABAGE or omeprazole showed decreased GSH and increased MDA as, ethanol leads to oxidative stress and mitochondrial depolarization in the cell, and destroy of gastric mucosal cells. Moreover, ethanol-induced oxidative stress in the gastric mucosa resulted in an elevated MDA concentration and a reduced GSH levels. Guler et al.; 2022 [46] and Jin et al**.;** 2019) [47] stated that alcohol affects oxidative stress which decreases SOD.



a. Intact mucosa and submucosa arrow (x100)

b. Severe exfoliations in the mucosal cells arrow (x100)

c. Ulceration of the surface mucosa with massive necrotic debris mixed with inflammatory cells arrow (x200)

d. Desquamation of middle lamina propria with reduction of gastric glands arrow (x100)

e. Atrophy of gastric folds and necrosis of gastric gland arrow (x200)

f. Sloughing of the surface epithelial lining of gastric mucosa with mucosal leukocytic infiltration arrow (x100)

g. Organization of gastric folds with few numbers of necrotic mucosal glands arrow (x200)

h. Desquamation of surface epithelial lining of gastric folds with intact subepithelial glands arrow (x100)

i. Submucosal edema and leucocytic infiltration arrow (H&Ex200)

Fig (5): Histopathological Photomicrograph of gastric mucosa

Lipid peroxidation increases the deterioration of the redox equilibrium (MDA). Since lipid peroxidation produces radical species, it has been commonly believed that it causes tissue damage and illnesses. [48]. Pretreatment with ABAGE and omeprazole reduced lipid peroxidation (MDA) and restored depleted GSH in ulcerated gastric mucosa. On the contrary, pretreatment with ABAGE is very effective in avoiding oxidative damage caused by free radicals by rise antioxidant enzymes (SOD and GSH) activities and boosting GSH while simultaneously lowering MDA as same as results of Elhadidy *et al*.; 2020 [49].

NO, which is generated by inducible NOS, contributes to tissue damage during an inflammatory reaction. Numerous studies demonstrated that NO can prevent gastric ulcers by increasing mucosal blood flow, triggering gastric mucus secretion, and minimizing leukocyte infiltration [50]. Inhibitory effects on NO production had a significant correlation with total phenolics [24].

According to a prior publication, the inhibition of TNF- α substantially reduced the severity of gastric injury in cirrhotic rats [51]. We further examined the effects of ABAGE on the production of NO, and histopathological changes gastric mucosal injured rats caused by ethanol. This is supported by a research by Ma and Kinneer, 2002 [52] that demonstrated that phenolic antioxidants have a strong inhibitory effect on signal-induced TNF-transcription and suggested an antioxidant-mediated anti-inflammatory mechanism via regulation of cytokine production during inflammation. The study's findings made it clear that ABAGE 400 and Omeprazole had effects that were almost identical to each other and superior to those of ABAGE 200 in terms of boosting GSH and SOD and lowering MDA and NO.

5. CONCLUSIONS

In conclusion, our present work revealed that LC/MS of *Malpighia glabra* leaves methanol extract showed the presence of biologically active fifteen compounds. Among them seven phenolic acids, seven flavonoids of different classes one iridoid. This research demonstrated the significance of natural antioxidants. ABAGE demonstrated a significant gastroprotective effect against acute gastric ulcers produced by ethanol in rats. This gastroprotective effect may be a result of a strengthened antioxidant defence system. In Moreover, ABAGE was devoid of hepatotoxicity and renal toxicity. Additional and detailed research is required for higher concentrations of ABAGE to investigate the gastrointestinal protective mechanism of ABAGE and the optimum concentration. This will open new aspects in treatment using natural plants as alternatives to synthetic drugs such as gastroprotective and antioxidant drugs

Supplementary Materials:

Funding: No fund was received for this work

Acknowledgments: NA.

Conflicts of Interest: All authors declared no conflict of interest.

Ethical Statement: The study was conducted in accordance with international guidelines for animal experiments and approved by the Ethical Committee at faculty of Pharmacy, Al-Azhar University, Cairo, Egypt (No: 350-2022).

Author Contribution: SAZ carried out the extraction and contributed in the pure compounds chromatographic separation; MMH participated in elucidation of the structure of isolated chemicals and supervised the work; MAE carried out the biological activities; MTI formulated the research point and supervised the work; RHE contributed in elucidation of the structure of isolated chemicals and supervised the work. All contributors contributed to the drafting and editing of the book, which is now ready for publication.

List of Abbreviations: 2D-PC: Two Dimension Paper Chromatography; ^{13}C-NMR: Carbon-13 Nuclear Magnetic Resonance; ^{1}H-NMR: Proton nuclear magnetic resonance; ABAGE: Abscisic acid O-β-D-glucopyranoside; ALT: Alanine transaminase; AST: Aspartate aminotransferase; BAW: n- butanol acetic acid water; DMSO: Dimethyl sulfoxide; DPPH: Diphenyl-1-picrylhydrazyl; ESI: Electrospray ionization; GSH: Glutathione; HPLC: High-performance liquid chromatography; LD: Lethal dose; M. glabra: *Malpighia glabra*; MDA: Malondialdehyde; MS: Mass spectrometry; NO: Nitric oxide; OD: Optical density; Q-TOF: Quadrupole Time-of-Flight; SDBS: Spectral Database system for Organic Compounds; SOD: Superoxide dismutase; TBA: Thiobarbituric Acid; TLC: Thin Layer Chromatography; TNF: Tumor necrosis factor; UV: Ultraviolet; XO: Xanthine oxidase.

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