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New Flavonoid Glycoside and Pharmacological Activities of *Pteranthus dichotomus* Forssk.

Emad M. Atta[1*], Adel A. Nassar[2], Nawal M. Hasan[3] and Ahmad R. Hasan[4]

[1] *Current address: Chemistry Depart. Fac. of Sci. Jazan Univ. Jazan, KSA. 114*
[1]*Permanent address: Genetic Eng. and Biotech. Res. Inst., Menoufia univ. Egypt. 79/22857*
[2] *Chemistry Depart. Fac. of Science, Menoufia Univ. Menoufia, Egypt. 79/22857*
[3] *Current address: Chemistry Depart. Fac. of Sci. Girls Section, Jazan Univ. Jazan, KSA. 114*
[3] *Permanent address: Aromatic and med. plant Depart. Desert Res. Center, Egypt. 11753*
[4] *Aromatic and medicinal plant Depart. Desert Research Center,Cairo, Egypt. 11753*

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Abstract: Luteolin 6-rhamnosyl (1``→ 4``) rhamnoside was isolated from *Pteranthus dichotomus* for the first time as a natural product along with eight compounds; kaempferol, quercetin, quercetin-7-glucoside, isoorientin, orientin-7-methoxide, luteolin, kaempferol-3-rhamnoside-7-glucouronic acid and myricetin-3-glucoside. The plant contained phenol, protocatechuic, *p*-hydroxy benzoic, gallic, *p*-coumaric and *o*-coumaric acids; they were identified by HPLC, the flavonoid compounds were purified by chromatographic methods, identified by chemical and physical methods including UV, ^{1}H, ^{13}C and 2D- NMR. The tested extract was highly safe as LD_{50} (4 g/kg b.wt.), it has anti-inflammatory, moderate analgesic effect and caused increase in urine volume, it also had no effect on liver functions of animals. Kidney functions were impaired after large dose (100 mg/kg^{-1}). It has Anti-tumor activity against Ehrlish Ascites Carcinoma. The new isolated compound showed antipyretic effect and increased the urine volume while the tested extract had moderate antipyretic activity in rats.

Keywords: Flavonoids; phenolic; biological activity; *Pteranthus; dichotomus.*

1. Introduction

Plant natural products are involved in many aspects of human existence. These natural products may be used as purified compounds or as components of complex mixtures which serve as medicines, pesticides, flavorings, herbicides, etc. Family Caryophyllaceae (pink family) is one of the largest families in the plant kingdom known to be rich in medicinal plants. Many species of this family had medicinal values and used in folk medicine. The whole plant of *Polycarpaea repens* is used as an antidote for snake bite. The ash or crushed leaves are used to treat sarcoptic mange of camels. α-1-barrigenol , camelliagenin and stigmasterol have been isolated from *Polycarpaea corymbosa* [1-4]. The roots of *Dianthus deserti* are used for sprains and as an ingredient in making soup. Glycosides and triterpenoid saponins are reported in *Dianthus superbus* [5,6].

Powdered leaves of *Polycarpaea corymbosa* are used externally and internally for bites of venomous reptiles and of animals; also over boils and swellings as poultice. Internally it is used in the form of a pill in jaundice and as an expectorant in pulmonary tuberculosis and hypochondria; it

* Corresponding author: E-Mail: emadata@yahoo.com
Tel. Current (00966552358374), Home (0020165575870), Fax: 0096673245212

contains camelliagenin A, barrigenol and stigmastanol [7]. The decoction of *Herniaria cinerea* is used in sour throat, as diuretic, powerful narcotic and stomach irritant. The aqueous extract of *Pteranthus dichotomus* exhibited strong cytotoxicity (above 97%) against cultured melanoma cell lines [8].

2. Materials and Methods

Plant Material

The aerial part of *Pteranthus dichotomus* Forssk. was collected from South Sinai, Egypt during spring season and identified by Botany Department, Faculty of Science Cairo University, Egypt and Desert Research Centre (12/08/11).The plant material was air dried in shade and grinded to fine powder for active constituents and pharmacological investigation.

2.1. Phytochemical studies

2.1.1. Identification of phenolic acids by HPLC

Phenolic compounds of *Pteranthus dichotomus* plant sample were extracted [9], identified on a Hewlett-Packard HPLC (Model 1100), using a hypersil C_{18} RP column (250 x 4.5 mm) with 5 µm particle size and comparing their relative retention times with those of standard mixture chromatogram. The concentration was calculated on the basis of peak area measurements, and then converted to µg phenolic g^{-1} dry weight.

2.1.2. Isolation and identification of flavonoid compounds: Extraction and purification

The air-dried powder of *Pteranthus dichotomus* Forssk. (1 kg) was extracted by percolation in 70 % methanol and filtered off; the marc lifted was extracted by the same way (this process repeated four times).

The combined methanol extracts were concentrated under reduced pressure at temperature not exceeding 40 ^{o}C till dryness (230 g), dissolved in hot water and filtered to remove chlorophyll and lipoidal matters, concentrated till dryness and then dissolved in small amount of methanol with stirring to remove salts; concentrated till dryness. The dried extract was dissolved in small amount of water (500 ml) and extracted successively using diethyl ether, chloroform, ethyl acetate and n-butanol by separating funnel. Each extract was dried over anhydrous sodium sulphate and concentrated again as before, to give 2.2 g, 1.2 g, 8.5 g and 22 g from diethyl ether, chloroform, ethyl acetate and n-butanol extracts respectively.

Qualitative Analysis

About 0.1 ml of alcoholic and successive extracts were separately chromatographed on Whatman No.1 filter paper (PC) and silica gel TLC (silica gel on aluminum sheets, Riedal-De Haen Ag, Sellze-Hannover, Germany) alongside with the available reference samples using the following systems:

For Paper Chromatography (PC)

a. n-Butanol: acetic acid: water (BAW) (4: 1: 5 v/v/v) upper phase [10].
b. Acetic acid: water (15 % AcOH) (15: 85 v/v) one phase.

For Thin Layer Chromatography (TLC)

c. Ethyl acetate: methanol: water (30: 5: 4).

d. Ethyl acetate: methanol: water (70: 5: 4).
e. Chloroform: methanol (95: 5).

The chromatograms were air-dried at room temperature; location of spots was carried out by

1. Examination under UV light before and after exposure to ammonia vapors.
2. Spraying with aluminum chloride and dried the chromatograms in an oven at 105 °C for 5 minutes and examined under UV.

It is shown that ethyl acetate containing the major and promising spots revealed to flavonoids.

Isolation and identification of flavonoid compounds

Ethyl acetate (8.5 g) was subjected to flash liquid chromatography (FLC) contained silica gel G mesh 60-120 (E. Merck) and eluted with 100 % chloroform and the polarity increased gradually with ethyl acetate. Fractions (200 ml each) were collected; collective fractions were obtained according to TLC manner using system; ethyl acetate: methanol: water (30: 5: 4). Six collective fractions (A (0.35 g), B (0.10 g), C (0.15 g), D (0.34 g), E (0.9 g) and F (1.5 g) were obtained containing nine major flavonoid compounds. These collective fractions were purified on column sephadex LH-20, the eluting system was methanol and water. The sub-fractions were subjected to thin layer chromatography (TLC) using system c and 2D paper chromatography. Final purification of some compound was carried out using HPLC (Agilent 1200 series) equipped with Diode Array Detector (DAD) using C_{18} RP column, the eluting system started with water and gradual increase with acetonitrile. The pure compounds were crystallized from methanol.

Identification and structure elucidation of the purified flavonoid compounds were done by, R_f values in PC, spectral data UV (Thermo spectronic, UniCam UV- 300 spectrophotometer) and NMR (Varian 400 MHz). The sugar moiety was identified by partial and complete acid hydrolysis using PC with authentic samples.

2.2. Pharmacological studies

2.2.1. Antitumor activity (cytotoxic activity)

A preliminary screening for cytotoxic activity towards Ehrlich ascities carcinoma using a set of sterile test tubes; where 2.5×10^5 tumor cells /ml were suspended in phosphate buffer saline. 25, 50, 100 µg /ml of the extract were added to the suspension, kept at 37º C for 4 hrs. Trypan blue dye exclusion test was carried out to calculate the percentage of non viable cells [11].

2.2.2. Determination of Median Lethal Dose (LD_{50})

LD_{50} of the alcoholic extract (70%) of *Pteranthus dichotomus* was determined [12]. Albino mice (25-30 g) were divided into groups each of 5 animals. Preliminary experiments were done to determine the minimal dose that kills all mice and the maximal dose that fails to kill any animal. Animals were kept under observation for 24 h during which symptoms of toxicity and rate of mortality were recorded.

2.2.3. Anti-inflammatory Activity

Anti-inflammatory effect of the alcoholic extract (70%) of *Pteranthus dichotomus* was studied [13]. 20 rats were divided into 4 equal groups. The thickness of the left hind paw of each rat was measured in mm. The 1st group was kept as a control while the 2nd was subcutaneously injected with diclofenac sodium in a dose of 5 mg/kg b.wt. The 3rd and 4th groups were orally administered the tested extracts in doses of 50 and 100 mg/kg b.wt, respectively. After 30 min of extracts administration, inflammation was induced by subcutaneous injection of 0.1 ml of 6% formalin in normal saline into the left hind paw. The paw thickness was measured hourly for a period of 4 h. and the magnitude of the paw swelling in the treated animals compared with that of the control.

2.2.4. Antipyretic Activity

Antipyretic effect of the alcoholic extract (70%) of *Pteranthus dichotomus* and the new compound was studied [14]. 20 rats of both sexes weighing 150-180 gm were divided into 5 equal groups. Hyperthermia was induced by subcutaneous injection of Brewer's yeast in physiological saline solution in a dose of 150 mg/g b.wt. After 17 h, the elevated body temperature of each rat was rectally measured. The 1st group was used as a control, the 2nd one was subcutaneously injected with paracetamol (100 mg/kg b.wt.), while the remaining groups were orally given the tested extract in doses of 50 and 100 mg/kg b.wt and the isolated compound. Rectal temperature of each rat was then recorded for 4 h at 1 h interval. The antipyretic effect was determined on the basis of the difference in the mean temperature between the control and the tested extracts.

2.2.5. Analgesic Activity

Analgesic effect of the tested extract was evaluated using the writhing method [15]. 20 mice of both sexes weighing 25-30 gm were divided into 4 equal groups. The 1st group was kept as a control while the 2nd was subcutaneously injected with diclofenac sodium in a dose of 5 mg/kg b.wt. Other groups were orally given the tested extract in doses of 50 and 100 mg/kg b.wt. After 30 min, each mouse was intraperitonealy injected with 0.25 ml of p-benzoquinone aqueous solution (0.1 mg/ ml). Thereafter, mice in all groups were observed for writhing hourly for 4 h. animals devoid of writhing in each group were counted and the analgesic potency of the tested extract was determined as % protection against writhing.

$$\% \text{ Protection} = \frac{\text{No. of animals that didn't writhe}}{\text{Original no. of animals}} * 100$$

2.2.6. Effect on Urine Volume

Twenty five adult rats (180-200 gm) were divided into 5 groups. The 1st group was kept as a control whereas the 2nd was orally given furosemide in a dose of 20 mg/kg b.wt. Rats of the 3rd 4th and 5th groups were used for studying the effect of the tested extracts and the new compound on urine volume in doses of 50 and 100 and 25 mg/kg b.wt respectively.

2.2.7. Effect on Liver and Kidney Functions

Mature rats of 150-180 gm were divided into 3 equal groups. The 1st group was left as a control, while the 2nd and 3rd were orally given the plant extract in doses of 50 and 100 mg/kg b.wt. for 21 days. Blood samples were collected from the orbital plexus of each rat and sera were separated. The sera were used to determine the activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) [16]. Levels of total proteins [17], albumine [18], globulin, urea [19] and creatinine were estimated [20].

3. Results and Discussion

3.1. Phytochemical studies

3.1.1. Identification of Phenolic acids by HPLC

The identified phenolic compounds were phenol, protocatechuic acid, *p*-hydroxy benzoic acid, gallic acid, *p*-coumaric acid and *o*-coumaric acid. 10 unknown compounds are also detected as shown in Table (1).

Table 1. HPLC of phenolic compounds of *Pteranthus dichotomus*.

Peak no.	RT	phenolic compound	Relative %
4	23.557	gallic acid	0.5
8	30.025	protocatechuic acid	0.2
10	33.898	*p*-hydroxy benzoic acid	0.6
14	38.206	*p*-coumaric acid	14.3
15	40.231	Phenol	3.8
16	41.896	*o*-coumaric acid	4.2

Where RT: Retention Time

3.1.2. Isolation and identification of flavonoid compounds

The flavoniod pattern of *Pteranthus dichotomus* is somewhat similar to that of other species of Caryophyllaceae. The structures of known compounds were identified by chemical methods; partial and complete acid hydrolysis and confirmed by physical analysis including UV, ^{1}H, ^{13}C and 2D NMR. The isolated compounds (H1-H8) were compared with previous published data [21-24], they are kaempferol, quercetin, quercetin-7-glucoside, isoorientin, orientin-7-methoxide, luteolin, kaempferol-3-rhamnoside-7-glucouronic acid and myricetin-3-glucoside; fig. (1)

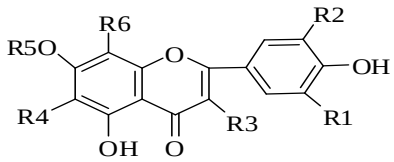

Figure 1. Isolated flavonoid compounds (H1-H8) of *Pteranthus dichotomus*

H1= Kaempferol	R1=R2=R4= R6=H, R3=OH, R5=H
H2= Quercetin	R2=R3=OH, R1=R4=R5=R6=H
H3= Luteolin	R2=OH, R1=R3=R4=R5=R6=H
H4= Myricetin-3-glucoside	R1=R2=OH, R3=O-glucoside, R4=R5=R6=H
H5= Isoorientin	R2=OH, R1=R3=R5= R6= H, R4=glucoside
H6= Orientin-7-methoxide	R2=OH, R1=R3=R4=H, R6=glucoside, R5 =Me
H7= Quercetin -7-glucoside	R2= R3= OH, R1=R4=R6=H, R5=glucoside
H8= Kaempferol -3-rhamnoside -7-glucouronic acid	R1=R2=R4=R6=H, R5=glucouronic acid, R3=O-rhamnoside

3.1.3. Structure elucidation of the new compound (H9):

UV spectral analysis of the new compound H9; luteolin-6-dirhamnoside exhibited band I at 343 nm and band II at 268 typical for a flavone the addition of shift reagents confirm the structure of

luteolin with orthodihydroxy groups at 3` and 4`(Table 2). ^{1}H NMR of the compound was coinciding with that of luteolin with two rhamnose moiety, while H-6 is substituted (Table 3). ^{13}C NMR showed signals (δ ppm) at 164.5, 103.3, 182.2, 162.1, 109.2, 161.7, 94.2, 157.9, 104.2, 122.1, 113.8, 146.2, 150.2, 116.4, 119.3, C-6 (109.2 ppm) more downfield; while C-7 appears more upfield by 2 ppm than normal which indicates the occupation at C-6 position. The two sugars also showed signals (δ ppm) at 82.1, 70.3, 70.1, 76.8, 69.9, 17.7 and 75.9, 70.4, 70.8, 72.2, 68.3, 18.8. The structure elucidation was done through 2-D NMR (H-H Cosy, HSQC and HMBC), H-3 make correlation with C-2 (164.5 ppm) and C-1` (122.1 ppm), the anomeric proton (4.6 ppm) make a correlation with C-6 (109.2 ppm), C-7 (161.7 ppm) and C-5 (162.1 ppm) indicates the substitution at C-6 position. From HMBC, correlation of the rhamnosyl H-l`` (4.4 ppm) to the rhamnosyl C-4`` (75.9 ppm), so the absence of H6 from ^{1}H NMR and the upfield shift of C-7, C-1``` and C-4`` compared with the corresponding data [21-26]; confirm the C6 substitution and 1``` - 4`` linkage between these two sugar moieties (Fig. 2).

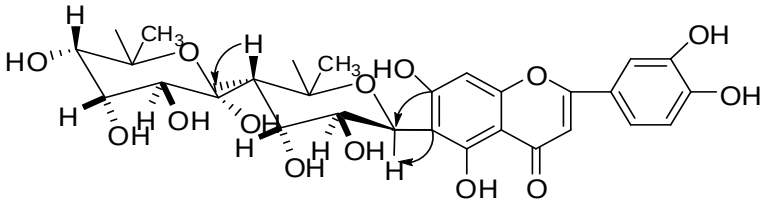

Figure 2. Luteolin-6-dirhamnoside (H9)

Table 2. UV spectral data of the isolated flavonoids.

Cpd	MeOH	NaOMe	NaOAc	NaOAc + H_3BO_3	$AlCl_3$	$AlCl_3$/HCl
H1	267.367	275.416	274.303.387	367.302.372	268.350.424	269.348.424
H2	255.301.371	276.425	275.326.389	263.386	272.302.456	270.357.425
H3	242.253.349	266.329.401	269.326.384	259.370	274.328.426	275.355.358
H4	262.351	265.306.405	277.367	257.372	262.428	257.360.408
H5	242.268.349	267.337.405	276.324.386	264.378.430	274.330.420	265.276.383
H6	268.294.349	267.335.403	264.323.385	264.374.430	270.329.406	265.296.380
H7	258.268.358	273.409	258.267.372	259.370	270.323.415	272.352.374
H8	271.346	271.396	273.324.408	373.349	276.353.393	279.359.393
H9	268.343	267.324.398	275.323.393	264.368.373	269.300.398	295.351.363

3.2. Pharmaclogical studies

3.2.1. Anti-tumor Activity

Alcoholic extract (70%) of *Pteranthus dichotomus* was investigated as anti-tumor against Ehrlich Ascites Carcinoma cells. Results indicated that the alcoholic extract (70%) induced inhibition for cell viability by 10%, 40% and 60% for concentrations of 25µg/ml, 50µg/ml and 100µg/ml, respectively as shown in Table (4).

Table 3. ^{1}H-NMR spectral data of the isolated flavonoids**.**

Cpd	H6`	H2`	H3`	H5`	H8	H3	H6	H1``	CH3rha	R.S.P	OCH3
H1	7.60,d, 8.0Hz	7.60,d, 8.0Hz	6.89,d 8.0Hz	6.89,d,8.0 Hz	6.41,d, 1.5Hz	-	6.22,d, 1.5 Hz	-	-	-	-
H2	7.49,dd, 8.5; 2.5Hz	7.45,d, 2Hz	-	6.89,d, 8.5Hz	6.53,d, 1.5Hz	-	6.21,d, 1.5 Hz	-	-	-	-
H3	7.92,dd,2.8;7.5Hz	7.84, d, 2.5Hz	-	7.48,d, 8.4Hz	6.72,d, 2.5Hz	6.63, s	6.15,d, 2.5 Hz	-	-	-	-
H4	7.24,s	7.24,s	-	-	6.52,d, 2.0Hz	-	6.24,d, 2.5Hz	5.79,d, 6.4Hz, glo.	-	3.21-4.51,m	-
H5	7.37,dd, 8.5;2.0Hz	7.32,d, 2.0Hz	-	6.82,d, 8.4Hz	6.46, s	6.57, s	-	4.53,d,8.0 Hz glo.	-	3.06, 3.90,m	-
H6	7.35,dd, 2.8;8.8Hz	7.32 d, 2.8Hz	-	6.79,d, 8.4Hz	-	6.57 ,s	6.30, s	4.51,d,8.0Hz glo.	-	3.04-4.01	3.61,s
H7	7.35,dd, 8.5;2.5Hz	7.32,d, 2.0Hz	-	6.81,d, 8.0Hz	6.59,d, 1.5Hz	-	6.38,d, 1.5 Hz	5.11,d,10.0Hz, glo.	-	3.04-4.07,m	-
H8	7.36,d, 8.0Hz	7.36,d, 8.0Hz	6.81, d, 8.0Hz	6.81,d,8.0Hz	6.60, d,2.5Hz	-	6.40,d, 2.5 Hz	5.11,d, 1.6Hz, rha, 4.90,d, 7.3Hz, glo	0.8,d	3.03-4.05,m	-
H9	7.37,dd, 2.8;7.5Hz	7.35, d, 2.0Hz	-	6.92,d, 8.4Hz	6.45, br. s,	6.60, s	-	4.61,1.6Hz,4.46,d,1.6Hz rha.	0.8,d 0.9,d	3.10-4.32,m	-

Where: rha: rhamnose, R.S.P: remaining sugar protons, glo: glucose and br: broad.

Table 4. Anti-tumor Activity of *Pteranthus dichotomus* (70%) alcoholic extract.

Concentration of extract (µg/ml)	**100**	**50**	**25**
Inhibition of cell viability (%)	60	40	10

3.2.2. Determination of Median Lethal Dose (LD_{50})

Oral administration of (70%) alcoholic extract of *P. dichotomus* in doses up to 4000 mg/Kg b. wt. failed to kill mice within 24 h. The tested extract is considered highly safe, since substances possessing LD_{50} higher than 50 mg/Kg b.wt. are considered non toxic [27].

3.2.3. Anti-inflammatory Activity

It was noticed that (70%) alcoholic extract of *P. dichotomus* in a dose of 100 mg/Kg b.wt. significantly decreased the paw thickness in comparison to the control and standard groups. The effect appeared at 2h and persisted for a period of 3 hours post-administration (Table 5) while a dose of (50 mg/Kg b.wt) has a slightly effect.

Table 5. Anti-inflammatory effect of *Pteranthus dichotomus* (70%) alcoholic extract and diclofenac sodium in rats. (n=5).

Groups	**Dose (mg/Kg^{-1})**	**Thickness of paw in mm after extract administration**			
		1 h	**2 h**	**3 h**	**4 h**
Control	0	6.78±0.16	6.86±0.18	6.74±.0.19	6.71±0.18
Diclofenac sodium	5	6.20±0.18*	6.11±0.18**	5.13±0.19***	5.80±0.19**
Extract	50	6.62±0.19	6.58±0.17	6.30±0.18	6.52±0.17
	100	6.50±0.17	6.31±0.18*	6.16±0.19*	6.51±0.17

* Significant at P ≤ 0.05 ** Significant at P ≤ 0.01 *** Significant at P ≤ 0.001

3.2.4. Antipyretic Activity

The results in Table (6) showed that the tested extract in dose of 100 mg/Kg b.wt had moderate antipyretic activity in rats while dose of 50 mg/Kg b.wt of the isolated compound showed highly activity.

Table 6. Antipyretic effect of *Pteranthus dichotomus* and paracetamol in hyperthermic rats. (n=5).

Groups	**Dose mg/Kg^{-1}**	**Rectal temperature (˚C) after compounds administration**			
		1 h	**2 h**	**3 h**	**4 h**
Control	0	38.98±0.56	38.97±0.50	38.88±0.50	38.75±0.51
Paracetamol	100	37.10±0.59**	37.00±051**	37.15±0.59**	37.22±0.50**
Extract	50	38.14±0.58	37.98±0.68	38.08±0.56	38.39±0.60
	100	37.97±0.64	37.72±0.50*	37.80±0.69	38.32±0.54
New cpd.	50	37.20±0.57**	37.15±0.50**	37.25±0.60**	37.50±0.58*

* Significant at P ≤ 0.05 ** Significant at P ≤ 0.01

Table 7. Analgesic effect of *Pteranthus dichotomus* (70%) alcoholic extract and diclofenac sodium in mice using writhing method. (n=5).

Groups	**Dose (mg/Kg^{-1})**	**% Protection against writhing after compounds administration**			
		1 h	**2 h**	**3 h**	**4 h**
Control	0	0	0	0	0
Diclofenac sodium	5	100	100	80	40
Extract	50	20	20	0	0
	100	40	40	20	0

Table 8. Effect of *Pteranthus dichotomus* (70%) alcoholic extract and furosemide on urine volume in rats. (n=5).

Groups	Dose (mg/Kg^{-1})	Volume of urine (ml) during 24 h
Control	00	2.6±0.11
furosemide	20	4.5±0.17 ***
Extract	50	2.9±0.15
	100	3.1±0.16 *
New cpd.	25	3.7±0.14**

* Significant at $P \leq 0.05$ ** Significant at $P \leq 0.01$ *** Significant at $P \leq 0.001$

3.2.7. Effect on Liver and Kidney Functions

Both doses of (70%) alcoholic extract of *P. dichotomus* didn't affect liver functions (Table 9), while kidney functions were impaired after oral administration of the high dose for 21 days (Table 10).

Table 9. Effect of *Pteranthus dichotomus* (70%) alcoholic extract on liver function.

Groups	Dose (mg/Kg^{-1})	AST (U/L)	ALT (U/L)	Proteins (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G ratio
Control	0	53.6±2.64	31.4±1.19	8.6±0.28	4.32±0.11	4.28±0.10	1.03±0.08
Extract	50	53.0±3.45	32.6±2.11	8.6±0.24	4.34±0.11	4.32±0.13	1.00±0.07
	100	52.3±2.80	34.6±2.24	8.7±0.25	4.38±0.13	4.32±0.13	1.01±0.08

Table 10. Effect of *Pteranthus dichotomus* (70%) alcoholic extract on kidney function.

Groups	Dose (mg/Kg^{-1})	Urea (mg/dl)	Creatinine (mg/dl)
Control	0	18.98±0.60	0.81±0.03
Extract	50	19.37±0.74	0.83±0.04
	100	22.64±0.65**	0.95±0.03*

* Significant at $P \leq 0.05$ ** Significant at $P \leq 0.01$

4. Conclusions

Investigation of *Pteranthus dichotomus* revealed that it is highly safe, had anti-inflammatory effect while it had moderate antipyretic effect and caused significant increase in the urine volume. It had no effect on liver functions of the animals. While kidney functions were impaired after oral administration, this may be attributed to high content of proteins in plant extract. It also observed that the *Pteranthus dichotomus* alcoholic extract had Anti-tumor activity against Ehrlish ascites carcinoma cells; the anti-tumor activity observed can be attributed to the presence of flavonoids. Certain dietary flavonoids posses' anti-tumor activity and this is directly linked to the hydroxylation pattern of the B-ring of the flavones and flavonols (such as luteolin and quercetin) seems to critically influence their activities, especially the inhibition of protein kinase activity and anti-proliferation [28]. The anti-inflammatory and analgesic effect observed can be attributed to the presence of flavonoids and tannins and consequently to their free radical scavenging activities [29]. In general the flavonoids have potent inhibitory activity against a wide of enzymes such as lipo-oxygenases, cyclo-oxygenases and others

[30-32]. It was reported that the flavonoids prevent the generation or action of the free radicals which cause tissue damage during the inflammation [33]. A number of flavonoids have been reported to produce analgesic activity. Also, there are few reports on the role of tannins in analgesic activity [34].

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