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ANTIOXIDANT, HEPATOPROTECTIVE AND ANTIMICROBIAL ACTIVITIES OF THE AERIAL PARTS OF *POLYGONUM BELLARDII* All.

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اظهر مستخلص الميثانول واجزاؤه المختلفة بالإضافة الى المركبات الفينولية (**1-6**) التي تم فصلها من نبات البوليجونم بيلاردى الين (الأجزاء الهوائية المزهرة) فاعلية ملحوظة كمضادات للاكسدة وذلك عن طريق تقنية الدى بى إتش،وقد أظهرت الدراسة أن المستخلص الميثانولى آمن حتى جرعة 4 جم/كجم، كما أنها أدت الى تأثيرات واضحة فى حماية كبد فئران التجارب من التدمير بإستخدام رابع كلوريد الكربون، كما أنها أظهرت ايضا تاثيرات مضادة للبكتريا موجبة والسالبة الجرام بالإضافة الى نشاطها كمضادات للفطريات.

وقد أظهر تعيين المحتوى الكلى لمركبات الفلافونيدات وإستخدام مادة الكوارسيتين كمقياس لذلك وجـود 185.2 ± 1.03 مجم/جم من وزن المستخلص الجاف عن طريق التحليل الطيفى.

*The methanolic extract of Polygonum bellardii All. (flowering aerial parts) and its fractions in addition to the isolated compounds (**1-6**) showed significant antioxidant potential by DPPH˙ scavenging activity technique. It also showed hepatoprotective activity against carbon tetrachloride (CCl_4) induced hepatic injury in albino rats. The fractions exhibited antibacterial activity against both Gram positive and Gram negative bacteria in addition to antifungal activity. The total methanolic extract of Polygonum bellardii All. did not show signs of toxicity and mortality up to 4 g/kg dose.*

Determination of total flavonoid content with respect to quercetin spectrophotometrically indicated the presence of 185.2±1.03 mg/g dried extract.

INTRODUCTION

The genus *Polygonum* (Polygonaceae) comprises about 200 species mostly distributed in the North temperate countries, few are tropical or in the Southern hemisphere[1.] Many species of the genus *Polygonum* had been reported to exhibit a variety of interesting biological activities[2-4]. A variety of chemical constituents such as flavonoids[5], anthraquinones[6], sesquiterpenoids[7], lignans[8], coumarins[9] and stilbenes[10] have been reported in the genus *Polygonum.*

Flavonoids are known for their powerful free radical scavenging activity[11]. Reactive oxygen species such as superoxide anion and hydroxyl radical, which play an important role in the toxic effects of numerous foreign compounds (xenobiotics), have been shown to be effectively detoxified by a variety of flavonoids[12]. In liver cells, the antioxidant activity of flavonoids has been related to an observed hepatoprotective effect of these compounds. Flavonoids are thought possibly to exert a membrane-stabilizing action, thus inhibiting radical-induced lipid peroxidation[13]. Carbon tetrachloride (CCl_4), a well-known potent hepatotoxic agent, is being used extensively to investigate hepatoprotective activity on various experimental animals[14]. Hepatic damage induced by CCl_4 resulted in an increase in serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT). The elevation of concentrations of these two serum enzymes is generally regarded as one of the sensitive markers of hepatic damage[14].

The chemical composition of genus *Polygonum* especially its flavonoidal content as well as its reported pharmacological and antimicrobial activities provoked us to investigate the biological and antimicrobial activities of *Polygonum bellardii* All. together with its total flavonoid content spectrophotometrically.

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MATERIAL AND METHODS

Plant material

The aerial parts of *Polygonum bellardii* All. were collected during flowering stage in April 2007 from Assiut governorate and kindly identified and authenticated by Prof. Dr. Moamen. M. Zarea, Professor of Plant Taxonomy, Department of Botany, Faculty of Science, Assiut University, Assiut, Egypt. A voucher specimen was kept in the Department of Pharmacognosy, Faculty of Pharmacy, Al-Azhar University, Assiut, Egypt, (Pb001).

Instruments

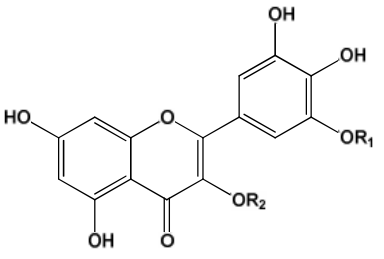
Ultrospec 1000, UV/visible Spectrometer, Pharmacia Biotech, Cambridge, England, Table-Top low speed Centrifuge L-500 (max speed 5000 r/min.), Xiang Yi, Human Lab Instrument Co., Korea, and Digital Ultrasonic Cleaner , MTI Corporation, USA. Rotary microtome HM 355 S, Germany and Light Multihead Microscope, Miken Instrument, Ambala Cantt-133001, India.

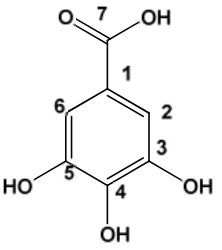
Chemicals

Ascorbic acid and quercetin as an antioxidant standard (were obtained from Sigma-Aldrich Chemicals Co, Germany), Sylimarin as standard hepatoprotective drug (obtained from Pharco Pharmaceutical Co. Egypt) and 2,2-Diphenyl-1-picryl hydrazyl (DPPH) (obtained from Sigma-Aldrich Chemicals Co. Germany). Other chemicals used were of high analytical grade and obtained from Sigma-Aldrich and Merck companies.

Extraction, fractionation and isolation of phenolic compounds

The air-dried powdered flowering aerial parts (2 Kg) of *Polygonum bellardii* All. were extracted by maceration and percolation with methanol till complete exhaustion (1:4 ratio) [four times each 8 L, overnight]. The combined methanolic extracts were concentrated under reduced pressure till constant weight to give a syrupy residue (150 g). The extract was subjected to successive solvent fractionation on VLC with *n*-hexane, chloroform, ethyl acetate, *n*-butanol and methanol till complete exhaustion in each case to give *n*-hexane (20 g), chloroform (25 g), ethyl acetate (20 gm), *n*-butanol (10 g) and methanol (50 g). Using different chromatographic techniques (C.C., LH-20 and RP-18), compounds **1** and **2** were isolated from the chloroform fraction and identified as gallic acid[15] and quercetin[16], respectively, while compounds **3-6** were obtained from the ethyl acetate fraction and identified as quercetin-3-*O*-(5"-acetyl-α-arabinofuranoside)[16] **3**, quercetin-3-*O*-β-D-glucopyranoside[16] **4**, myricetin-3-*O*-(5"-acetyl-α-arabinofuranoside[16] **5**, and myricetin-3-*O*-α-arabinofuranoside[16] **6**. The structures of isolated (tested) compounds (**1-6**) are illustrated in (Fig. 1).





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Compound	R_1	R_2
2	H	H
3	H	acetyl arabinose
4	H	glucose
5	OH	acetyl arabinose
6	OH	arabinose

Fig. 1: Structures of tested compounds **1-6**.

Animals

Male albino rats (each 100-120 g), were bred and housed under standardized environmental conditions in the pre-clinical animal house, Pharmacology Department, Faculty of Medicine, Assiut University, Assiut, Egypt. The animals were fed with standard diet and free access to water. They were kept at 24-28°C temperature, 60-70% relative humidity, and 12 hrs day and night cycle for one week to acclimatize to the environmental conditions.

Test organisms

Bacterial strains used in this study were: *Escherichia coli*, *Pseudomonas aeruginosa* as Gram-negative bacteria and *Staphylococcus aureus*, *Bacillus subtilis* as Gram-positive bacteria. *Candida albicans* was used for determination of antifungal activity. All strains were clinical isolates obtained from the Microbiological Laboratory, Microbiology Department, Faculty of Pharmacy, Al-Azhar University, Assiut, Egypt. All bacterial strains were cultivated in nutrient agar medium and incubated at 37°C for 24 hrs, while *Candida albicans* was cultivated in potato dextrose agar at 28°C for 48 hrs. Nystatin was used as reference antifungal (discs), and Cefotax as reference antibiotic (discs), (Oxoid Co. England).

I- Evaluation of antioxidant activity[17-20]

The antioxidant activity was determined firstly by TLC procedure[17] then by 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$), radical scavenging method[17]. The investigated extracts were dissolved in methanol (1:20, *w/v*) and applied (10 µL) spotwise to TLC plate with silica gel as adsorbent. The plates were developed in an ethyl acetate - formic acid - acetic acid - water (100:11:11:26, *v/v/v/v*) as solvent system. The plates were sprayed with 0.2% (*w/v*) DPPH$^{\bullet}$ in methanol reagent and observed in day light. DPPH$^{\bullet}$ radical scavenging activity was measured by spectro-photometric method[18&19]. One milliliter of methanolic solution of compounds (1-6) isolated from the flowering aerial parts of *Polygonum bellardii* All. of various concentrations (10-200 µg/ml) was mixed with 1 ml of methanolic solution of DPPH$^{\bullet}$ (200 µM). Similarly; 1 ml methanolic solution of ascorbic acid and quercetin of various concentrations (10-200 µg/ml) were mixed with 1 ml of DPPH$^{\bullet}$ solution. A mixture of 1 ml of methanol and 1 ml of methanolic solution of DPPH$^{\bullet}$ (200 µM) served as control. After mixing, all the solutions were incubated in dark for 30 min and then absorbance was measured at 517 nm[20]. The experiments were performed in triplicate using ascorbic acid and quercetin as a positive control standards and % scavenging activity was calculated by using the following formula[20].

$$\%Scavenging = \frac{absorbance\ of\ control - absorbance\ of\ test}{absorbance\ of\ control} \times 100$$

Results of antioxidant activity of total methanolic extract and its fractions are recorded in (Table 1) and illustrated in (Fig. 2)

II- Acute toxicity studies[21]

The acute toxicity study for *Polygonum bellardii* All. was performed using albino rats (120 - 150 g) of either sex. The animals were fasted overnight prior to the experiment and maintained under standard conditions. The total methanolic extract of *Polygonum bellardii* All. was dissolved in 2% tween 80 and administered orally at doses increasing (1, 2, 4 and 8 g/kg b.w.). The animals were remained for 48 hr under observation for any signs and symptoms of toxicity.

III- Hepatoprotective activity[22]

Previously prepared extracts (ethyl acetate, *n*-butanol and total alcohol) were separately taken in weighed amounts (2 and 4 g each) and solubelized in normal saline with the aid of 2% tween-80 to obtain concentrations of 200 and 400 mg/ml.

Experimental design: Rats were divided into 9 groups, each of 4 animals:

Group I Control (vehicle): Received 1% v/v Tween-80 in distilled water (5 ml/kg body weight, p.o.) single daily dose for 7 consecutive days.

Group II (CCl_4 induced): Received 1% v/v Tween-80 in distilled water (5 ml/kg body weight, p.o.) single daily dose for 7 consecutive days.

Group III (standard): Received standard drug Sylimarin (100 mg/ kg body weight

p.o.) single daily dose for 7 consecutive days.

Group IV, V (test-1 and 2): Received total alcohol extract (200 and 400 kg body weight, p.o.) single daily dose for 7 consecutive days.

Group VI, VII (test-3 and 4): Received ethyl acetate extract (200 and 400 kg body weight, p.o.) single daily dose for 7 consecutive days.

Group VIII, IX (test-5 and 6): Received *n*-butanol extract (200 and 400 kg body weight, p.o.) single daily dose for 7 consecutive days.

Groups from II to IX have received CCl_4 in olive oil (1:1 v/v, 0.7 ml/kg body weight i.p.) single dose on the 4th day only in addition to their basic treatments.

Sample collection

All rats were sacrificed by cervical decapitation separately after 24 hrs of the last treatment. Blood samples of each group were collected into sterilized dry centrifuge tubes and rotated at 3000 r/min. for 10 minutes to obtain clear serum. Liver was excised immediately for histopathological examination.

Estimation of biochemical parameters

The clear serum obtained after centrifugation was used for the estimation of serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) and total bilirubin (TB). All the biochemical parameters were measured spectrophotometrically.

Estimation of SGOT and SGPT

It is based on the reaction of ketoglutrate with aspartate in case of AST (=SGOT) to form glutamate and oxaloacetate. In case of ALT (=SGPT) it is based on the reaction of ketoglutrate with alanine to form glutamate and pyruvate. The formed keton (pyruvate or oxaloacetate) reacts with 2,4-dinitro phenyl hydrazine hydrochloride in alkaline solution to give colored complex that determined spectrophotometrically at 505 nm.[23].

Estimation of total bilirubin

It is based on that, the serum bilirubin reacts with diazotized sulphanilic acid to give a purple azo-bilirubin dye, which measured colorimetrically at 540 nm[24&25]. Results of effect of the total extract and fractions of *Polygonum bellardii* All. on SGOT, SGPT and total bilirubin of CCl_4 treated Rats are recorded in (Table 2).

Histopathological study[22]

Liver is dissected out and the liver samples were excised from the experimental animals of each group and washed with normal saline. Initially the materials were fixed in 10% buffered neutral formalin. They were processed for paraffin embedding following the microtome technique. The sections were taken at 5µ thickness processed in alcohol-xylene series and were stained with alum-haemato-xylin and eosin. The sections were examined microscopically for the evaluation of histo-pathological changes of hepatic cells and results are illustrated in (Fig. 3).

IV- Evaluation of antimicrobial activity

Preparation of the extracts for antimicrobial activity

Concentrations of 5, 10, 25, 50, 75, 100, and 200 mg/ml of total methanolic extract and its fractions, *n*-hexane, chloroform, ethyl acetate and *n*-butanol were prepared separately in dimethyl sulphoxide (DMSO)

Determination of antimicrobial activity[26]

Agar cup diffusion method was used to detect inhibition zones caused by the total extract and fractions of the aerial parts of *Polygonum bellardii*. For determination of antibacterial activity bacterial cultures were adjusted to 0.5 McFarland turbidity standards and inoculated on 15 cm diameter nutrient agar plates. For the determination of antimycotic activity, the *C. albicans* was firstly adjusted to the concentration of 10^6 cfu/ml. By using a sterile cork porer and under aseptic conditions, cups were made in the medium in which fixed volumes from the different concentrations of the tested fractions were dispensed to fill the cups, using sterile micropipette. Plates were placed carefully in the incubators at 37°C for 24 hrs for bacteria and at 28°C for 48 hrs for fungi. After incubation the diameter of the clear zone of inhibition surrounding the samples was taken as a measure of the inhibitory power of the sample against the particular test organisms. Any zone of

inhibition was recorded as positive results. Results of the antimicrobial activity of total extract and fractions of *Polygonum bellardii* All. on tested micro-organisms are recorded in (Table 3).

Determination of MIC[27]

The minimum inhibitory concentration (MIC) of the extracts was determined for each of the test organisms in triplicates. To 0.5 ml of varying concentrations of the extracts (0.5, 1, 2.5, 5, 10, 25, and 50 mg/ml), 2 ml of nutrient broth was added and then a loopful of the test organism previously diluted to 0.5 McFarland turbidity standard for (bacterial isolates) and 10^6 cfu/ml (for fungal isolates) was introduced to the tubes. The procedure was repeated 6 times on the test organisms using the standard antibiotic (cefotax for bacteria and nystatin for fungal isolates). A tube containing nutrient broth only was seeded with the test organisms as described above to serve as control. Tubes containing bacterial cultures were then incubated at 37°C for 24 hrs, while tubes containing fungal spore cultures were incubated for 48 hrs at room temperature (28°C). After incubation the tubes were then examined for microbial growth by observing turbidity. Results of minimum inhibitory concentration (MIC) of the total extract and fractions of *Polygonum bellardii* All. are recorded in (Table 4).

V- Estimation of total flavonoids content

Aluminum chloride colorimetric method was used for total flavonoids determination[28]. The total extract of the plant material (0.5 ml of 1:10 g/ml) in methanol was separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. The mixture was kept at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 415 nm. Calibration plot was generated by using quercetin solutions at concentrations from 12.5-100 µg/ml in methanol. Experiments were performed in triplicates and results are recorded as mean in figure 4.

Statistical analysis

Data were analyzed by comparing values for different treatment groups with the values for individual controls. Results are expressed as mean ±S.E (n= 4 animals). The significant differences among values were analyzed using analysis of variance (one-way Anova) followed by Dunnett's t test for comparison between different groups. [$p< 0.05$ was considered as significant, $p< 0.01$ was considered as very significant].

RESULTS AND DISCUSSION

I- Antioxidant activity

DPPH$^{\bullet}$ is a relatively stable free radical which when encounters proton donors such as antioxidants, it gets quenched and the absorbance decreases[29]. In the present study, the direct measurement of radical scavenging activity was used for evaluation of antioxidant activity. Some isolated compounds from the flowering aerial parts of *Polygonum bellardii* exhibited good radical quenching activity against DPPH$^{\bullet}$. Results (Table 1 and Fig. 2) indicated good scavenging activity of the methanol extract and its fractions towards DPPH$^{\bullet}$ in comparison with ascorbic acid and quercetin. The ethyl acetate fraction showed maximum activity in comparison with total methanolic extract and other fractions, followed by *n*-butanol and chloroform fractions successively. The isolated compounds exhibited higher activity than total methanolic extract and fractions. Compounds **1** and **2** (gallic acid and quercetin) showed activity closely related to the references (ascorbic acid and quercetin), followed by compounds **6**, **4**, **5** and **3**. The obtained high antioxidant activity results of the isolated flavonoid compounds (Table 1, Fig. 2) are closely related to the chemical structure of their aglycone moieties and also the position of hydroxyl groups. The antioxidant activity of the aglycone (compound **2**) is more potent than their corresponding glycosides (compounds **3** and **4**) that are in good agreement with the published data[30&31]. The presence of ortho-dihydroxylation of the B-ring of the flavonoid molecule, C2-C3 double bond and 4-oxo group of the ring C in addition to the presence of both 3-and 5-hydroxyl moiety of the rings C and A, play an important role in radical scavenging activity of the flavonoids[16,30&31].

Table 1: Antioxidant activity of the total methanolic extract, fractions and isolated compounds (**1-6**) of *Polygonum bellardii* All.

Extract/Fraction or Compound	Concentration (µg/ml)					
	10	25	50	100	200	-
	% inhibition					IC_{50}
Ascorbic acid	47.1±2.03%	66.3±1.79%	86.9±3.12%	98.8±1.54%	99.6±3.10%	14.5±2.92
Quercetin	45.0±2.95%	65.0±2.88%	85.0±3.62%	97.3±0.91%	99.1±3.22%	15.1±2.25
Total methanolic extract	10.0±0.97%	19.8±1.24%	25.0±1.07%	42.9±2.14%	64.8±1.09%	135.4±2.05
n-Hexane Fr.	05.8±0.45%	13.8±1.21%	20.8±1.13%	35.9±3.14%	41.9±1.45%	253.4±1.91
Chloroform Fr.	15.8±3.20%	21.8±1.65%	36.8±1.88%	50.9±2.98%	67.9±0.78%	98.1±3.02
Ethyl acetate Fr.	29.9±3.12%	51.9±3.10%	68.2±3.51%	73.3±1.56%	82.5±3.21%	20.51±1.87
n-Butanol Fr.	26.8±2.66%	44.9±2.88%	60.1±2.43%	71.1±3.32%	77.3±1.88%	34.7±2.32
Compound 1	43.1±1.18%	63.3±2.19%	88.9±2.17%	99.8±2.24%	100±2.23%	15.8±3.35
Compound 2	42.0±1.35%	61.0±2.11%	83.0±2.12%	94.3±1.41%	97.1±2.52%	18.5±4.30
Compound 3	28.4%±2.13	43.5±0.09%	68.6±1.62%	76.3±0.98%	85.6±2.04%	23.4±2.31
Compound 4	26.6±1.67%	44.9±1.53%	69.1±1.66%	78.6±2.05%	88.7±2.22%	24.1±1.85
Compound 5	27.7±1.32%	46.6±1.52%	72.8±1.43%	77.6±3.00%	86.3±1.76%	22.4±4.11
Compound 6	29.2±1.77%	45.9±2.11%	75.9±1.92%	79.8±3.02%	90.1±1.88%	22.2±2.76

Notes: Compound **1**: Gallic acid, **2**: quercetin, **3**: quercetin-3-*O*-(5"-acetyl-α-arabinofuranoside), **4**: quercetin-3-*O*-β-D-glucopyranoside, **5**: myricetin-3-*O*-(5"-acetyl-α-arabin-ofuranoside, and **6**: myricetin-3-*O*-α-arabinofuranoside.

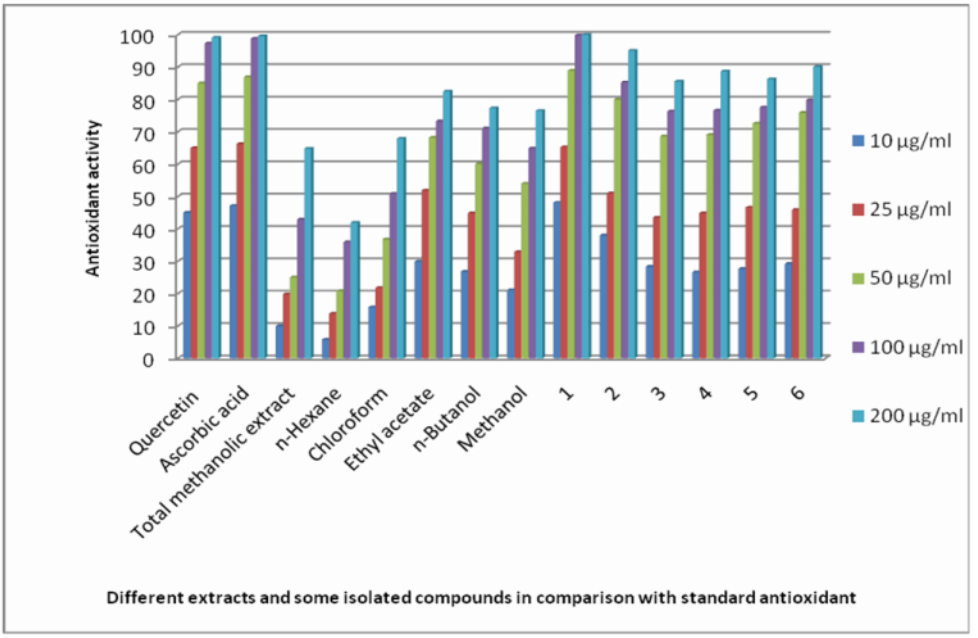


Fig. 2: Antioxidant activity of total methanolic extract, fractions and isolated compounds from *Polygonum bellardii* All.

Notes: **1**: Gallic acid, **2**: quercetin, **3**: quercetin-3-*O*-(5"-acetyl-α-arabinofuranoside), **4**: quercetin-3-*O*-β-D-glucopyranoside, **5**: myricetin-3-*O*-(5"-acetyl-α-arabinofuranoside, and **6**: myricetin-3-*O*-α-arabinofuranoside.

II- Acute toxicity studies

The symptoms of toxicity were characterized by irritability, writhing, hypothermia, loss of motor coordination, sedation and deep sleep, followed by death. The total methanolic extract of *Polygonum bellardii* All. did not show signs of toxicity and mortality up to 4 g/kg dose.

III- Hepatoprotective activity

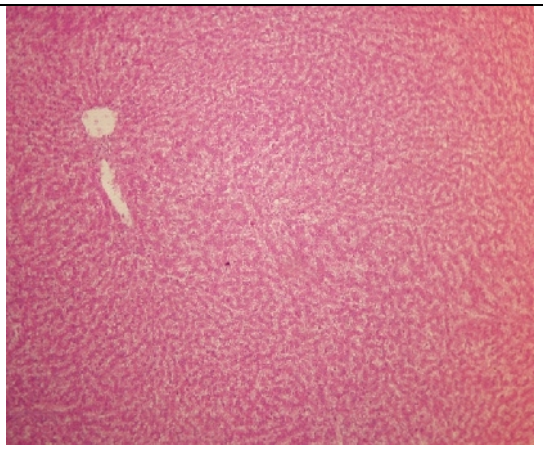
CCl_4 is one of the most commonly used hepatotoxin in the experimental study of liver disease[32]. The hepatotoxic effects of CCl_4 are largely due to generation of free radicals[33]. CCl_4 is biotransformed by the cytochrome P450 system to produce the trichloromethyl free radical ($\bullet CCl_3$) that reacts rapidly with oxygen to form a trichloromethyl-peroxy radical ($\bullet CCl_3O_2$). This metabolite possibly attack membrane polyunsaturated fatty acids thereby causing lipid peroxidation leading to impairment of membrane function and liver injury[34]. Damage of liver cell is reflected by an increase in the levels of hepatospecific enzymes; these are cytoplasmic and are released into circulation after cellular damage[35]. In this study significant increase in the SGOT and SGPT enzymes in the CCl_4 treated group could be taken as an index of liver damage. Bilirubin is one of the most useful clinical clues to the severity of necrosis and its accumulation is a measure of binding, conjugation and excretory capacity of hypatocyte. The obtained results (Table 2) showed that; the total methanolic extract in addition to ethyl acetate and *n*-butanol fractions of *Polygonum bellardii* exhibited significant decrease in SGOT and SGPT enzymes and total bilirubin as compared with CCl_4 treated group. Decrease in serum enzymes and bilirubin after treatment with the total extract and some fractions in liver damage induced by CCl_4, indicated the effectiveness of the total extract and fractions in normal functional status of the liver. The *n*-butanol fraction (400 mg/kg body weight) exhibited hepatoprotective activity nearly the same as sylimarin against carbon tetrachloride (CCl_4) induce hepatic injury in albino rats, while ethyl acetate fraction (400 mg/kg body weight) showed hepatoprotective activity higher than sylimarin. The obtained activity attributed to the presence of phytoconstituents like flavonoids and phenolic acids are known to possess hepatoprotective activity[36].

Table 2: Effect of the total methanolic extract and fractions of *Polygonum bellardii* All. on SGOT, SGPT and total bilirubin of CCl_4 treated rats.

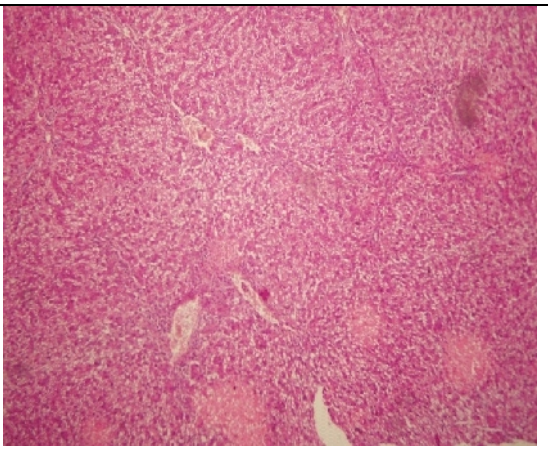
Animal group	Concentration mg/kg	SGOT (IU/L)	SGPT (IU/L)	Total bilirubin Mg/dL
Control (vehicle)	-	28.2±1.86	23.6±1.17	0.59±0.017
CCl_4 treated	0.7	114.0±9.82$^{\#\#}$	94.8±5.79$^{\#}$	1.31±0.083$^{\#}$
Sylimarin	100	42.2±2.83**	35.2±2.58**	0.62±0.020**
Total methanolic extract	200	68,4±3.91*	58.6±2.39*	0.80±0.024*
	400	49.3±2.87**	39.8±2.13*	0.71±0.017*
Ethyl acetate Fr.	200	56.6±2.68*	42.1±1.89*	0.76±0.028*
	400	34.4±1.93**	28.9±1.87**	0.54±0.027**
n-Butanol Fr.	200	54.2±2.22*	47.6±3.29*	0.79±0.022*
	400	41.8±1.96**	33.7±1.88**	0.62±0.020**

Notes: Values are mean ± SEM; number of rats used in each group= 4; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ Compared with the control group, $*p < 0.05$, $**p < 0.01$ Compared with the CCl_4 group.

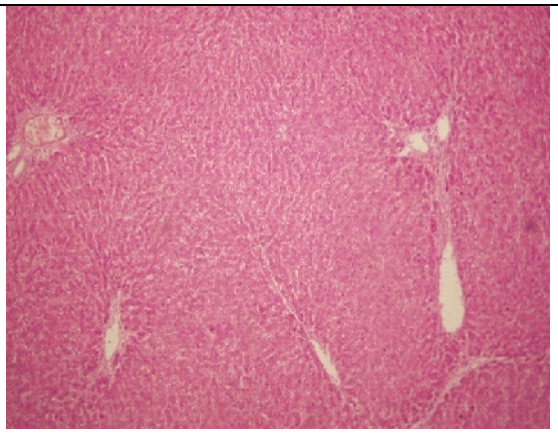
Histological Changes: Histology of liver section of control group showing normal histological architecture (Fig 3-A), whereas that of CCl_4 treated rat liver sections showed cloudy swelling and degeneration of hepatocytes. Necrosis of cells was also observed with broken cell pieces, irregular appearance due to oozing of cell materials and cell death (Fig 3-B). The histogram of groups administered CCl_4 and *Polygonum bellardii* (total extract at 200 mg/kg and *n*-butanol fraction at 200 mg/kg) showed normal and affected areas of liver patches adjacent to each other. The degenerative changes, necrosis and haemorrhage were less observed (Fig 3-D and 3-E). Furthermore, the degenerative changes and necrosis were obscured in the standard (sylimarin) group; in addition to groups administered other fractions of *Polygonum bellardii* (ethyl acetate at 200 and 400 mg/kg and *n*-butanol at 400 mg/kg) (Fig. 3F-H) thus confirming the hepatoprotective effect of *Polygonum bellardii*. The remarkable hepatoprotective effect in CCl_4-induced liver damage could be due to the phenolic constituents as flavonoids present in the plant[37]



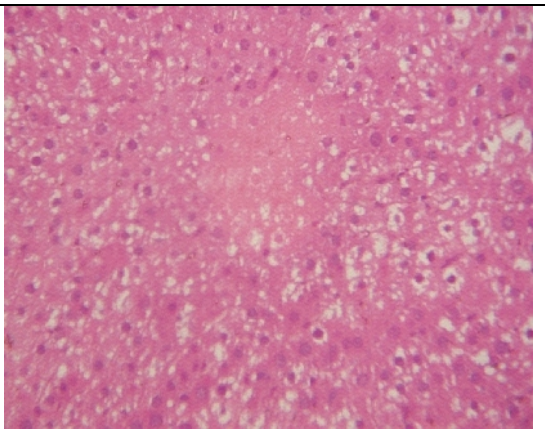
A- Control (X100)
Normal hepattocytes with no pathological findings



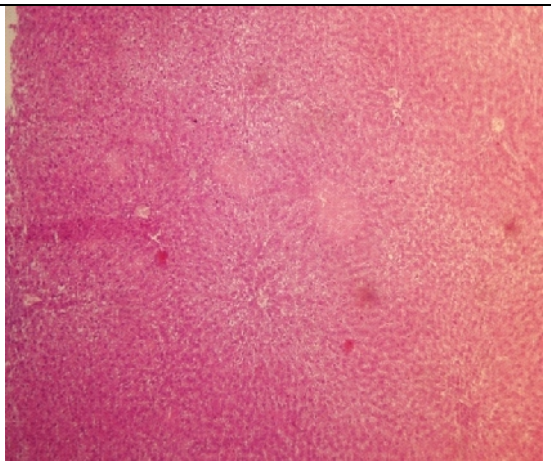
B- CCl_4 (X100)
Severe congestion, vascular degeneration extensive cloudy swelling, necroinflammatory foci and extensive lobular necrosis



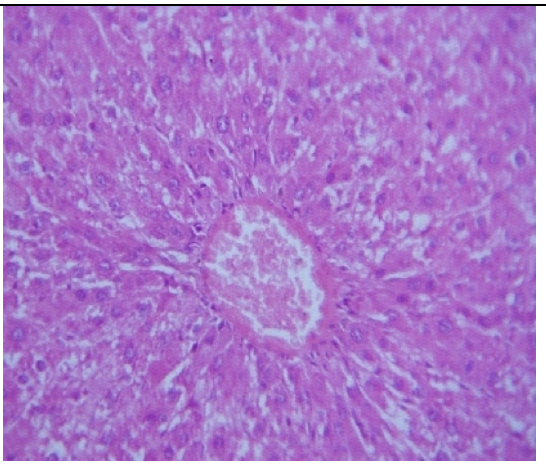
C- Sylimarin 100 mg (X100)
Cloudy swelling and mild congestion



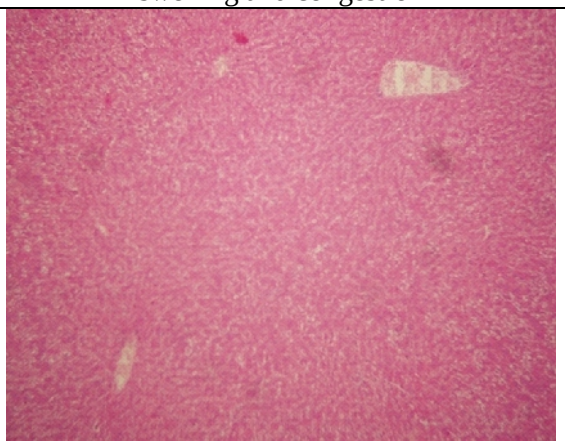
D- *n*-Butanol fraction 200 mg (X400)
Lobular necrosis and moderate hydropic degeneration of hepatocytes with pyknotic nuclei



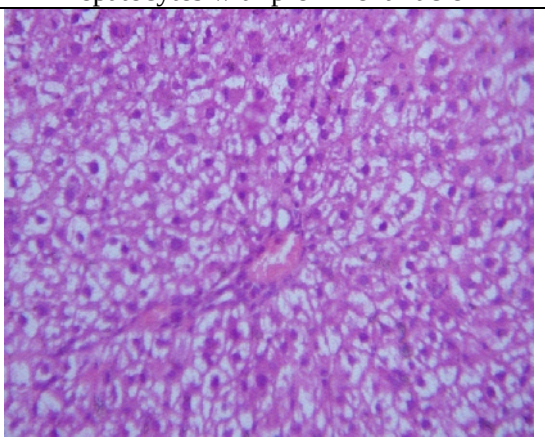
E-Total methanolic extract 200 mg (X100) Multiple foci of piecemeal necrosis at the periphery of hepatic lobule, mild cloudy swelling and congestion



F- Ethyl acetate fraction 200 mg (X400) Dilated sinusoids, dilated central vein with severe congestion and cloudy swelling of hepatocytes with prominent nuclei



G- Ethyl acetate fraction 400 mg (X100) No necrosis but severe congestion with mild hydropic degeneration



H- *n*-Butanol fraction 400 mg (X400) Severe hydropic degeneration and congestion

Fig. 3: Histopathological findings in rat liver sections after administration of total methanolic extract and fractions of *Polygonum bellardii* in comparison with hepatoprotective reference substance (sylimarin) and hepatotoxic agent CCl_4 (Hematoxylin-eosin staining).

IV- Antimicrobial activity

The results (Tables 3 and 4) showed remarkable antibacterial activity of the different extracts under investigation against Gram positive and Gram negative bacteria especially with ethyl acetate and *n*-butanol fractions which showed good antibacterial activity. Also the different extracts exhibited antifungal activity compared with Nystatin (antifungal drug). The maximum antifungal activity was obtained with ethyl acetate and *n*-butanol fractions. This antimicrobial activity may be due to the presence of phenolic constituents as flavonoids, lignans, and phenolic acids in different extracts of the flowering aerial parts of the plant.

V- Total flavonoids

Polyphenolic compounds have an important role in stabilizing lipid oxidation and are associated with antioxidant activity. Results (Fig. 4) indicated that, the total flavonoids of the methanolic extract obtained from the flowering aerial parts of *Polygonum bellardii* All. were 185.2±1.03 mg/g dried extract. The obtained results confirmed the above results of antioxidant and hepatoprotective activity of the plant.

Table 3: Results of the antimicrobial activity of total methanolic extract and fractions of *Polygonum bellardii* All. on tested micro-organisms.

Sample	Conc. (mg/ml)	Inhibition zone diameter (mm / sample)				
		Staphylococcus aureus	*Bacillus subtilis*	*Escherichia coli*	*Pseudomonas aeurginosa*	*Candida albicans*
Total methanolic extract	100	20	25	20	20	20
	50	18	23	17	19	15
	25	15	19	15	13	13
n-Hexane Fr.	100	17	17	18	15	15
	50	13	14	15	13	13
	25	11	13	13	11	11
Chloroform Fr.	100	19	15	18	17	17
	50	16	13	17	15	15
	25	13	13	14	11	13
Ethyl acetate Fr.	100	24	25	23	25	30
	50	21	23	18	23	27
	25	17	15	15	18	22
n-Butanol Fr.	100	22	23	21	20	15
	50	20	18	19	18	12
	25	17	15	15	14	11

Table 4: Minimum inhibitory concentration (MIC) of the total methanolic extract and fractions of *Polygonum bellardii* All.

Organism	MIC (mg/ml)					MIC (µg/disc)	MIC (µg/disc)
	Tot.	Hex	Ch.	Eth.	But.	Cefotax	Nystatin
Staphylococcus aureus	5.0	25	10	2.5	2.5	30	-
Bacillus subtilis	2.5	10	10	5.0	5.0	15	-
Escherichia coli	5.0	10	5.0	5.0	5.0	30	-
Pseudomonas aeurginosa	10	25	25	2.5	5.0	30	-
Candida albicans	10	25	10	1.0	25	-	25

Notes: Tot: total methanolic extract, Hex: *n*-hexane fraction, Ch: chloroform fraction, Eth: ethyl acetate fraction, and But: *n*-butanol fraction.

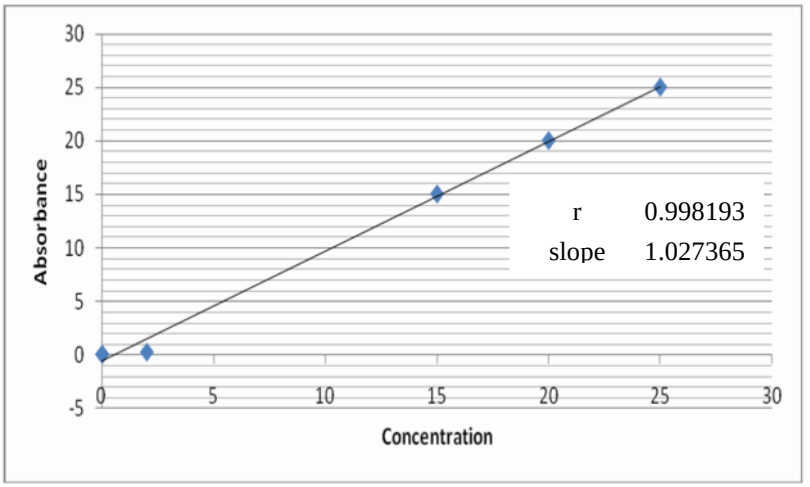


Fig. 4: Calibration curve of quercetin in MeOH at 415 nm.

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