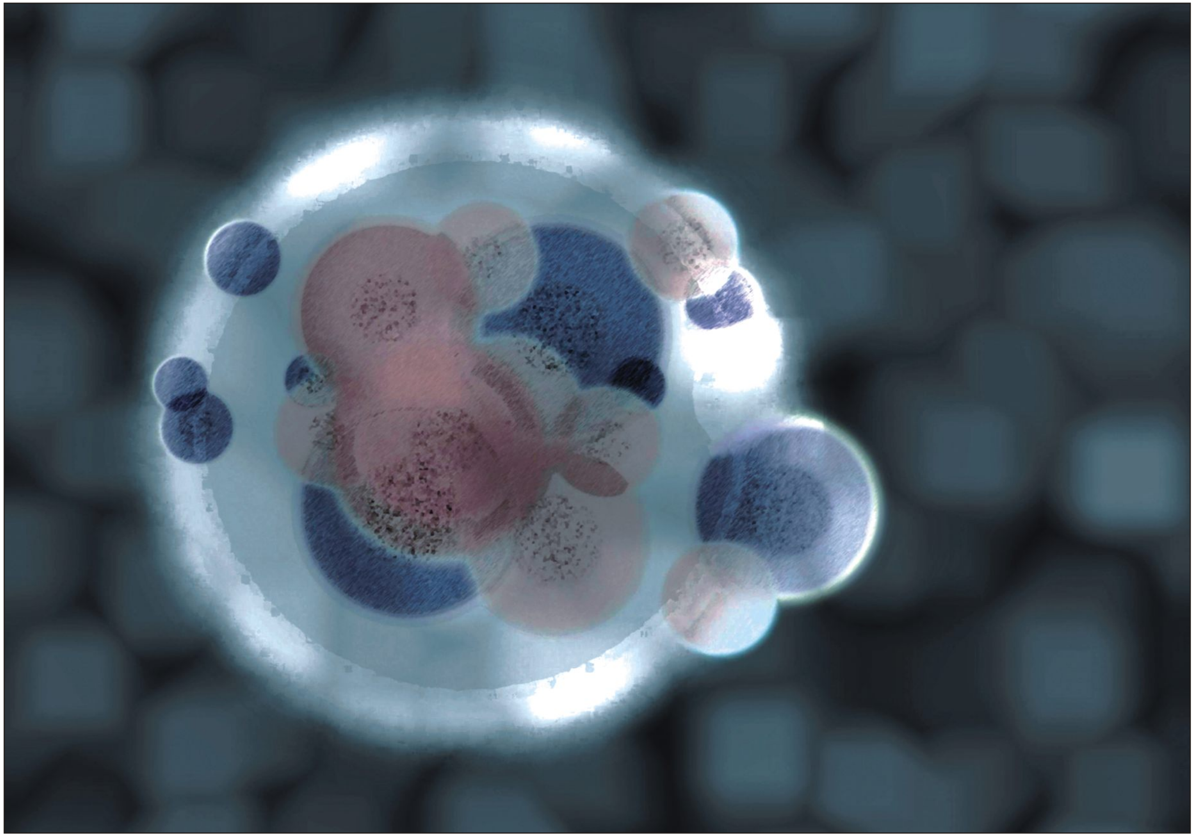

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Research Article

Evaluation of Hepatoprotective, Antioxidant and Cytotoxic Properties of Isolated Flavonoids from *Breynia disticha* J.R.Forst. and G.Forst

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Abstract

Background and Objective: *Breynia disticha* J.R.Forst. and G.Forst is a tropical shrub widely grown in tropical Africa. The leaves have characteristic green, purple and pink color, found in gardens and public areas. The aim of the study was to investigate phytochemical and biological activities of successive extracts of aerial parts of *Breynia disticha* J.R.Forst. and G.Forst, cultivated in Egypt. **Materials and Methods:** Collection and taxonomical identification of the plant. Biological screening of successive extracts of *Breynia disticha* J.R. Forst. and G. Forst. and structural elucidation of compounds isolated by using different spectral techniques. **Results:** Two new flavonoids, quercetin 3-O-β-D-xylopyranoside (1→6) β-D-galactopyranoside along with its analogue kaempferol 3-O-β-D-xylopyranoside (1→6) β-D-galactopyranoside were isolated from 70% aqueous methanol extract of aerial parts of *Breynia disticha* J.R.Forst. and G.Forst. The chemical structures of the two compounds were elucidated by NMR spectroscopic analysis. Quercetin 3-O-β-D-xylopyranoside (1→6) β-D-galactopyranoside showed significant antioxidant and cytotoxic activities against hepatocellular carcinoma compared by its analogue. Also the polar fraction showed hepatoprotective activity by reducing elevated serum ALT level, AST, ALP, total bilirubin and decreased liver content of NO and normalized MDA, GSH and SOD. **Conclusion:** The results of this study revealed the presence of two new compounds and the polar fraction of the plant represent a promising hepatoprotective activity.

Key words: *Breynia disticha*, flavonoids, acute toxicity, hepatoprotective, antioxidant, cytotoxic activity

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Breynia disticha J.R.Forst. and G.Forst (*B. disticha*) belongs to family Phyllanthaceae is a tropical shrub characterized by its leaves with white, green and red coloration commonly found in gardens and public areas. Ethnomedically, *B. disticha* is used as chewing stick and the treatment of fever and malaria in Nigeria. Also it is used in treating headaches, toothaches and tooth infections[1]. Few studies have been published regarding the phytochemistry and biological activity of *B. disticha* which showed the isolation of two amide derivative breynivosamides A and B and two dioxopiperazine derivatives breynivosines A and B along with asperphenamate, cristatin A, breynioside A, robustaside A (E)-p-coumaric acid, blumenol A and lygodinolide were isolated from the leaves of BD[2]. Regarding its biological activity, studies have been reported for the leaves showed antifungal, antibacterial[3], antiplasmodial[1] and antioxidant activities[4]. Hence, further investigation of *B. disticha* biological activities and phytochemical investigation of compounds are required.

The aim of this study was to carry out a phytochemical study of the alcoholic extract of the aerial parts of *B. disticha* cultivated in Egypt as well as the evaluation of its antioxidant, cytotoxic and hepatoprotective activity.

MATERIALS AND METHODS

Phytochemical study

Plant material: The aerial parts of *Breynia distacha* J. R. Forst. and G. Forst. were collected from Al Zoharia gardens in Egypt and authenticated by Mrs. Trease Labib Consultant of Plant Taxonomy at the Ministry of Agriculture, Egypt. This study was conducted between November, 2017 and May, 2018 voucher specimen number (20.6.18.1) is kept in the department of Pharmacognosy, Faculty of Pharmacy, Cairo University. The aerial parts of *B. disticha* were air dried and grinded for extraction. The dried aerial parts of plant (900 g) were subjected to continuous extraction apparatus (Soxhlet) successively and exhaustively using solvents with increasing polarity in the following order petroleum ether 40-60°C, ether, dichloromethane, ethyl acetate, methanol and 70% aqueous methanol (Solvent used of HPLC grade). For each solvent the extraction was continued till exhaustion. The solvent was stripped off by distillation under reduced pressure at a temperature not exceeding 40°C, 150 g of the powdered plant were macerated with 100% methanol to be used as crude extract and distilled till dryness.

Chemicals: Polyamide Carl Roth, Sephadex LH-20 Sigma-Aldrich, Whatman paper No. 3. All chemicals used in the present study are fine chemicals and solvents used are HPLC grade.

Extraction and Isolation: About 70% aqueous methanol successive extract (75.38 g) was lyophilized to remove any traces of water. About 23 g of the dried extract was applied to a polyamide column chromatography and eluted with solvent system (water: methanol) step wise starting with 100% water till 100% methanol to afford 13 sub fractions after monitoring on paper chromatography using 5% acetic acid as eluent. Fraction 6 (0.7703 mg) was applied on whatman paper chromatography No. 3 using 5% acetic acid as eluent to yield compound I (14 mg) and compound II (10 mg).

General experimental procedure: Bruker High Performance Digital FT-NMR Spectrometer Avance III 400 MHz. UPLC MS/MS "Waters" 3100 "USA", TQ Detector (Acquity ultra performance LC), Mass lynx V 4.1. UV-visible spectrophotometer double beam UVD-3500.

General acid hydrolysis method: Acid hydrolysis was performed by using 6% aqueous hydrochloric acid. The 6% aqueous hydrochloric acid was evaporated and washed with water several times. The mixture was extracted with ethyl acetate, then the aqueous layer was neutralized (by repeated addition of MeOH and evaporation) for determination of the released sugar moiety by using whatman paper chromatography No. 1 with Butanol: Acetic acid: Water (4:1:5 v/v/v) as eluent and aniline phthalate was employed as a spray reagent for color detection of the sugars.

Biological studies

Chemicals: CCL_4 was obtained from El-Gomhouria Company for drug and chemicals, Egypt, silymarin was obtained from Sigma-Aldrich, USA. All other chemicals, used throughout the experiment were of the pro-analysis grade available. Kits used in measurement of serum aspartate transaminase (AST), alanine transaminase (ALT), malondialdehyde (MDA), Nitric Oxide (NO) and reduced glutathione (GSH) superoxide dismutase (SOD) were purchased from Biodiagnostic, Inc. (Egypt).

Animals: Swiss mice of 20-30 g body weight were used for the acute toxicity study. Wister male rats, weighing from 100-130 g were used throughout the hepatoprotective experiment. The rats were obtained from the animal house colony of the National Research Centre, Dokki, Giza, Egypt.

Animal procedures were performed in accordance with the Ethics Committee of the National Research Centre and followed the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals registration number 16045 and according to ethics committee of Faculty of Pharmacy, Cairo University serial number MP (1293).

Methods

Cytotoxic and antioxidant activities: The successive extracts and crude extract of aerial parts of *B. disticha* along with two new compounds were subjected to *in vitro* cytotoxicity bioassay using MTT method according to Mosmann[5] using Doxorubicin as positive control and to antioxidant activity using DPPH$^{\bullet}$ method using Ascorbic acid as positive control according to Ratty *et al.*[6].

Acute toxicity study: Selected 48 mice of uniform weight are taken and divided into 8 groups each of six for each male and female mice group. The successive extracts and crude methanolic extract of *B. disticha* were given orally and separately to mice in graded doses up to 2 g kg^{-1} [7]. The control group received the same volumes of distilled water. The percentage mortality for extracts was recorded 24 h later. Mice were observed for 14 days, for any changes in the skin and fur, respiratory, circulatory, autonomic, central nervous systems, somatomotor activity and behavioral pattern. Particular observation for tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma was done.

Hepatoprotective study

Experimental design: After 1 week of acclimation, the rats were fasted overnight before treatment and were divided randomly into six groups, each of 10 rats as follows:

- Group 1 : Normal healthy control rats
- Group 2 : CCl_4-intoxicated rats: rats injected intraperitoneally with a single dose of CCl_4 was as a mixture of corn oil and CCl_4 (1:1, 1 mL kg^{-1} body weight, 0.5 mL CCl_4+0.5 mL corn oil)[8]
- Group 3 : Rats treated with silymarin (200 mg kg^{-1}/day)[9] for 14 days then injected with CCl_4
- Groups 4-5 : Rats treated with polar and non polar fractions of *Breynia disticha* for 14 days then injected with CCl_4

The 24 h post CCl_4 injection, blood samples were collected from each animal in all groups into sterilized tubes for serum separation. Serum was separated for biochemical serum analysis. After blood collection, the rats of each group were sacrificed under ether anesthesia and their livers were collected, weighed and washed using chilled saline solution. The liver parts were minced and homogenized in ice-cold bi-distilled water to yield 10% homogenates. The homogenates were centrifuged and the supernatants were used for biochemical tissue analysis. Other liver parts were collected and were used for histopathological study.

Histopathological examinations: Livers of all animals were dissected immediately after death. Serial sections of 5µm thick were cut and stained with haematoxylin and eosin for histopathological investigation and polysaccharides were detected by PAS (periodic acid Schiff) according to Gamble[10], Jones *et al.*[11] and Myers *et al.*[12] method.

Statistical analysis: All the results were expressed as Mean±SEM. Analyses were processed using Graph Pad Prism software for Windows (version 6.0, Graph Pad Software, Inc., San Diego, CA). The significance of difference among the studied groups was determined using one-way analysis of variance (ANOVA) followed by Tukey *post-hoc* test. Values with $p<0.05$ were considered significant.

RESULTS AND DISCUSSION

Spectral studies of isolated compounds: Compound 1 isolated as yellow amorphous powder; UV (λmax) nm: (MeOH): 359, 258; (+NaOMe): 409, 272; (+$AlCl_3$): 412, 274; (+$AlCl_3$/HCl): 403, 269; (+NaOAc): 370, 269; (+NaOAc/H_3BO_3) 379, 262. The spectrums of compound 1 shown in Fig. 1-5.

The UV shifts indicated a C3-O-substituted flavonol with the presence of free 4'-OH and free hydroxyl group at position C7 and C5. ESI-MS showed molecular ion peak at (-ve) m/z: 595 $[M-H]^-$. The acid hydrolysis of compound 1 revealed the presence of xylose and galactose. The ^{13}C and ^{1}H NMR (DMSO-d_6) spectra of compound 1 (Table 1) showed the presence of characteristic signals of 3-O-substituted quercetin. The coupling constant of the anomeric proton of galactose at 5.38 ppm and xylose at 4.02 of 7.24 and 7.3, respectively, revealed the diaxial coupling of β-configuration of galactopyranoside and xylopyranosyl. Furthermore, the NMR spectra showed up field shift of C6'' at 68.06 ppm of galactose and H'''1 of xylose at 4.02 ppm indicating 1→6 attachment which is confirmed by HMBC correlation between C6 of galactose and H1''' of xylose. Thus, compound 1 is identified as new compound namely, Quercetin 3-O-ß-D-xylopyranoside (1?6)-ß-D-galactopyranoside as shown in Fig. 6.

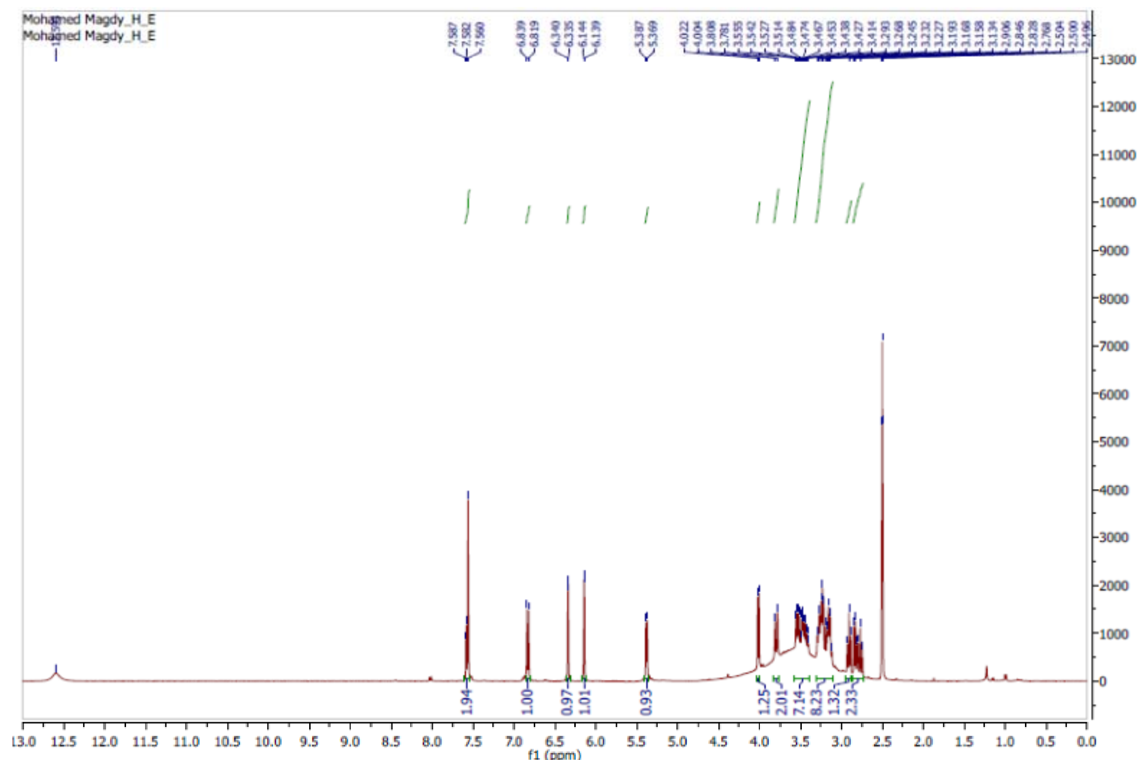

Fig. 1: ^{1}H-NMR spectrum of compound 1

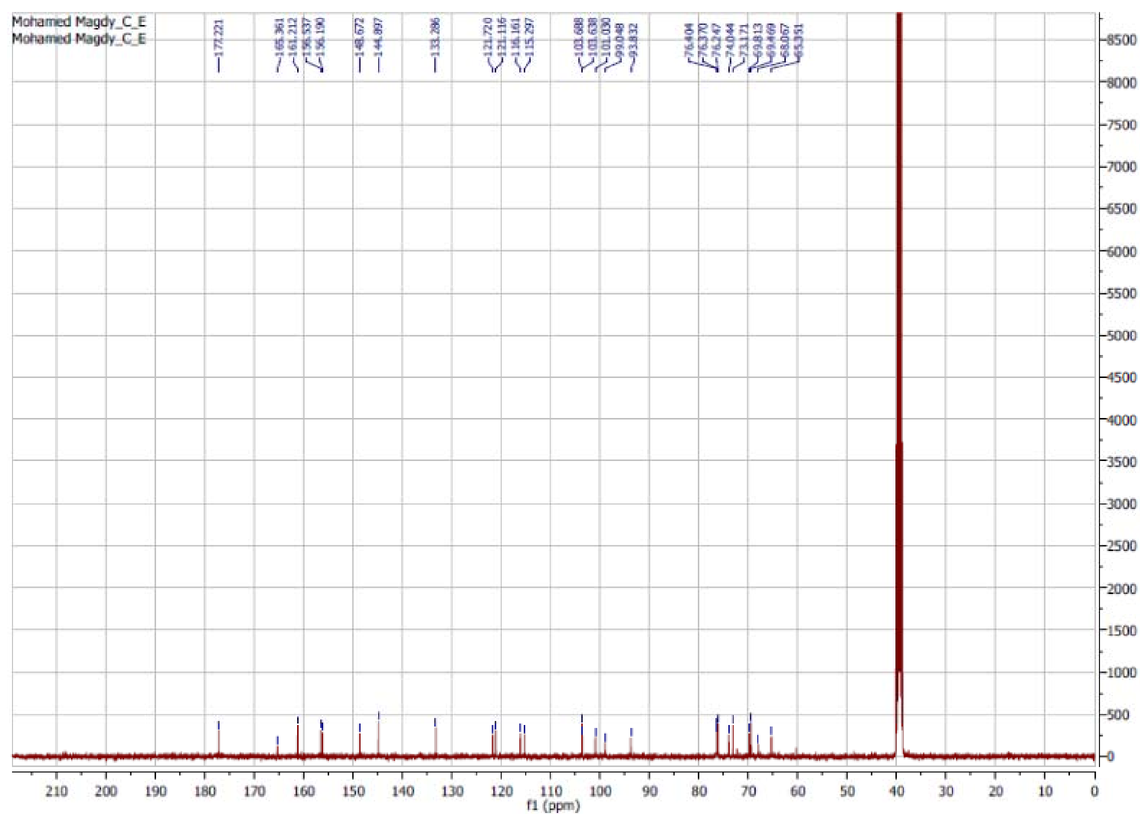

Fig. 2: ^{13}C NMR spectrum of compound 1

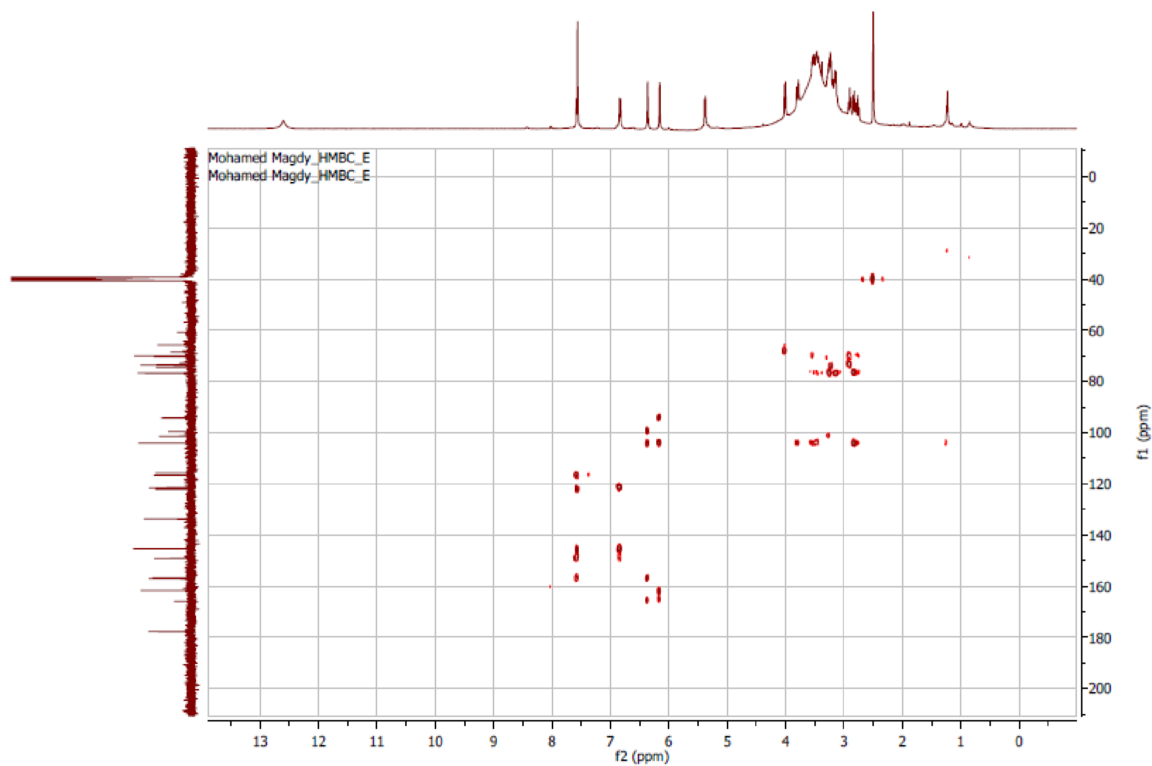

Fig. 3: HMBC spectrum of compound 1

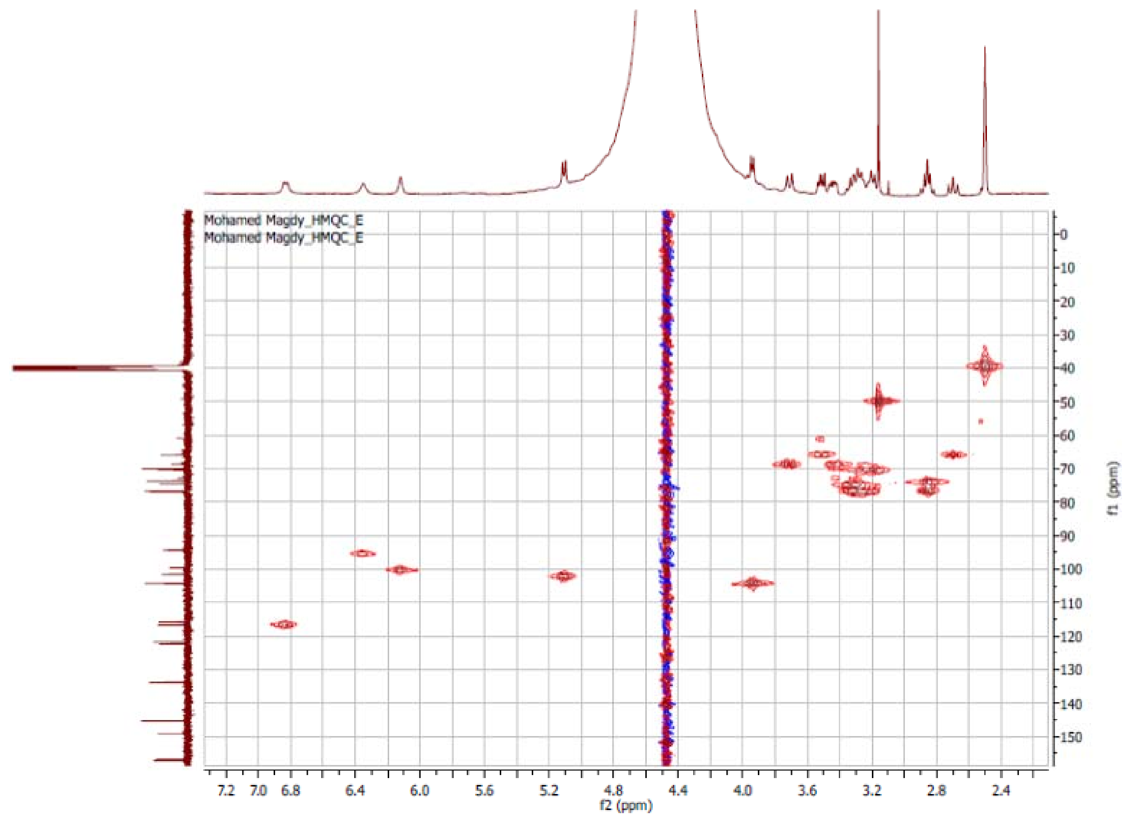

Fig. 4: HMQC spectrum of compound 1

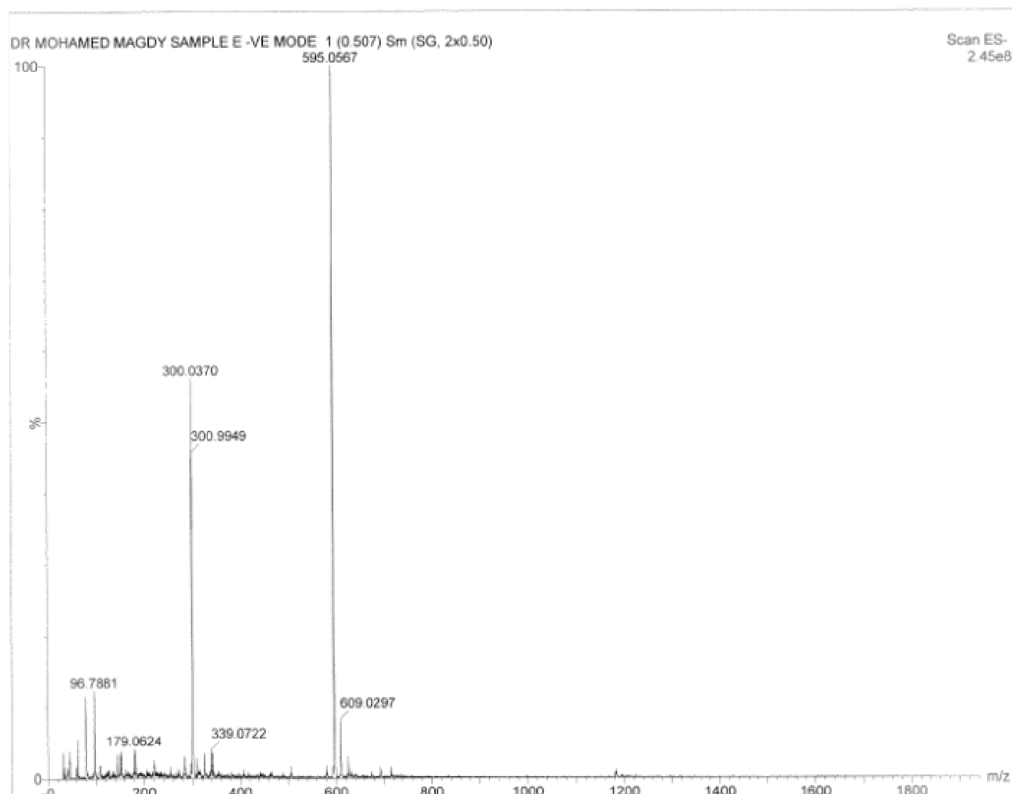

Fig. 5: m/z spectrum of compound 1

Table 1: ^{1}H and ^{13}C NMR data of compound 1

Compounds	δ [13]C	δ ^{1}H coupling constant
Chemical shift (δ, ppm)[a]		
C2	156.19	
C3	133.28	
C4	177.22	
C5	161.21	
C6	99.04	6.15, d (1.84)
C7	165.36	
C8	93.83	6.34, d (1.7)
C9	156.53	
C10	103.68	
1'	121.72	
2'	115.29	7.58, d (2.0)
3'	144.89	
4'	148.67	
5'	116.16	6.83, d (8.2)
6'	121.11	7.58, d (8.9)
1''	101.03	5.38, d (7.24)
2''	76.37	
3''	74.04	
4''	69.46	
5''	73.17	
6''	68.06	
1'''	103.63	4.02, d (7.3)
2'''	76.24	
3'''	76.40	
4'''	69.81	
5'''	65.35	

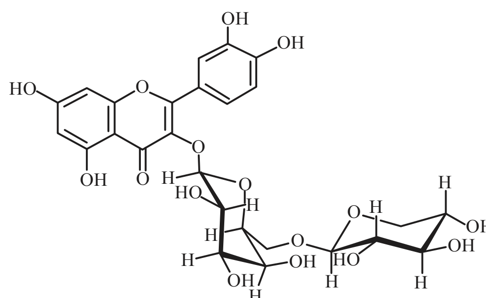

Fig. 6: Chemical structure of compound 1

Compound 2 is a yellow amorphous powder; UV (λmax) nm: (MeOH): 267, 354; (+NaOMe): 274, 402; (+$AlCl_3$): 275, 368; (+$AlCl_3$/HCl): 274, 350; (+NaOAc): 269,361; (+NaOAc/H_3BO_3) 265, 357. The spectra of compound 2 presented in Fig. 7-9. Similar to compound 1 the UV spectra of compound 2 after addition of shift reagents indicted that compound 2 is a 3-O-substituted flavonol. ESI-MS showed molecular ion peak at (-ve) m/z: 579 $[M-H]^-$. The acid hydrolysis of compound 2 indicated the presence of xylose and galactose.

The ^{13}C and ^{1}H NMR (DMSO-d_6) data of compound 2 (Table 2) showed the presence of characteristic resonances of kaempferol linked to 3-O-β-D-xylopyranoside (1→6) β-D-galactopyranoside as compound 1. Thus, compound 2 is

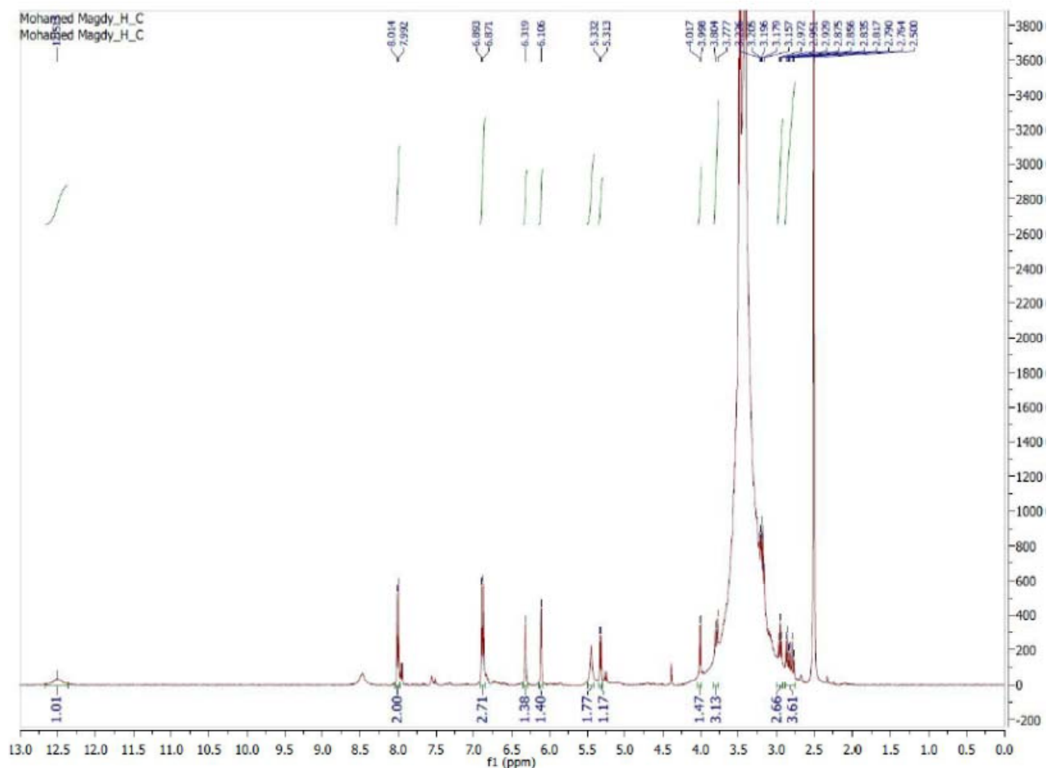

Fig. 7: ^{1}H NMR spectrum of compound 2

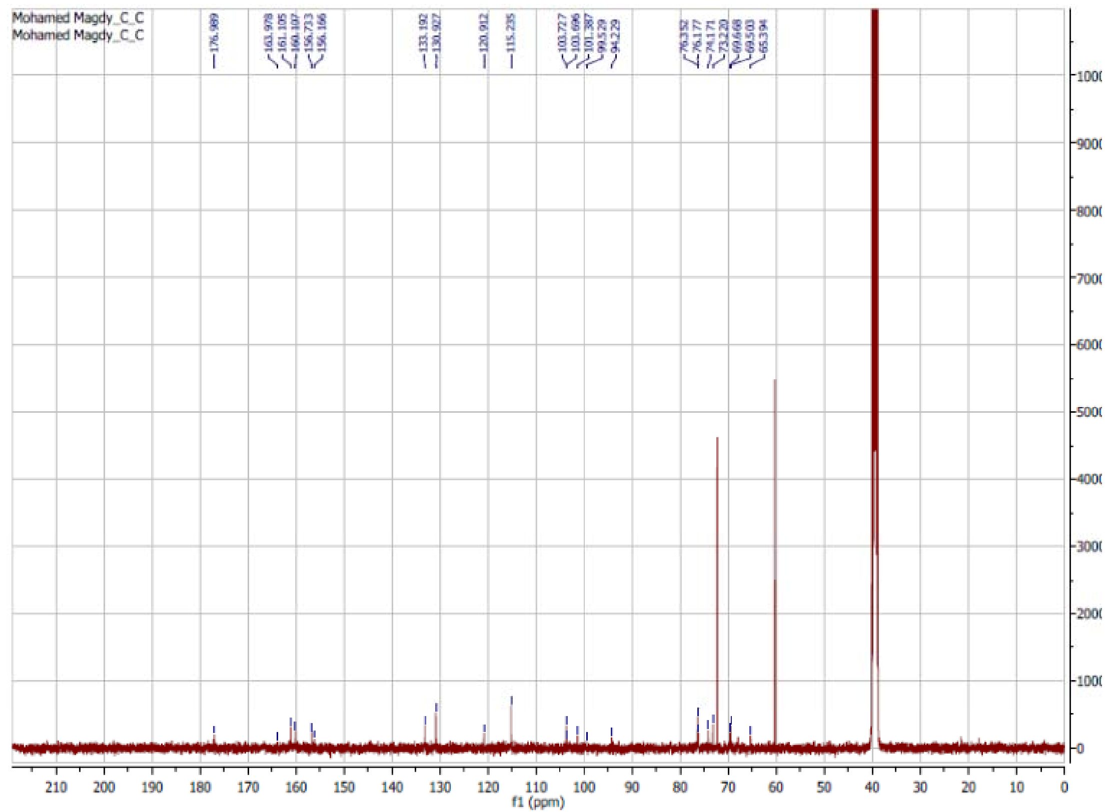

Fig. 8: ^{13}C NMR spectrum of compound 2

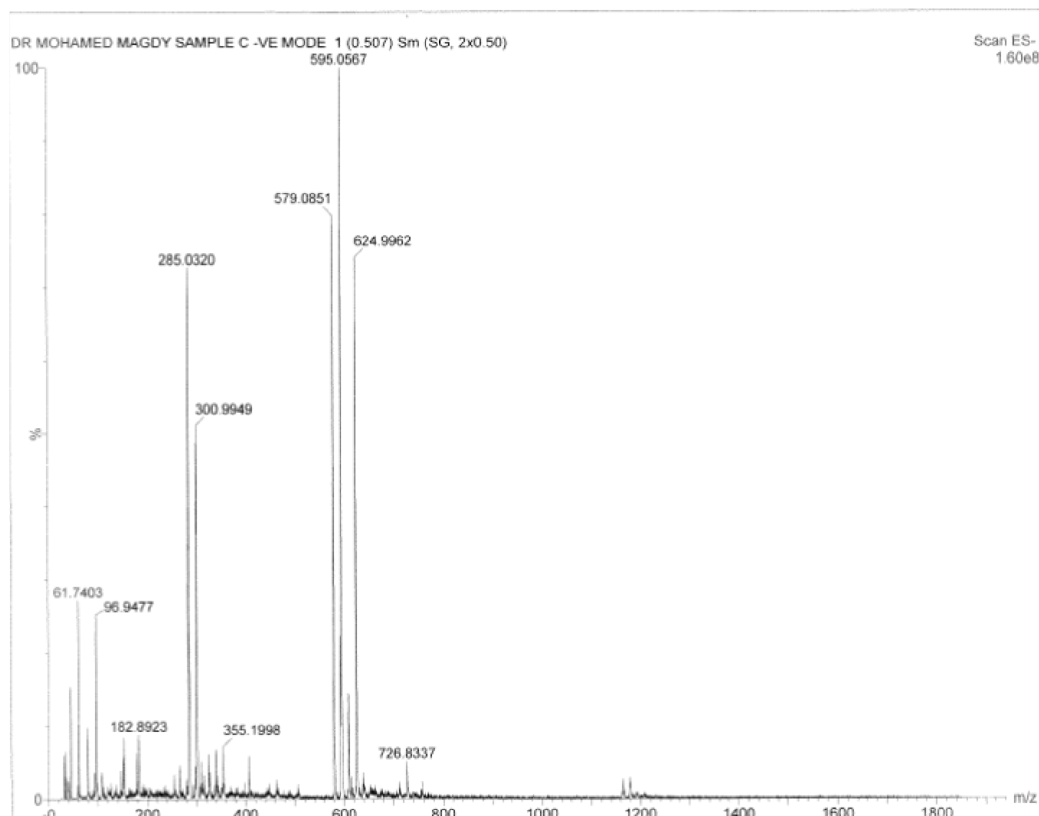

Fig. 9: m/z spectrum of compound 2

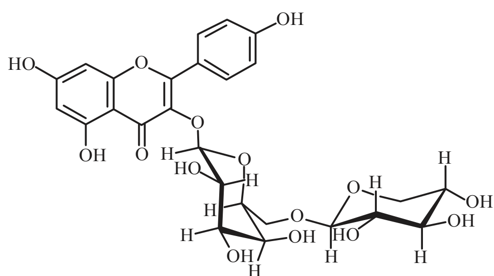

Fig. 10: Chemical structure of compound 2

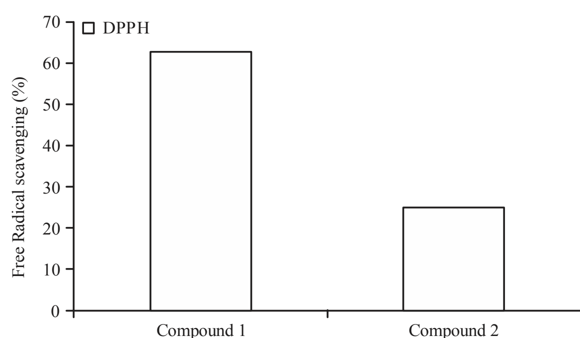

Fig. 11: Free radical scavenging activity of compound 1 and 2 on DPPH• at concentration 85 µM

identified as new compound namely, Kaempferol 3-O-β-D-xylopyranoside (1→6)-β-D-galactopyranoside as shown in Fig. 10.

Antioxidant activity: The crude methanolic extract and the successive extracts were preliminary screened at 50 ppm for their free radical scavenging activity and were further assayed at lower concentrations to calculate their EC_{50} values for each one.

The successive extracts of methanol and 70% aqueous methanol were the most active extracts among others with EC_{50} 37±1.03 and 47.1±0.05, respectively. On the other hand, compound 1 and 2 isolated from the 70% successive aqueous methanol results on DPPH• showed that compound 1 (63.1%) has more free radical scavenging activity compared to compound 2 (25.1%) (Fig. 11).

Cytotoxic activity: The study of *in vitro* screening of the antiproliferative activities of successive extracts and crude extract of *B. disticha* against human cancer cell line hepatocellular carcinoma (HepG2) showed no activity. However, screening of the new compounds 1 and 2 on different cell lines HepG2, MCF-7 (breast cell line) and A-549

(lung cell line) showed that compound 1 have significant cytotoxic activity on HepG2 in comparison with compound 2 as shown in Fig. 12.

Acute toxicity study: The results showed no mortality after 24 h of oral administration of the successive extracts as well as total crude methanolic extracts of *Breynia distacha* J.R.Forst. and G.Forst. at graded doses up to a 2 g kg^{-1}. So the experimental doses used were 1/10 of 2 g kg^{-1} of each extract (400 mg kg^{-1}). After 15 days of single oral administration of the crude and successive extracts, the results revealed that no significant change in skin and fur, respiratory, circulatory, autonomic, central nervous systems, somatomotor activity and behavioral pattern.

Hepatoprotective activity

Effect on liver enzymes: Intraperitoneal injection with a single dose of CCl_4 showed significant elevation in their serum enzyme levels of ALT, AST, ALP and total bilirubin after 24 h as compared with control group. Pre-treatment with *Breynia disticha* J.R.Forst. and G.Forst. non polar (petroleum ether, ether and dichloromethane mixed extracts) showed reduction in elevated serum ALT, AST levels and total bilirubin while *Breynia disticha* J.R.Forst. and G.Forst. polar (ethyl acetate, methanol and 70% aqueous methanol mixed extracts) showed reduction in elevated serum ALT, AST, ALP and total bilirubin as compared with CCl_4 treated group. Regarding, pretreatment with silymarin exhibited significant reduction in serum ALT, AST and total bilirubin as compared with CCl_4 treated group (Table 3).

Effect on oxidative stress biomarkers: The liver contents of NO and MDA were significantly (p<0.05) increased by 11.22 and 50.55% , while the liver contents of GSH and SOD were significantly decreased by 44.28 and 65.85%, respectively in rats injected with a single dose of CCl_4 as compared to the normal group. Pre-treatment with *Breynia disticha* J.R.Forst.

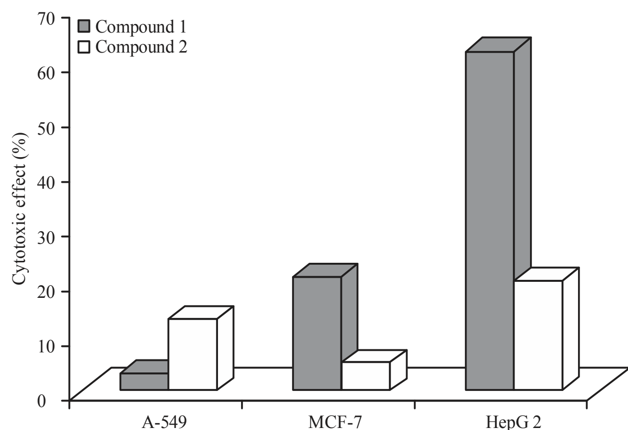

Fig. 12: Cytotoxic effect of compound 1 and 2 on 3 cancer cell lines, HepG 2, MCF-7 and A-549 at concentration 170 µM from the compound used

Table 2: ^{1}H and ^{13}C NMR data of compound 2

Compounds	δ ^{13}C	δ ^{1}H (coupling constant)
	Chemical shift (δ, ppm)[a]	
C2	160.10	
C3	133.19	
C4	176.98	
C5	161.10	
C6	99.52	6.11, s
C7	163.97	
C8	94.22	6.32, s
C9	156.73	
C10	103.72	
1'	120.91	
2'	130.92	8.01, d (8.8)
3'	115.23	6.89, d (8.8)
4'	156.16	
5'	115.23	
6'	130.92	
1''	101.38	5.33, d (7.3)
2''	76.35	
3''	74.17	
4''	69.50	
5''	73.22	
6''	68.40	
1'''	103.69	4.01,d (7.36)
2'''	76.17	
3'''	76.35	
4'''	69.66	
5'''	65.39	

Table 3: Effects of polar and non polar fractions of *Breynia disticha* J.R.Forst. and G.Forst. on liver enzymes and serum bilirubin concentration in rats treated with CCl_4

Parameters	Normal control	CCl_4	Silymarin	*Breynia disticha* non polar fraction	*Breynia disticha* polar fraction
ALT (U mL^{-1})	46.97±0.41	91.98±1.54[a]	68.78±0.72[a,b]	84.92±4.92[a]	74.78±5.36[a,b]
AST (U mL^{-1})	111.01±1.10	146.18±0.69[a]	130.35±0.84[a,b]	130.85±2.76[a,b]	123.18±4.93[a,b]
Alkaline phosphatase (IU L^{-1})	190.72±9.28	345.70±0.94[a]	313.12±3.11[a]	350.90±1.71[a]	258.37±15.90[a,b]
Total bilirubin (mg dL^{-1})	1.32±0.06	3.54±0.45[a]	2.28±0.09[b]	2.51±0.17[a]	1.71±0.18[b]

Data were expressed as Mean±SE (n = 6), Statistical analysis was carried out by one-way ANOVA followed by Tukey HSD test for multiple comparisons, [a]Significantly different from normal control at p<0.05, [b]Significantly different from CCl_4 control at p<0.05

Table 4: Effects of pretreatment with polar and non polar fraction of *Breynia distich*a J. R. Forst and G. Forst on oxidative stress and antioxidant status

Parameters	Normal control	CCl_4	Silymarin	*Breynia disticha* non polar fraction	*Breynia disticha* polar fraction
NO (µmol g^{-1} tissue)	0.041±0.000	0.046±0.001^a	0.045±0.001^a	0.041±0.001^b	0.039±0.001^b
MDA (nmol g^{-1} tissue)	6.000±0.18	9.03±0.17^a	6.68±0.17^b	7.120±0.64^b	6.040±0.19^b
GSH (mg g^{-1} tissue)	22.160±0.55	12.35±0.19^a	19.70±1.37^b	19.680±1.22^b	21.570±0.83^b
SOD (U g^{-1} tissue)	33.420±3.08	11.41±2.26^a	14.67±3.08^a	17.120±2.20	33.020±5.08^b

Data were expressed as Mean±SE (n = 6), statistical analysis was carried out by one-way ANOVA followed by Tukey HSD test for multiple comparisons, ^{a}Significantly different from normal control at $p<0.05$, ^{b}Significantly different from CCl_4 control at $p<0.05$

Table 5: Results of immuno-histo-morphometric measurements of Caspase-3 Area (%) and PAS Area (%) to the all tested groups

	Parameters	
Groups	Caspase-3 area (%)	PAS area (%)
Saline group	4.03±0.06^b	11.06±1.09^b
CCL_4 group	20.45±0.09^a	32.55±1.44^a
Silymarin group	8.06±1.09^{a,b}	16.31±1.29^{a,b}
B. disticha non polar extract	10.32±1.06^{a,b}	22.13±1.25^{a,b}
B. disticha polar extract	7.06±0.03^b	20.03±3.14^{a,b}

Data were expressed as Mean±SE. Statistical analysis was carried out by one-way ANOVA. ^{a}Significantly different from normal control at $p<0.05$, ^{b}Significantly different from CCl_4 control at $p<0.05$

and G.Forst. non polar showed a significant ($p<0.05$) reduction in liver content of NO by 10.49% and normalized MDA and GSH only while *Breynia disticha* J.R.Forst. and G.Forst. polar decreased liver content of NO by 13.51% and normalized MDA, GSH and SOD, as compared with CCl_4 treated group. Pretreatment with silymarin normalized liver content of MDA while normalized liver contents of GSH and SOD, as compared with CCl_4 treated group (Table 4).

Histopathological results: Histopathological examination of liver tissue for all examined groups revealed in the negative group which received only saline it has normal hepatic architecture with average hepatic cords thickness which are radiating from central vein centrally to portal tract at the periphery with healthy hepatocytes have intact cell membrane and nuclei (Fig. 13a). In contrast the pathological changes which happened in the positive control group which received CCL_4 showing destructive hepatic architecture and the central veins are congested (Fig. 13b). An improvement has been noticed in the 3rd group (reference) which is treated by silymarin has more or less normal hepatic architecture with few number of degenerated hepatocytes (Fig. 13c). The 4th group which is treated by *B. disticha* non polar extract showing signs of recovery as there are foci of regenerative hepatic tissue with residual degenerated hepatocytes (Fig. 13d). The much better improvement has been occurred in the 5th group which treated by *B. disticha* polar extract as there is total repair of hepatic tissue unless the dilated hepatic vessels (Fig. 13e).

Immunohistochemical assessments: Further histopathological evaluation was done using quantitative morphometric analysis of the pathological changes. The amount of liver tissue affected by degenerated hepatocytes were rather intensely cytoplasmic stained (damaged area) which was determined using a computer-assisted automated image analyzer. It has been noticed that the highest damaged area was in the 2nd group which received CCl_4 20.45±0.09 which is statistically significant in both 4th and 5th groups which received *Breynia disticha* whether non-polar 10.32±1.06 or the polar fraction 7.06±0.03 as shown in Table 5.

Assessment of polysaccharides area % using Periodic acid-Schiff stain (PAS): Histomorphometric analysis to determine the amount of polysaccharides was carried out on PAS stained sections, we found that the amount of polysaccharides was the greatest in the CCl_4 group 32.55±1.44. On the other hand, the groups which treated by *B. disticha* (non-polar extract) and *B. disticha* (polar extract) resulted in a significant reduction of polysaccharides amount in these groups by 22.13±1.25 and 20.03±3.14 respectively (Table 5).

DISCUSSION

Since ancient times and in traditional medicine natural products have been used to treat many diseases. Plants were used as concoctions or as concentrated extracts to treat different pathological conditions without identifying or knowing its active compounds. However, modern medicine targets the isolation and purification of biologically active compounds. Natural products have diverse of compounds approved by the FDA and used in treatment of several illnesses[13,14].

The chemical composition of *B. disticha* leaves were previously reported[2] for the first time. However, there is no reported data regarding its hepatoprotective activity.

In the present study, *B. disticha* successive extracts showed no acute toxicity up to 2 g kg^{-1} that confirm their use

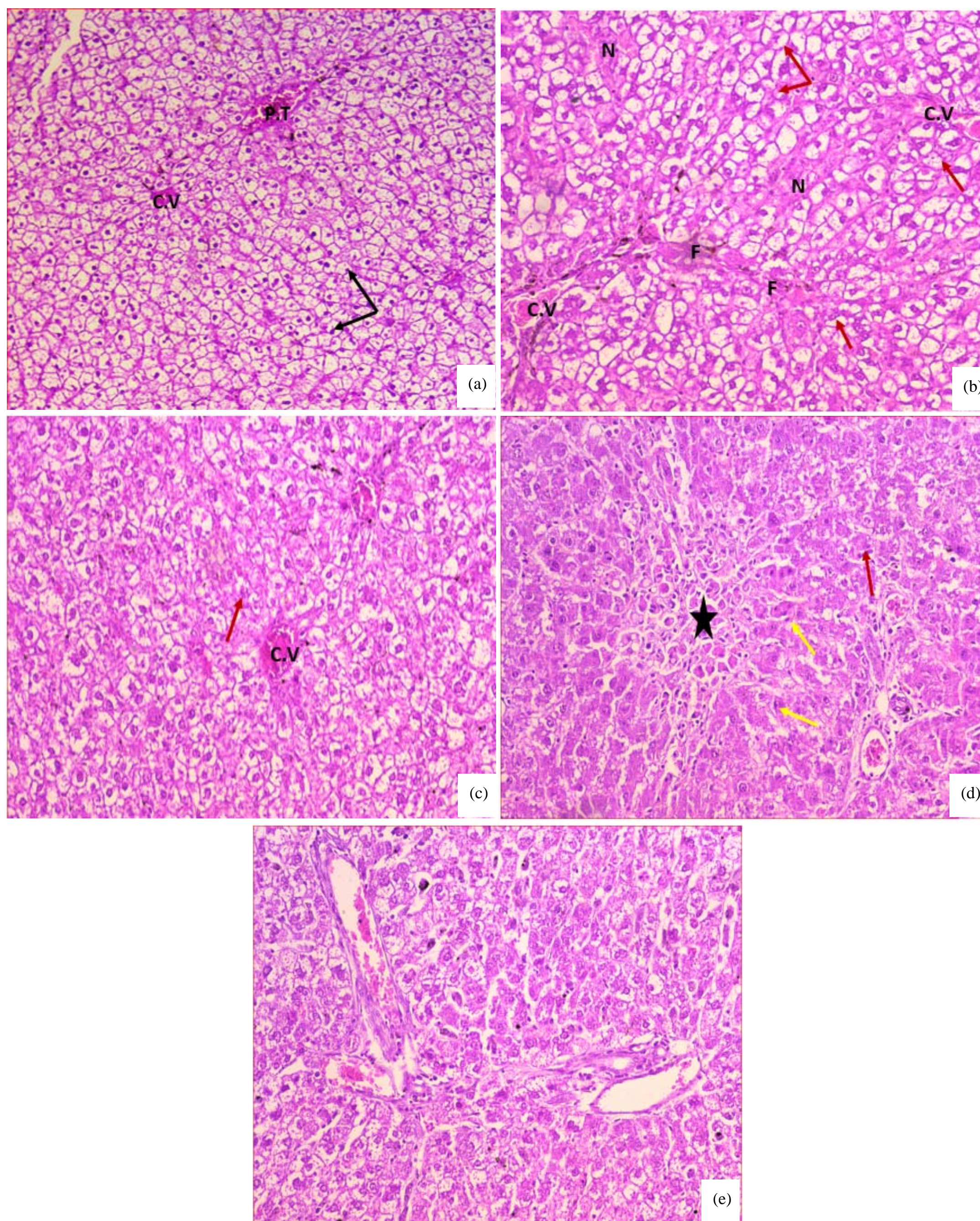

Fig. 13(a-e): A photomicrography of hepatic tissue for all examined groups: (a) Saline, (b) CCl_4, (c) Silymarin, (d) *B. disticha* (non polar extract) and (e) *B. disticha* (polar extract)

C.V: Central vein, P.T: Portal tract, Black arrows: Intact hepatocytes, Red arrows: Degenerated hepatocytes, Black star: Regenerated hepatic tissue

as chewing stick in traditional medicine[15]. The successive methanolic and 70% aqueous methanolic extract showed antioxidant activity indicating that the most promising activity was detected in the polar fractions[16].

Regarding cytotoxic activity of *B. disticha* against HepG2, no activity was detected this is in agreement with reported that previously reported[3] no cytotoxicity was observed by using Brine Shrimp Lethality Assay.

Upon studying the polar fraction of *B. disticha*, it showed hepatoprotective activity by reducing elevated serum levels of ALT, AST, ALP and total bilirubin showing the ability of *B. disticha* to prevent hepatocytes injury induced by CCL_4. Also

B. disticha polar fraction restores liver content of MDA, GSH and SOD and decreased content of NO as compared with CCl_4 group indicating the antioxidant properties of *B. disticha*. Pathological results revealed that *B. disticha* capable of repairing hepatic tissue and reduced toxic effect of CCL_4. Gowda *et al.*[17] stated that *Breyniavitis-idaea* (burm.f) c. fisher. showed significant increase in the cell viability (%) in evaluating its hepatoprotective activity using *in vitro* MTT assay. Phytochemical investigation of the successive 70% aqueous methanol extract using different tools of chromatography led to the isolation of two new compounds, quercetin 3-O-β-D-xylopyranoside (1→6) β-D-galactopyranoside and kaempferol 3-O-β-D-xylopyranoside (1→6) β-D-galactopyranoside. The antioxidant activity of quercetin is higher than that of Kaempferol due to 3',4'-dihydroxy substitution in the B ring, as in quercetin, increases the antioxidant activity substantially compared with a mono-hydroxy substituent as in kaempferol [18]. Also, Umeokolia *et al.*[2] isolates two new amide derivatives and two new dioxopiperazine derivatives from the ethyl acetate fraction of *Breynia nivosa*.

CONCLUSION

Breynia disticha showed no acute toxicity and possessed hepatoprotective and antioxidant activities, along with two new compounds isolated. Thus, *Breynia* is a promising natural source of new compounds that need more investigation it can be used in new biologically active drug discovery.

SIGNIFICANCE STATEMENT

This study discovers the hepatoprotective and antioxidant activity of *B. disticha* that can be beneficial as alternative treatment. The investigation of plant compounds is effective method for identification of new active compounds that can be used in drug industry. This study will help the researcher to uncover the critical areas of *B. disticha* that many researchers were not able to explore. Thus a new theory on chemical investigation and biological activity of *B. disticha* may be arrived at.

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