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Phenolic Metabolites and some Pharmacological Activities of *Cassia renigera* Benth

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ABSTRACT

Genus *Cassia*, comprise a huge variety of plants that are widely distributed worldwide and well known for their biological and pharmacological properties. The phytochemical investigation of *Cassia renigera* Benth. leaves resulted in the isolation of quercetin 3-*O*-β- glucopyranoside, kaempferol 3-*O*- β-glucopyranoside, kaempferol 3-*O*- β-rhamnoside, epiafzelchin, quercetin and kaempferol. The structures of the isolated compounds were established by chromatography, UV, ^{1}H' and ^{13}C NMR spectroscopy. Pharmacological studies showed that the three doses of 70% aqueous methanolic extract of *C. renigera* Benth. (125, 250 and 500 mg/kg orally) possessed an anti-diabetic, anti-inflammatory, analgesic, and moderate anti-oxidant activities in dose dependent manner.

Key words: *Cassia renigera*, flavonoids, anti-diabetic, anti-inflammatory, analgesic, antioxidant

Introduction

The use of natural products with therapeutic properties is an ancient as human civilization. Recently, herbal medicines have increasingly been used to treat many human diseases (Ahmed *et al.,* 2014). Natural antioxidants, especially phenolics and flavonoids are well known for their safety and ability to retard lipid oxidative rancidity in foods and combating the progress of many chronic diseases (Williams et al, 2004). Numerous studies were carried out on plants with anti-oxidant properties (Vivek and Surendra , 2006; Supriya et al, 2013). However, there is still great interest in finding new anti-oxidants from natural sources. The *Cassia* genus (Family: Fabaceae) represents one of the largest and most diverse group of flowering plants, including herbs to trees. Plants of the genus *Cassia* are widely distributed in most tropical and subtropical countries. (Marazzi, 2006). Phytochemical analysis of certain *Cassia* species led to the isolation of flavonoids, anthraquinones, anthracene derivatives and naphthalene derivatives (Kitanaka and Takido, 1994; Choi et al, 1995; Sayed *et al.,* 2012). Previous phytochemical investigation of *Cassia renigera* Benth. isolated anthraquinones from the seeds (Tiwari and Anjali, 1979; Rani and Kalidhar, 1997). Moreover, two flavonoids (5-hydroxy-3`, 4`, 5`, 6, 7 pentamethoxy flavonone-5-O-α-L- rhamnopyranoside and quercetin -3, 6-dimethylether) were isolated from the stem bark (Tiwari and Bajpai, 1977). Genus *Cassia* has been reported to have various biological and medical properties such as anti-oxidant, analgesic, anti-pyretic, anti-inflammatory, expectorant, antibacterial, anti-parasitic, anti-mutagenic, anti-tumor, hepatoprotective and anti-diabetic activities (Muller *et al*., 1989; Caceres *et al.,* 1993; Jain *et al.,* 1997; Bhakta *et al.,* 2001; Villasenor *et al.,* 2002; Tona *et al.,* 2004; Cheng *et al.,* 2006; Yin *et al.,* 2013). The present study deals with the isolation of phenolic constituents of *C. renigera* Benth. leaves as well as assessment of the anti-oxidant, anti-inflammatory, analgesic and anti-diabetic activities of its aqueous-methanol extract.

Materials and methods:

General methods

^{1}H and ^{13}C NMR spectra were obtained on Bruker AMX- 400, Avance 400, and Avance 300 spectrometers with standard pulse sequences operating at 400 MHz in ^{1}H NMR and 100 MHz in ^{13}C NMR. Chemical shifts are given in d values (ppm) using tetramethylsilane as the internal standard. UV spectra were

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recorded with Schimadzu UV-1601. Column chromatography (CC) was carried out on Polyamide 6S and Sephadex LH20.

Plant material

Leaves of *C. renigera* Benth. were collected in December 2013, from Alexandria desert road, Egypt. Identification of the plant was confirmed by Mrs. Tereez Labib Consultant of Plant Taxonomy at the Ministry of Agriculture and ex-director of Orman botanical garden, Giza, Egypt. Voucher Specimens were kept in herbarium, Agricultural museum, Cairo, Egypt.

Extraction and isolation

Air dried leaves of *C. renigera* Benth. (1.8 kgs) were extracted with aqueous methanol 70%. The combined extracts were filtered, evaporated under reduced pressure (600 g), defatted with petroleum ether, and the sugars were precipitated. The resulted aqueous methanolic extract weighed (280 g); 30gms of which were used for Pharmacological Studies. Weighed samples of *C. renigera* Benth. extract were used to prepare the solutions, which were diluted with distilled H_2O to the appropriate concentration for the experiment. The remaining extract (250 g) was dissolved in least amount of H_2O to be applied on polyamide 6S column chromatography (150×10 cm). The column was eluted with H_2O, and mixtures of H_2O-CH_3OH according to decreasing polarity and 10 combined fractions were collected after examination on paper chromatography. Fractions 4-5(3.2 g), which were eluted from the polyamide column by 40% CH_3OH:H_2O, further purified on sephadex LH-20 sub column using a mixture of H_2O: ethanol as eluent with decreasing polarity to yield pure compounds 1 (20 mg), 2 (17 mg), 3 (22 mg) and 4 (25 mg). Fraction 9 (2.6 g), which was eluted from the polyamide column by 60% CH_3OH:H_2O, after further purification on sephadex LH-20 sub column using Bu/ H_2O saturated as an eluent yielded two compounds; compound 5 (30mg) and 6 (28mg).

Pharmacological investigations

In-vitro study of the antioxidant activity:

C. renigera Benth. leaves extract was screened for the possible anti-oxidant activity by DPPH free radical-scavenging activity. DPPH free radical scavenging activity was evaluated by measuring the scavenging activity of the extract on stable 2.2-diphenyl-1-picryl hydrazylradical (DPPH) (Bozin et al, 2006). A 0.5 mM solution of DPPH in methanol was prepared. A stock solution of sample (1.0 mg/ml) in methanol was prepared. Different concentrations (100–1000 ug/ml) were added to 1.0 ml (0.5 mM DPPH) and final volume was made to 3.0 ml with methanol. The mixture was shaken vigorously and kept standing at room temperature for 10 min. Then the absorbance of the mixture was measured at 517 nm on UV-spectrophotometer. The decrease in the absorbance indicates an increase in DPPH-radical scavenging activity. The percentage inhibition was calculated by the following equation. [DPPH radical scavenging (%) = (1− AS ⁄ AC) × 100] where AC is the absorbance of control and AS is absorbance of sample. IC_{50} value was calculated as the concentration of sample required to scavenge 50% of DPPH free radicals. The experiment was done in triplicate and mean values were calculated. Vitamin C was used as reference standard.

In-vivo investigations

Animals

Animals used were adult male albino mice weighing 20-25 g. and adult male albino rats weighing 120-150 g. purchased from the animal house at the National Research Center (NRC, Giza, Egypt). All the procedures described below were carried out in accordance with the guidelines of the EU Directive 2010/63/EU for animal experiments. Upon arrival, the animals were kept in a quiet place, housed eight per cage and acclimatized to a colony room with controlled ambient temperature (22±1 °C), humidity (50±10%) and a 12 hour natural light/dark cycle. They were fed a standard diet, water was provided and they were acclimated for 7 days before entry into the subsequent study. They were allowed free access to water and food throughout the period of investigation. The experiments were performed with 8 animals per treatment group according to a randomized schedule.

Determination of LD_{50}:

The LD_{50} of *C. renigera* Benth. leaf extract was determined using albino mice and rats. The extract dissolved in distilled water was given orally in graded doses, with the control group received the same volumes of distilled water. The percentage mortality for the extract was recorded 24 hours later. (Armitage, 1971). No mortalities were recorded up the dose of 5gm/ kg which was the maximum dose that could be suspended in a reasonable ingestible amount of water. The experimental doses used in the biological tests were 1/10, 1/20 and 1/40 of this dose.

Analgesic activity:

The writhing test

Pain was induced by injection of irritants into the peritoneal cavity of mice. The animals react with a characteristic stretching behavior which is called writhing (Bozin et al, 2006). The extract was administered subcutaneously in the three dose levels (125, 250 and 500mg/kg). After 30 min.; 0.6% acetic acid in distilled water (10 ml/Kg) was injected intraperitoneally and the stretching reaction was evaluated (Koster et al, 1959; Anuja et al, 2010). The number of writhes (muscular contractions) were counted for 30 min. immediately after the acetic acid injection and expressed as writhing numbers. Acetyl acetic acid (Aspirin) was used as standard reference drug in a dose of 100 mg/kg.

Anti-inflammatory activity:

Carrageenan- induced rat paw edema:

The method developed by (Winter et al, 1962) was employed. Albino wistar male rats (130- 150 g) were divided into various groups of eight animals each. Animals were deprived of food for 12 h prior to experiment and only water was given *ad-libitum*. First group was used as a control group and received 1 ml of 20% v/v DMSO solution; the second group received indomethacin orally (10 mg/kg) dissolved in 20% v/v DMSO solution. Other groups received the extract at doses of 125, 250 and 500 mg/kg in distilled water orally. One hour after the administration of the corresponding treatments; carrageenan suspension (0.1 ml of 1% w/v suspension in 0.9% saline solution) was injected into the sub planter region of right hind paw of animals. Immediately before carrageenan injection, the paw volume was measured (initial paw volume) using plethysmometer (Harvard Apparatus Co. Model No.LE7500, USA). The paw volume was measured after 1, 2 and 3 h after carrageenan administration. The difference between initial (Vb) and subsequent readings (Vt) gave the change in edema volume for the corresponding time. % Edema of control (Ec) and of treated (Et) were used to calculate percentage (%) inhibition and (%) edema volume by using following formula:

%Edema = [(Vt–Vb)/Vb] x 100

%Inhibition = [1- (Et /EC)] x 100

Vt= edema volume after different time intervals, Vb= basal edema volume

Ec = % Edema of control (carrageenan), Et = % Edema of treated.

Anti-hyperglycemic activity:

Hyperglycemia was induced by a single i.p. injection of streptozotocin STZ (55 mg/kg) (Pushparaj *et al.,* 2000). Briefly, rats were weighed and injected with STZ dissolved in a citrate buffer (0.1 M, pH 4.5). After 48 h blood samples were withdrawn from the retro-orbital venous plexus from18 h food-deprived rats under light ether anesthesia and the plasma was separated by centrifugation for the determination of glucose level. Only rats with plasma glucose levels more than 250 mg/dl were selected and considered as hyperglycemic animals that have been subjected to further experimentation. Forty hyperglycemic rats were divided into 5 groups. Group 1: administered distilled water ingestions and served as diabetic control. Group 2: administered Gliclazide (20 mg/kg p.o) and used as standard group, Group 3, 4 and 5: administered *Cassia renigera* Benth. hydroalcoholic leaves extract at doses of 125, 250 and 500 mg/kg p.o. An additional group of eight normal rats served as normal control was kept in a separate cage and allowed food and water *ad-libidum* and administered oral distilled water ingestions daily. All groups were administered the corresponding treatments for 10 days. Twenty-four hours after the last dose; blood samples were withdrawn from the retro-orbital venous plexus from 18 h food-deprived rats then centrifuged at 3000 rpm for 10 min and the serum obtained was used for determination of glucose, total cholesterol and triglycerides levels using spectrophotometric diagnostic biochemical kits.

Statistical analysis:

Values were expressed as means ± S.E. Comparisons between means were carried out using Statistical analysis was done using non-parametric Kruskal-Wallis test followed by Dunn's multiple comparisons test for the analgesic activity and one way ANOVA followed by Tukey's multiple comparisons tests for the anti-inflammatory and anti-hyperglycemic . $P<0.05$ was accepted as being significant in all types of statistical tests. Graph prism software (version 6) was used to carry out all statistical tests.

Chemical Characterization of the Compounds isolated for the first time from C. renigera

The structure of the isolated compounds was established through chromatography as well as conventional chemical and spectroscopic methods of analysis (UV, ^{1}H and ^{13}C NMR). Compounds isolated were identified as follows:

Kaempferol-3-O –β-D-glucopyranoside (1):

Greenish yellow amorphous powder, UV λ *max* (nm): MeOH, 256, 292sh, 357; MeOH+NaOMe, 272, 325sh, 415; MeOH+NaOAc, 273, 315, 390; MeOH+NaOAc+H_3BO_3, 261, 320sh, 377; MeOH+$AlCl_3$, 274,

340sh, 430; MeOH+$AlCl_3$+HCl: 269, 340sh, 400. ^{1}H NMR (MeOH-d_6): 8.06 (2H, *d*, *J* = 8.76 Hz, H-2',6'), 6.88 (2H, *d*, *J* = 8.76 Hz, H-3',5'), 6.44 (1H, *d*, *J* = 1.64 Hz, H-8), 6.21 (1H, *d*, *J* =1.64 Hz, H-6), 5.37 (1H, *d*, *J* = 7.32 Hz, H-1'' of glucose), 3.09-3.67 (m, rest of glucose protons). ^{13}C NMR: 156.74 (C-2), 133.65 (C-3), 177.93 (C-4), 161.68 (C-5), 99.18 (C-6), 164.63 (C-7), 94.14 (C-8), 156.74 (C-9), 104.46 (C-10), 121.37 (C-1'), 131.36 (C-2', C-6'), 115.58 (C-3', C-5'), 160.40 (C-4'), 101.34 (C-1''), 74.68 (C-2''), 76.87 (C-3''), 70.35 (C-4''), 77.93 (C-5''),61.30 (C-6'').

Kaempferol 3-O-α -L-rhamnopyranoside (2):

Greenish yellow amorphous powder, UV *λ max* (nm): MeOH, 256, 292sh, 357; MeOH+NaOMe, 272, 325sh, 415; MeOH+NaOAc, 273, 315, 390; MeOH+NaOAc+H_3BO_3, 261, 320sh, 377; MeOH+$AlCl_3$, 274, 340sh, 430; MeOH+$AlCl_3$+HCl: 269, 340sh, 400. ^{1}H NMR (MeOH-*d6*): 7.66 (2H, *d*, *J* = 8.68 Hz, H-2',6'), 6.82 (2H, *d*, *J* = 8.72 Hz, H-3',5'), 6.28 (1H, *d*, *J* = 1.88 Hz, H-8), 6.11 (1H, *d*, *J* =1.84 Hz, H-6), 5.27 (1H, *d*, *J* = 7.32 Hz, H-1'' of rhamnose), 3.20-3.62 (m, rest of rhamnose protons).^{13}C NMR: 157.90 (C-2), 134.81 (C-3), 178.23 (C-4), 161.83 (C-5), 98.46 (C-6), 164.56 (C-7), 93.37 (C-8), 157.17 (C-9), 104.51 (C-10), 121.23 (C-1'), 130.50 (C-2', C-6'), 115.13(C-3', C-5'), 160.20 (C-4'), 102.11 (C-1''), 70.64 (C-2''), 70.72 (C-3''), 71.77 (C-4''), 70.51 (C-5''), 16.24 (C-6'').The presence of L-rhamnosyl moiety was further supported by the presence of a secondary methyl group as a doublet at δ 0.83 (*J*=5.2 Hz) as well as the absence of oxymethylene group at C-5 position of the sugar unit.

Quercetin-3-O –β-D-glucopyranoside (3):

Yellow amorphous powder; UV *λ max* (nm): MeOH, 253, 263sh, 294 sh, 352; MeOH+NaOMe, 271, 328sh, 410; MeOH+NaOAc, 273, 321, 375; MeOH+NaOAc+H_3BO_3, 262, 300sh, 377; MeOH+$AlCl_3$, 275, 305sh, 332sh, 435; MeOH+$AlCl_3$+HCl: 275, 305sh, 361sh, 403. ^{1}H NMR (MeOH-*d6*):*δ* 7.53 (1H, *d*, *J* = 2.1 Hz, H-2'), 7.66 (1H, *dd*, *J* = 2.1 Hz and 7.80 Hz, H-6'), 6.83 (1H, *d*, *J* = 8.8 Hz, H-5'), 6.31 (1H, *d*, *J* = 1.8 Hz, H-8), 6.11 (1H, *d*, *J* = 1.8 Hz, H-6), 5.08 (1H, *d*, *J* = 7.76 Hz, H-1'' of glucose),3.20-3.81 (m, rest of glucose protons). ^{13}C NMR: *δ* 156.80 (C-2), 133.60 (C-3), 177.50 (C-4), 161.60 (C-5), 98.90 (C-6), 164.60 (C-7), 93.80 (C-8), 156.60 (C-9), 104.00 (C-10), 121.60 (C-1'), 115.80 (C-2'), 145.80 (C-3'), 148.80 (C-4'), 116.20 (C-5'), 122.00 (C-6'), 101.20 (C-1''), 71.60 (C-2''), 74.40 (C-3''), 70.02 (C-4''),77.70 (C-5''), 61.50 (C-6'').

Epiafzelchin (4):

Yellow amorphous powder ; ^{1}H NMR (MeOH- d_6, 400 MHz) δ 9.14 (1H, br s, Ar-OH), 9.13 (1H, br s, Ar-OH), 9.08 (1H, br s, Ar-OH), 7.32 (2H, d, *J* = 8.4, Ar-H-20), 6.79 (2H, d, *J* = 8.4, Ar-H-30), 5.94 (1H, d, *J* = 1.2, Ar-H-8), 5.34 (1H, d, *J* = 1.2, Ar-H-6), 4.87 (1H, s, CH (Ar) O), 4.66 (1H, d, *J* = 4.7, 2-OH), 4.19 (1H, m, CH (OH) CH2), 3.61 (1H, dd, *J* = 16.4, 4.5, CH2), 3.36 (1H, dd, *J* = 16.2, 3.4, CH2); ^{13}C NMR (MeOH-d6, 400 MHz) δ 156.6 (C, C-7), 156.4 (2C, C-5 overlap with C-8a), 156.2 (C, C-40), 130.2 (C, C-10), 128.1 (2CH, C-20), 114.6 (2CH, C-30), 98.6 (C, C-4a), 95.1 (CH, C-8), 94.5 (CH, C-6), 78.5 (CH, C-2), 64.5 (CH, C-3), 27.9 (CH2, C-4).

Quercetin (5):

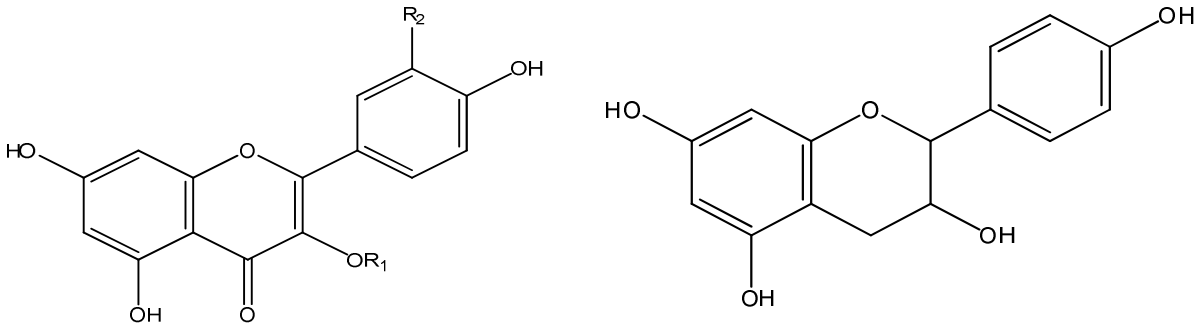
Yellow amorphous powder, UV λ_{max} nm: MeOH, 253, 268sh, 297sh, 368; MeOH+ NaOMe, 247sh, 321; MeOH + NaOAc, 257sh, 274, 329, 390. ; MeOH+NaOA+ H_3BO_3, 261, 303sh, 388;MeOH+AlCl3, 272, 304sh, 333, 458; MeOH+$AlCl_3$+HCl: 265, 301sh, 359, 428.: ^{1}H NMR (MeOH -d_6): δ 7.69(1H, d, *J* = 2.1, H-2'), 7.55 (1H, dd, *J* = 2.1 Hz, and 8.4 Hz, H-6'), 6.90 (1H, d, *J* = 8.4 Hz, H-5'), 6.42 (1H,d, *J* = 1.8 Hz, H-8), 6.20 (1H, *d*, *J* = 1.8 Hz, H-6).^{13}C NMR: *δ* 147.50 (C-2), 136.44 (C-3), 176.55 (C-4),161.43 (C- 5), 98.88 (C-6), 164.59 (C-7), 94.05 (C-8), 156.83 (C-9), 103.71, (C-10), 122.66 (C-1'), 116.31 (C-2'), 145.76 (C-3'), 148.40 (C-4'), 115.76 (C-5'), 120.68 (C-6').

Kaempferol (6):

Greenish yellow powder, UV λ_{max} nm: MeOH, 254 sh, 268, 322sh, 365; MeOH+ NaOMe, 275,320, 412 (dec.); MeOH + NaOAc, 275, 300, 385; MeOH+NaOAc+ H_3BO_3, 269, 295sh, 370; MeOH+$AlCl_3$, 262sh, 270, 353, 426; MeOH+$AlCl_3$+HCl: 260, 271, 350, 428.: ^{1}H NMR (MeOH -*d6*): *δ* 7.9 (2H, *d*, *J* = 8.1, H-2',6'); 6.90 (2H, *d*, *J* = 8.2 Hz, H-3', 5'), 6.42 (*d*, *J* = 2.4 Hz, H-8), 6.20 (1H, *d*, *J* = 2.4 Hz, H-6).

Results and Discussion

Fractionation of the 70 % aqueous methanolic extract of *C. renigera* Benth. resulted in the isolation and identification of six compounds (1-6) (Fig. 1). The structure of the isolated compounds was established through chromatography as well as conventional chemical and spectroscopic methods of analysis: UV, ^{1}H and ^{13}C NMR (Shahat *et al.,* 2005; Bezanger-Beanquesne *et al.,* 1968; Rao *et al.,* 2000; kafui *et al.,* 2010; Mabry *et al.,* 1970).



1 *R1 = glucoside*
2 R_1 = *rhammnoside*
3 R_1 = *glucose*
5 R_1 = H
6 R_1 = *R2 = H*

R_2 = *H* *4*
R_2 = H
R_2 = OH
R_2 = OH

Fig. 1: Chemical structure of the isolated compounds

In-vitro Anti-oxidant activity

DPPH test is a direct and reliable method for determining radical scavenging action. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized this color fading can be quantitatively measured from the changes in absorbance using spectrophotometric technique. The extract was subjected to screening for the possible antioxidant activity. IC50 value was calculated as the concentration of sample required to scavenge 50% of DPPH free radicals. *C. renigera* Benth. extract showed mild antioxidant activity where IC 50% was (417.15 ug). Vitamin C was used as reference standard and its IC 50 % was (16.77 ug). (Table 1). This finding is in rhythm with previous investigations on *Cassia* genus; The antioxidant activity of *C. spectabilis* was evaluated by Sangetha et al. using (DPPH) radical-scavenging assay and reported that the flower, stem, leaf and pod extracts exhibited 54.29%, 53.28%, 45.17% and 6.18% of radical-scavenging activities, respectively, at 1.0 mg/mL of extract tested. Moreover; it was reported that the water extract from whole plants of *C. tora* L. showed strong antioxidant activity (Zhang *et al.,* 2007).

Table 1: Anti-oxidant activity of *Cassia renigera* Benth.

	Conc. ug	% Scavenging
Cassia renigera Benth.	100	28.16311
	250	40.06288
	500	61.69017
	750	69.75038
	1000	79.75419
Vitamin C	5	36.15511
	10	43.49172
	15	44.1744
	20	50.7282
	25	64.2363

In-vivo investigations

Our study revealed the presence of analgesic, anti-inflammatory and anti-hyperglycemic potentials for the extract. Regarding the analgesic activity; Irritating agent (acetic acid) resulted in severe writhes in mice. Aspirin administration inhibited writhes by 73-77.5 %. Oral administration of *C. renigera* dose dependently inhibited the writhes where the highest dose showed 60.55% writhes inhibition and its result was comparable to those of Aspirin as shown in table (2).

Table 2: Analgesic activity of *Cassia renigera* Benth.

Groups	C+ve	Aspirin (100 mg/kg)	*C. renigera* (125 mg/kg)	*C. renigera* (250 mg/kg)	*C. renigera* (500 mg/kg)
Count Mean± S.E.	87.13 ± 1.3 #	23.5 ± 0.9 *	77.88 ± 0.6 #	44.38 ± 0.6	34.38 ± 0.5 *
% Inhibition		73.03%	10.62%	49.07%	60.55%

Values are expressed as means ± SEM (n = 8). Significantly different from control group at P < 0.05. # Significantly different from Aspirin group at P < 0.05.*
Statistical analysis was done using non-parametric Kruskal-Wallis test followed by Dunn's multiple comparisons test.

As for the anti-inflammatory potential; carrageenan injection resulted in severe paw edema. Indomethacin administration inhibited edema by 50%, 47% and finally 73% along the time interval of the experiment. Oral

administration of *C. renigera* Benth. caused a dose dependent anti-inflammatory effect as the higher dose significantly inhibited paw edema % to (32.04%, 47.2% and 72.55% in 1, 2 and 3 hrs, respectively). These results were comparable to those of indomethacin as shown in table (3).

Table 3: Anti-inflammatory activity of *Cassia renigera* Benth.

% Edema				% Inhibition		
Time(h) Groups	1st	2nd	3rd	1st	2nd	3rd
Control	37.02 ± 1.5 #	72.23 ± 5.5 #	84.04 ± 2.2 #			
Indomethacin	18.49 ± 1.8 *	37.88 ±3.7 *	22.24 ±1.8 *	50.05	47.56	73.54
C. renigera 125	28.92 ± 2.2 *#	45.23 ± 2.7 *	71.43 ± 3.5 *#	21.88	37.38	15.01
C. renigera 250	26.73 ± 1.7 *#	41.45 ± 2.5 *	35.2 ± 2.7 *#	27.8	42.61	58.12
C. renigera 500	25.16 ± 2.2 *	38.14 ± 2.7 *	23.07± 0.9 *	32.04	47.2	72.55

Values are expressed as means ± SEM (n = 8). Significantly different from control group at P < 0.05. # Significantly different from indomethacin group at P < 0.05.*

Finally Anti-hyperglycemic property was elucidated; the effect of *C. renigera* Benth. leaves extract at the doses of 125 mg/kg, 250 mg/kg and 500 mg/kg significantly reduced blood glucose as shown in table 4. Fasting blood glucose levels in diabetic control rats were significantly higher than those in normal rats (248.2 ± 5.1 vs. 77.4 ± 1.7) mg/dl. A significant (P<0.05) dose dependent decrease in blood glucose level was observed after 10 days of treatment with the three dose levels of *C. renigera* (102.9 ± 2.2, 100.9 ± 2.6 and 97.79 ± 2.1 mg/dl respectively). Table 4 also shows the effect of hydroalcoholic extract of *C. renigera* Benth. leaf extract on the levels of serum triglycerides and cholesterol. The levels of cholesterol and triglycerides were significantly increased in diabetic rats compared to those in normal group (102.2± 2.6 vs. 59.85±2.1 and 101.9±3.03 vs. 67.98± 1.4 mg/dl, respectively). Administration of the hydroalcoholic extract at a doses of 125, 250 and 500 mg/kg significantly reduced triglycerides to (72.29 ± 2.97, 82.75 ± 2.8 and 84.72 ± 2.5) mg/dl and cholesterol to (71.53 ± 2.7, 72.21 ± 2.3 and 72.52 ± 3.2) mg/dl respectively.

Previous researches performed on genus of *Cassia* had nearly similar results. The anti-inflammatory activity of *C. tora* against acute rat paw edema showed significant anti-inflammatory activity (Maity *et al.,* 1998). Meanwhile; the anti-nociceptive and anti-inflammatory effects of *C. spectabilis* were evaluated by (Da Silva *et al.,* 2012). The results of their work demonstrated pronounced anti-inflammatory and anti-nociceptive properties. *C. auriculata* extract revealed antioxidant effects both in vitro and in vivo (Juan-Badaturuge *et al.,* 2011, Rajagopal *et al.,* 2003 and Gupta *et al.,* 2009a). The leaves extract has been shown to possess anti-hyperglycaemic effect in experimentally diabetes (Rajagopal *et al.,* 2003; Gupta *et al.,* 2009a, 2009b, 2010) The alcoholic extract of flowers possessed antidiabetic properties as reported by Pari and Latha, (2002). Further studies revealed that *C. auriculata* extract possess antihyperlipidemic effect as discussed by Gupta *et al.,* 2009c and Gupta *et al.,* 2011).

Table 4: Anti-diabetic activity of *Cassia renigera* Benth.

Parameters Groups	Glucose Mg/dL	Triglycerides Mg/dL	Cholesterol Mg/dL
Control	77.4 ± 1.7#	59.85 ± 2.1#	67.98 ± 1.4#
Diabetic	248.2 ± 5.1*	102.2 ± 2.6*	101.9 ± 3.03*
Gliclazide (20 mg/kg)	83.67 ±2.5#	60.31 ± 2.5#	70.38 ± 2.02#
C.renigera (125 mg/kg)	102.9 ± 2.2*#	72.29 ± 2.97*#	71.53 ± 2.7#
C.renigera (250 mg/kg)	100.9 ± 2.6*#	82.75 ± 2.8*#	72.21 ± 2.3#
C.renigera (500 mg/kg)	97.79 ± 2.1*#	84.72 ± 2.5*#	72.52 ± 3.2#

Values are expressed as means ± SEM (n = 8). Significantly different from normal group at P < 0.05. # Significantly different from diabetic group at P < 0.05.*
Statistical analysis was done using one way ANOVA followed by Tukey's multiple comparisons test.

Conclusion

The antioxidant, analgesic, anti-inflammatory and anti-hyperglycemic properties of *C. renigera* extract may offer a potential therapeutic source for the treatment of multiple diseases such as inflammation, pain, hyperglycemia and hyperlipidaemia.

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