Bioactivity and chemical constituents of *Gleditsia triacanthos* L. leaves

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Received 19 December 20107
Accepted 8 March 2018

Egyptian Pharmaceutical Journal
2018, 17:70–76

Background and objective
Gleditsia triacanthos L. is a deciduous tree belonging to the family Fabaceae. It is used in folk medicine as it possesses important biological activities: antimutagenic, anticancer, cytotoxic, and rheumatoid arthritis-treating properties. The study aimed to evaluate the anti-inflammatory, analgesic, and antipyretic activities of the nonpolar extract of the plant in addition to investigation of its chemical composition.
Materials and methods
The dried powdered leaves of *G. triacanthos* were successively extracted with solvents of increasing polarities: petroleum ether (PE), chloroform, ethyl acetate, and aqueous ethanol; moreover, the total ethanol extract was also prepared. The PE extract was fractionated into unsaponifiable matter (USM) and fatty acids, which were methylated to give FAME fractions, which are subjected to GC/MS analysis.
Results
GC/MS analysis of USM and FAME revealed that 24 compounds represented 76.86% of the total fraction of USM, with squalene (22.68%) as the major compound followed by nonacosane (21.03%) and isophytol (14.70%). Twenty-one compounds of FAME fraction representing 85.87% were identified, with methyl 9,12,15-octadecatrienoate (31.37%) as the major compound followed by methyl hexadecanoate (19.35%) and methyl 9,12-octadecadienoate (13.52%). The unsaturated fatty acid represented 45.89% of the total fraction. GLC of the sterol fraction isolated from the PE revealed the identification of campesterol (51.23%), stigmasterol (42.34%), β sitosterol (4.88%), and cholesterol (1.55%). The PE extract exhibited the highest analgesic and antipyretic activities (81.73 and 91.10% potency, respectively) in comparison with tramadol and paracetamol (100% potency), correspondingly, whereas total ethanol extract exhibited the highest anti-inflammatory activity followed by ethyl acetate and PE extract (74.60, 66.66 and 62.69% potency, respectively) in comparison with indomethacin (100% potency).
Conclusion
The results of our study suggest that the PE extract of *G. triacanthos* leaves extracts can be used as an alternative therapy for their analgesic, anti-inflammatory, and antipyretic activities.

Keywords:
analgesic antipyretic and anti-inflammatory activities, fatty acids, *Gleditsia triacanthos*, unsaponifiable matter

Egypt Pharmaceut J 17:70–76

1687-4315

Introduction

Gleditsia triacanthos L. (Honey locusts) is a deciduous tree belonging to the family Fabaceae. It flowers in July, and the seeds ripe from October to November. The plant comprises 12 species [1], and it can reach a height of 20–30 m; their life spans are ~120 years. The Honey locust is not a honey plant; the name was derived from the sweet taste of the legume pulp, which was used for food by native American people, and it can be fermented to make beer. The pulp on the insides of the pods is edible, but the black locust is toxic. *Gleditsia* spp. have been used in folk medicine, and the anomalous fruits produced by old or injured plants of *Gleditsia sinensis* L have long been known in traditional Chinese medicine as saponin-rich herbal medicine and as pesticide [2]. *G. triacanthos* has been used in folk medicine: pods have been used as a folk remedy for dyspepsia, measles, indigestion, and as antiseptic; bark for dyspepsia and in the treatment of whooping cough, measles, and smallpox; seeds as a natural source of phenolic compounds and antioxidants; and leaves used as a potential source of anticancer compounds [3]. *G. triacanthos* was reported

to contain triterpene glycoside triacanthoside C in addition to oleanolic and echinocystic acids isolated from the pericarps [4] gleditschosides A, B, C, D, and E were detected in pericarp and leaves of *G. triacanthos* [4]. Different *Gleditsia* fruits species were found to contain saponin triacanthosides A1, G, and C together with gleditschosides A, B, C, D, and E, which were isolated from *G. triacanthos* [4–7]. Triacanthine alkaloid was isolated from the leaves of *G. triacanthos* [8,9]. Vitexin, luteolin, isovitexin, and quercetin are reported to be isolated from *G. triacanthos* [10,11]. *G. triacanthos* leaves were studied for their flavonoid content and cytotoxic and antioxidant activities [12]. In this view, it is imperative to carry out more pharmacognostical and pharmacological investigations on *G. triacanthos* leaves to find out the unexploited potential of this medicinal plant under study. This plant is chosen for study because it is in abundance, easily available, safe, and widely used in traditional medicine.

Materials and methods

General experiment

Silica gel 60 for column chromatography (E. Merck, Darmstadt, Germany), aluminium sheets G60 F_{254} (Silica gel, layer thickness 0.2 mm; Fluka Chemie AG, Switzerland); solvent system: benzene–acetone (9 : 1 v/v); chloroform (Ch)–methanol (9.5 : 0.5 v/v); benzene–ethyl acetate (EA) (3 : 1 v/v), reagent for detection sterols and terpenes: 1% vanillin–sulfuric acid reagent. Apparatus for GC/MS analysis of unsaponifiable matter (USM) FAME was gas chromatograph coupled with a mass spectrometer (Agilent 6890 gas chromatograph coupled with an Agilent mass spectrometric detector, 70 eV). Agilent Technologies 6890 N (Network GC System, USA) was used for GLC analysis of sterol fraction. Indomethacin (Kahira Pharmaceuticals Industries Company, Cairo, Egypt) was used as a standard anti-inflammatory agent; carrageenan (Sigma Co.) was used for induction of acute inflammation in rats; paracetamol was used as a standard antipyretic drug and tramadol as a standard analgesic drug (Misr Co. for Pharmaceuticals Industries, Cairo, Egypt). Solvents used were petroleum ether (PE) (BP 60–80°C), benzene, diethyl ether, EA, Ch, dichloromethane, 70% and 95% ethanol, methanol, and distilled water. Solvents were distilled and dehydrated before use.

Conditions of GC/MS analysis

Capillary column of Thermo Scientific TR-5MS (5% phenyl polysilphenylene siloxane), 30 m length, 0.25 mm, internal diameter, and 0.25 μm thickness, was used. Carrier gas was helium at 13 psi, injector temperature was 200°C, detector temperature was 280°C, and temperature programming was 60°C for 5 min, 60–280°C at a rate of 5°C/min, and 280°C isothermal for 10 min for USM and 5 min for FAME. The detector was mass spectrometer.

Conditions of GLC analysis

The conditions for GLC analysis were as follows: capillary column HP-5 (5% phenyl methyl siloxan), 30 m length, 0.32 mm, internal diameter, and 0.25 μm thickness, initial temperature:80°C, initial time: 1.00 min, rate: 8°C/min, final temperature: 300°C, inlet temperature: 250°C, detector: 300°C flame ionization detector (FID), flow: 2 ml/min, carrier gas: N_2 30 ml/min, H_2 30 ml/min, and air 300 ml/min.

Plant material

Fresh leaves of *G. triacanthos* L. were obtained from the Zoo in May 2007. The plant was identified by Terase Labib, the plant taxonomist of Orman Garden, Giza, Egypt, and confirmed by the taxonomist Dr M. El-Gebaly, NRC. A voucher specimen (M 96) was deposited by Dr Mona Marzouk and kept in the herbarium of Pharmacognosy Department, NRC.

Animals

In this study, adult albino rats of Sprague Dawley strain weighing 130–150 g and albino mice weighing 25–30 g were used. Animals were obtained from the Animal House Colony of the National Research Centre, Dokki, Egypt. They were kept under the same hygienic conditions with well-balanced water and diet. Normal diet consisted of vitamin mixture 1%, mineral mixture 4%, corn oil 10%, sucrose 20%, cellulose 0.2%, and casein (95% pure) 10.5%, and starch 54.3%. Medical research ethical committee in the National Research Center has approved the work (09/130) 2009.

Investigation of nonpolar extract

Preparation of plant extracts

The different extracts of the dried powdered leaves were previously prepared as reported [12].

Preparation of the USM and FAME

Five grams of the PE extract of the leaves was subjected to saponification, and the USM and the FAME were prepared adopting the reported methods [13] to give 2.5 g of the USM and 1.5 g of the fatty acids (FAs).

GC/MS analysis of USM matter and FAME

The USM matter and FAME were subjected to GC/MS analysis adopting the aforementioned conditions.

The identification of the compounds was accomplished by comparing their retention times and mass spectral data with those of the library and published data [14,15]. Quantitative determinations were carried out based on peak area measurements. The results are listed in Tables 1 and 2.

Isolation of the sterol fraction by column chromatography

Five grams of the PE extract was subjected to fractionation on silica gel column (G 60 F 254; Merck) using PE (60–80)/Ch as the mobile phase. Ten fractions were collected and monitored by TLC in comparison with authentic β-sitosterol using benzene–acetone (9 : 1 v/v) solvent system spraying with 10% H_2SO_4 and heating in oven at 110°C. The fractions proved to contain β-sitosterols (R_f 0.5) were mixed and subjected to preparative TLC using the same solvent system. The sterol band was scratched and eluted by Ch and then analyzed by GLC analysis (Table 3).

Biological study

Acute toxicity test (LD_{50})

The total ethanol (TE) extract of *G. triacanthos* leaves was subjected to LD_{50} determination according to the reported method [16]. Male albino mice (25–30 g) were divided into groups of six animals each. Preliminary experiments were done to determine the minimum dose that kills all animals (LD_{100}) and the maximum dose that fails to kill any animal. Several doses at equal logarithmic intervals were chosen in between these two doses, and each dose was injected subcutaneously into a group of six animals. The mice were then observed for 24 h, and symptoms of toxicity and mortality rates in each group were recorded and LD_{50} was calculated.

Analgesic activity test

The analgesic activity was determined by acetic acid-induced writhing method using Swiss albino mice (20–25 g). Animals were acclimatized to the laboratory conditions for at least 1 h before testing and were used once during the experiment. Forty-eight albino mice were divided into eight groups, with six animals each. First group albino mice that received 1 ml saline serving as a control, second and third group mice received 50 and 100 mg/kg, body weight, of TE extract. Fourth, fifth, sixth, and seventh groups mice received 100 mg/kg, body weight, of PE, Ch, EA, and aqueous ethanol (AE) extracts, respectively. Eighth group mice received 4 mg/kg, body weight, of the standard analgesic (tramadol), and

Table 1 Results of GC/MS analysis of the USM of PE extract of *G. triacanthos* L. leaves

Nos	Compounds	RR_t	BP	M^+	Rel. %	Molecular formula
1	2-Ethylhexanol	0.18	57	130	0.15	$C_8H_{18}O$
2	*n*-Undecane	0.22	57	156	0.08	$C_{11}H_{24}$
3	*n*-Dodecane	0.28	57	170	0.17	$C_{12}H_{26}$
4	Dihydroactinidiolide	0.51	111	180	0.75	$C_{11}H_{16}O_2$
5	Neophytadiene	0.63	95	278	0.15	$C_{20}H_{38}$
6	6,10,14-Trimethyl-2-pentadecanon	0.64	58	268	0.75	$C_{18}H_{36}O$
7	Loliolide	0.64	111	196	0.35	$C_{11}H_{16}O_3$
8	Farnesylacetone	0.67	69	262	0.18	$C_{18}H_{30}O$
9	Isophytol	0.75	71	269	14.70	$C_{20}H_{40}O$
10	Phytol	0.77	71	296	0.27	$C_{20}H_{40}O$
11	*n*-Docosane	0.78	57	310	0.08	$C_{22}H_{46}$
12	*n*-Tricosane	0.82	57	324	0.16	$C_{23}H_{48}$
13	*n*-Tetracosane	0.86	57	338	0.18	$C_{24}H_{50}$
14	*n*-Pentacosane	0.89	57	352	0.23	$C_{25}H_{52}$
15	*n*-Hexacosane	0.93	57	366	0.31	$C_{26}H_{54}$
16	*n*-Heptacosane	0.96	57	380	0.87	$C_{27}H_{56}$
17	*n*-Octacosane	0.99	57	394	0.85	$C_{28}H_{58}$
18	Squalene	1	69	410	22.68	$C_{30}H_{60}$
19	*n*-Nonacosane	1.02	57	408	21.03	$C_{29}H_{60}$
20	*n*-Triacontane	1.05	57	422	1.50	$C_{30}H_{62}$
21	*n*-Hentriacontane	1.05	57	436	5.29	$C_{31}H_{64}$
22	α-Tocopherol (vitamin E)	1.07	430	430	3.54	$C_{16}H_{34}$
23	Amyrin β	1.17	218	426	1.23	$C_{29}H_{50}O$
24	Urs-12-en-3-ol	1.20	218	426	1.36	$C_{30}H_{50}O$
Total identified constituents					76.86	
Unidentified constituents					23.14	

BP, base peak; *G. triacanthos*, *Gleditsia triacanthos*; M^+, molecular weight; PE, petroleum ether; RR_t, retention time relative to squalene (R_t=56.01 min); R_t, retention time.

Table 2 Results of GC/MS analysis of FAME of PE extract of *G. triacanthos* L. leaves

Nos	Compounds	RR_t	BP	M^+	Rel. %	Molecular formula
1	Methyl dodecanoate (methyl laurate)	0.65	74	214	0.23	$C_{13}H_{26}O_2$
2	Methyl tetradecanoate (methyl myristate)	0.78	74	242	1.55	$C_{15}H_{30}O_2$
3	Methy l,9-pentadecenoate	0.83	41	254	0.25	$C_{16}H_{30}O_2$
4	Methyl penatdecanoate	0.84	74	256	0.34	$C_{16}H_{32}O_2$
5	Methyl-7-hexadecenoate	0.88	79	268	0.25	$C_{17}H_{32}O_2$
6	Methyl-9-hexadecenoate (methyl pamitoleate)	0.89	55	268	0.50	$C_{17}H_{32}O_2$
7	Methyl hexadecanoate (methyl palmitate)	0.90	74	270	19.35	$C_{17}H_{34}O_2$
8	Methyl heptadecanoate (methyl margarat)	0.95	74	284	0.75	$C_{18}H_{36}O_2$
9	Methyl 9,12-octadecadienoate (methyl linoleate)	0.99	81	294	13.52	$C_{19}H_{34}O_2$
10	Methyl-9,12,15-octadecatrienoate	1	79	292	31.37	$C_{19}H_{32}O_2$
11	Methyl/octadecanoate (methyl stearate)	1.00	74	298	8.52	$C_{19}H_{38}O_2$
12	Methyl nonadecanoate	1.05	74	312	0.31	$C_{20}H_{40}O_2$
13	Methyleicosanoate (methyl arachate)	1.09	74	326	1.98	$C_{21}H_{42}O_2$
14	Methyl heneicosanoate	1.14	74	340	0.25	$C_{22}H_{44}O_2$
15	Methyl docosanoate (methyl behenate)	1.18	74	354	1.69	$C_{23}H_{46}O_2$
16	Methyl tricosanoate	1.22	74	368	0.62	$C_{24}H_{48}O_2$
17	Methyl tetracosanoate	1.26	74	382	2.28	$C_{25}H_{50}O_2$
18	Methyl pentacosanoate	1.30	74	396	0.41	$C_{26}H_{52}O_2$
19	Methyl hexacosanoate (methylcerotate)	1.34	74	410	0.64	$C_{27}H_{54}O_2$
20	Methyl octacosanoate	1.42	74	438	0.37	$C_{29}H_{58}O_2$
21	Methyl nonacosanoate	1.47	74	452	0.69	$C_{30}H_{60}O_2$
Total identified constituents					85.87	
Unidentified constituents					14.13	

BP, base peak; *G. triacanthos*, *Gleditsia triacanthos*; M^+, molecular weight; PE, petroleum ether; RR_t, retention time relative to methyl-9,12,15-octadecatrienoate (R_t=36.23 min); R_t, retention time.

Table 3 Results of GLC analysis of sterol fraction of *G. triacanthos* L. leaves

Nos	Compounds	R_t	Area (%)
1	Cholesterol	30.49	1.55
2	Campesterol	31.21	51.23
3	Stigmasterol	31.70	42.34
4	β-Sitosterol	33.09	4.88

G. triacanthos, *Gleditsia triacanthos*.

30 min later 0.6% acetic acid was injected intraperitoneally (0.2 ml/mice). Each mouse was then placed in an individual clear plastic observed chamber, and the total number of writhes/30 min was counted for each mouse [13]. The numbers of writhing and stretching were recorded and compared with the control drug. The results were illustrated in Table 4.

The percent was calculated using the following ratio:

$$\text{Percentage of protection} = \frac{\text{Control mean} - \text{treated mean}}{\text{Control mean}} \times 100.$$

Antipyretic activity test [13]

Forty-eight male albino rats, with average body weight of 100 g, were divided into eight groups, with six animals each. The normal rectal temperature was recorded before starting the experiment. Pyrexia was induced by intramuscular injection of 1 ml/100 g, body weight of 44% yeast suspension. The site of injection was massaged to spread the suspension beneath the skin. After 18 h, the rectal temperature was recorded for all groups to serve as the baseline of elevated body temperature, to which the antipyretic effect will be compared. A single oral administration of a dose of

Table 4 Analgesic activity of TE and successive extracts of *G. triacanthos* L. leaves

Groups	Dose (mg/kg, body weight)	Number of abdominal constriction	Percentage of inhibition	Percentage of potency
Control	1 ml saline	49.2±1.1	–	–
Total ethanol	50	41.3±1.1	16.06	26.25
Total ethanol	100	28.6±0.2*	41.86	68.43
Petroleum ether	100	24.6±0.9*	50.00	81.73
Chloroform	100	29.8±0.6*	39.43	64.45
Ethyl acetate	100	31.3±0.4*	36.38	59.47
Aqueous ethanol	100	25.9±0.6*	47.35	77.40
Tramadol	4 mg/kg	19.1±0.3*	61.17	100

G. triacanthos, *Gleditsia triacanthos*; TE, total ethanol. *$P<0.01$, statistically significantly different from control group.

each tested extract, paracetamol or saline, was given. One and two hours later, other records of rectal temperature were measured and the results are compiled in Table 5.

Anti-inflammatory activity test

The anti-inflammatory test was evaluated adopting the carrageenan-induced rat hind-paw edema test [16]. This model uses carrageenan as an irritant to induce paw edema. NSAIDs such as indomethacin, reduce paw swelling in a dose-dependent manner to a maximum of 60%. Test materials are assessed for acute anti-inflammatory activity by examining their ability to reduce or prevent the development of carrageenan-induced paw swelling. Forty-eight adult male albino rats, divided into eight groups, with six animals each, were orally treated with one of the following: 50 and 100 mg/kg body weight of the TE extract, 100 mg/kg body weight of each successive extract, 20 mg/kg body weight of indomethacin, and saline (control), as shown in Table 6. One hour after oral administration, all animals were given a subplantar injection of 0.1 ml of 1% carrageenan solution in saline in the right hind paw and 0.1 ml saline in the left hind paw. Four hours after oral administration, the rats were killed. Both hind paws were, separately, excised and weighed. Edema percentage and edema inhibition percentage were calculated according to the following equations. Results are illustrated in Table 5.

$$\text{Percentage of edema} = \frac{\text{Weight of right paw} - \text{weight of left paw}}{\text{Weight of left paw}} \times 100,$$

$$\text{Percentage of edema inhibition} = \frac{M_c - M_t}{M_c} \times 100,$$

M_c: the mean edema in the control group; M_t: the mean edema in the drug-treated group.

The results of the aforementioned three experiments were statistically analyzed using the Student's *t*-test [17]. Results with *P* value of less than 0.01 were considered statistically significant.

Results

GC/MS analysis of the USM of PE extract of *G. triacanthos* leaves (Table 1) revealed identification of 24 compounds constituting 76.86% of the total composition. The oxygenated compounds constituted 23.28% whereas the nonoxygenated compounds constituted 53.58%. Squalene (22.68%), *n*-nonacosane (21.03), and isophytol (14.70%) were the major compounds. Analysis of the FA methyl esters by GC/MS (Table 2) revealed the identification of 21 compounds representing 85.87% of the total FA, the unsaturated FAs constituted 45.89%, whereas the saturated constituted 39.98%. 9,12,15-Octadecatrienoic acid was the major polyunsaturated compound (31.37%), followed by hexadecanoic acid (19.35%), and 9,12-octadecadienoic acid (13.52%). Campesterol (51.23%), stigmasterol (42.34%), β sitosterol (4.88%), and cholesterol (1.55%) were identified by GLC analysis of the sterol fraction of the PE extract (Table 3).

LD_{50} of the TE extract of leaves of the plant was found to be 6.6 g/kg, body weight (the plant is safe); this explains the use of different parts (seeds, young leaves and fruits) of the plant as food in some countries [18–20]. The highest analgesic activity was exhibited by 100 mg of PE, AE, TE, Ch, and EA (81.73, 77.40, 68.43, 64.45 and 59.47% potency, respectively) in comparison with tramadol (100% potency).

Table 5 Antipyretic activity of TE and successive extracts of *G. triacanthos* L. leaves

Groups	Dose (mg/kg, body weight)	Induced rise (zero time) (deg.)	Body temperature change					
			1 h			2 h		
			Mean±SE	Percentage of change	Percentage of potency	Mean±SE	Percentage of change	Percentage of potency
Control	1 ml saline	38.6±0.3	38.6±0.3	–	–	39.2±0.4	–	–
Total ethanol	50	38.8±0.3	38.5±0.4	0.77	20.21	38.1±0.3	1.80	30.82
Total ethanol	100	39.1±0.4	38.4±0.5*	1.79	46.99	37.8±0.1*	3.32	56.85
	100	39.1±0.4	38.4±0.5*	1.79	46.99	37.8±0.1*	3.32	56.85
Petroleum ether	100	39.5±0.4	38.6±0.2*	2.28	59.84	37.4±0.1*	5.32	91.09
Chloroform	100	39.3±0.5	38.5±0.2*	2.04	53.54	37.7±0.2*	4.07	69.69
Ethyl acetate	100	39.1±0.4	38.3±0.3*	2.05	53.81	37.8±0.1*	3.32	56.85
Aqueous ethanol	100	38.2±0.2	38.1±0.2	0.26	6.82	38.0±0.2	0.52	8.90
Paracetamol	20	39.4±0.4	37.9±0.2*	3.81	100	37.1±0.2*	5.84	100

G. triacanthos, *Gleditsia triacanthos*; TE, total ethanol. *$P<0.01$, statistically significantly different from control group.

Table 6 Anti-inflammatory activity of TE and successive extracts of *G. triacanthos* L. leaves

Groups	Dose (mg/kg/body weight)	Percentage of paw edema		Percentage of potency
		Mean±SE	Percentage of inhibition	
Control	1 ml saline	59.4±1.9	–	–
Total ethanol	50	43.7±1.2	26.43	41.53
	100	31.2±1.1*	47.47	74.60
Petroleum ether	100	35.7±0.7*	39.89	62.69
Chloroform	100	39.8±0.5*	32.99	51.84
Ethyl acetate	100	34.2±1.1*	42.42	66.66
Aqueous ethanol	100	38.1±1.5*	35.85	56.34
Indomethacin	20	21.6±0.7*	63.63	100

G. triacanthos, *Gleditsia triacanthos*; TE, total ethanol. *$P<0.01$, significantly different from control group.

The PE (100 mg) exhibited significant antipyretic activity after 2 h (91.10% potency) followed by 100 mg of Ch, TE, and EA (69.69, 56.85 and 56.85% potency, respectively) in comparison with 20 mg of paracetamol (100% potency). The AE exhibited very low activity. All extracts exhibited anti-inflammatory activity as indicated by inhibition of the rat paw edema weight induced by carrageenan. The significant anti-inflammatory activity was found to be dose dependent and the highest activity was found to be exhibited by 100 mg of the TE, EA, PE, AE, and Ch (74.6, 66.66, 62.69, 56.34 and 51.84% potency, respectively) in comparison with indomethacin (100% potency).

Discussion

Pain is one of the basic symptoms of all human ailments and is a sensorial modality and primary protection response; analgesics relieve pain without affecting its cause [21]. Acetic acid writhing test along with electrical stimulation of the tail of the mice is a sensitive way to measure the analgesic activity [22]. The normal body temperature of an individual is controlled by hypothalamus gland which regulates rising or decreasing body temperature through adjusting the balance between heat production and heat loss. So any disturbance of hypothalamic thermostat results in a complaint of fever [23]. Pyrexia or fever is caused as a result of infection, tissue damage, inflammation, graft rejection, malignancy, or other diseased states. It is the body's natural defense to create an environment where infectious agent or damaged tissue cannot survive. The infected or damaged tissue initiates the formation of proinflammatory mediators (cytokines like interleukin 1β, α, β, and tumor necrosis factor-α). Yeasts induce pathogenic pyrexia, which leads to the production of PGE2 that affects the thermoregulatory center, leading to elevation of body temperature [24]. So the principal action of antipyretics is the ability to inhibit the enzyme cyclooxygenase (COX) and interrupt the synthesis of inflammatory prostaglandins [23]. Moreover, the synthetic antipyretic agents irreversibly inhibit COX-2 with high selectivity but are toxic to the hepatic cells, glomeruli, cortex of brain, and heart muscles, whereas natural COX-2 inhibitors have lower selectivity with fewer adverse effects [25]. In our study, the plant extracts may reduce pyrexia by reducing brain concentration of prostaglandin E2, especially in the hypothalamus. Inflammation is a complex biological response of vascular tissues to harmful stimuli. The primary signs of acute inflammation were pain, heat, redness, and swelling, and may lead to loss of function [26]. Inflammation is the first physiological defense system which involves the activation of many enzymes such as COX-1 and COX-2 and lipooxygenase, which are responsible for the release of inflammatory mediators (prostaglandins and leukotrienes, which in turn lead to fluid exudation, cell migration, and tissue breakdown) [24,27]. Phytochemical screening of PE extract revealed presence of sterols and/or triterpenes. The unsaponifiable fraction contains appropriate percentage of α-tocopherol, β amyrin, and isophytol. α-Tocopherol has been shown to have anti-inflammatory effects both *in vitro* and *in vivo*, and the use of high doses of α-tocopherol has been shown to decrease release of proinflammatory cytokines. β-Amyrin is a triterpenoid and was found to be the precursor of boswellic acid and ursolic acid which have strong 5-lipooxygenase inhibitor and antielastase activity. Phytol and isophytol are volatile terpenoids reported for the treatment of rheumatoid arthritis and other chronic inflammatory diseases [28]. 9,12,15-Octadecatrienoate (linolenic acid) is the major compound in the saponifiable fraction. It is an ω-3 (18 : 3, *n*-3) FA which metabolized n the body to octadecatetraenoic acid (18 : 4, *n*-3), stearadonic acid (20 : 4, *n*-3), and eicosapentaenoic acid (20 : 5, *n*-3). The anti-inflammatory properties of ω-3 FAs, especially eicosapentaenoic acid, are due to competition with arachidonic acid (AA) as a substrate for COX and 5-lipoxygenase [29]. At present, the natural analgesics and anti-inflammatory agents of plant origin are being appreciated owing to possible toxicity of synthetic drugs. The results of this study suggest that *G. triacanthos* leaves can be used as a supplementaryor

alternative therapy for their analagesic, anti-inflammatory, and antipyretic activities.

Acknowledgements

The authors extend special appreciation to the National Research Centre, Dokki, Giza, Egypt for offering the facilities for this study. This work was extracted from the MSc Thesis of Wedian El-Sayed Mohamed Ashour, Pharmacognosy Department, National Research Centre.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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