Egypt. J. Chem. **Vol. 66,** No. 6 pp. 411- 418 (2023)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

New Glycosylated Flavone and Anthraquinone from *Cassia notabilis* Randell.: Antidiabetic, Anti-inflammatory and Analgesic Activity Evaluation

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Abstract

The phytochemical investigation of the leaves of *Cassia notabilis* Randell. revealed the isolation of two new glycosides, identified as 6-methoxyapigenin-4`-methyl ether 7- *O*-*α*-L-rhamnopyranoside-8-*C*-*β*-D-glucopyranoside (**1**) and 1,7,8-trihydroxyanthraquinone 3-*O*-*β* -D-glucopyranoside (**7**), along with apigenin 7-*O*- *β*-D-glucpyranosyl-(1→6)-*β*-D-glucopyranoside (**2**), acacetin 7- *O*-*α*-L-rhamnopyranoside (**3**), luteolin 7-*O*- *β*-D-glucpyranosyl-(1→6)-*β*-D-glucopyranoside (**4**), luteolin 7-*O*- *β*-D-glucpyranoside (**5**), diosmetin 7- *O*-*α*-L-rhamnopyranoside (**6**). The structures of the isolated compounds were established by chromatographic and spectroscopic evidences. Pharmacological studies showed that the three doses of 70% aqueous methanolic extract of *C. notabilis* leaves (125, 250 and 500 mg/kg) have antidiabetic, analgesic, anti-inflammatory and antioxidant activity.

Keywords: *Cassia notabilis*, flavonoids, antidiabetic, anti-inflammatory, analgesic, antioxidant.

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1. Introduction

Herbal medicines have been used through different civilizations in curing different diseases. Today, researches are carried out on these herbal medicines to document their uses on scientific basis. *Cassia*, a large genus belonging to family Fabaceae, is widely distributed in most tropical and subtropical countries [1]. The genus has been reported to have many biological activities which are attributed to a wide diversity of chemical compounds. Its biological activities include laxative, anti-oxidant, anti-inflammatory, analgesic, anti-pyretic, antibacterial, anti-parasitic, anti-tumor, hepatoprotective, and anti-diabetic activities [2-6]. *Cassia* species are characterized by the presence of flavonoids, anthraquinones, anthracenes, alkaloids and naphthalene derivatives [7-9]. *Cassia notabilis* Randell. is native to Australia. Reviewing the literature approved that no previous studies were carried out on *Cassia notabilis* Randell. In this work we reported the isolation of a new flavone diglycoside and a new anthraquinone glucoside together with five known flavonoids, in addition to the assessment of anti-oxidant, anti-inflammatory, analgesic, and antidiabetic activities of this species.

2. Experimental

General methods

^{1}H and ^{13}C NMR spectra were recorded with a Bruker AMX- 400, spectrophotometer with standard pulse sequences operating at 400 MHz in ^{1}H NMR and 100 MHz in ^{13}C-NMR. Column chromatography (CC) was carried out on Polyamide 6S and Sephadex LH20.

2.1. Plant material.

Leaves of *C. notabilis* Randell. were collected from Alexandria desert road, Egypt in May 2018. 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. A voucher specimen (C. n 98) is deposited in the Herbarium, the Agricultural Museum, Cairo, Egypt.

2.2. Extraction and isolation

Air dried leaves of *C. notabilis* Randell. (1.2 kg) were extracted with aqueous methanol 70%. The combined extracts were filtered, concentrated under reduced pressure (500 ml), defatted with petroleum

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Receive Date: 06 December 2022, Revise Date: 20 December 2022, Accept Date: 25 December 2022

DOI: 10.21608/EJCHEM.2022.178935.7271

ether and the free sugars were precipitated by methanol and filtered. The resulted aqueous methanolic extract was concentrated to give 200 g, 170 g from which was dissolved in the least amount of water and applied on polyamide 6S column chromatography (150×10 cm). The column was eluted with H_2O, and mixtures of H_2O-CH_3OH in order of decreasing polarity and 12 combined fractions were collected after examination on paper chromatography. Fraction 3 (3.6 g), eluted by 20% CH_3OH:H_2O was further purified on Sephadex LH-20 column, using a mixture of CH_3CH_2OH: H_2O (2:8) as eluent to yield pure compounds 1 (18 mg) and compound 2 (20 mg). Fraction 5 (2.8 g), eluted by 40% CH_3OH:H_2O was further purified on Sephadex LH-20, using a mixture of CH_3CH_2OH: H_2O (2:8) as eluent to yield pure compounds 3 (25 mg), 4 (30 mg), 5 (32 mg) and 6 (30 mg). Fraction 6 (3.8 g), eluted 60% CH_3OH was further purified on Sephadex LH-20 using a mixture of CH_3CH_2OH: H_2O (1:1) as eluent to yield pure compounds 7 (18 mg).

2.3. Pharmacological investigations

In-vitro study of the antioxidant activity

DPPH free radical-scavenging method was used for screening the antioxidant activity in the aqueous methanolic extract of *Cassia notabilis* leaves. The scavenging activity of the extract was measured on stable 2.2-diphenyl-1-picryl hydrazyl radical (DPPH) [10]. A solution of 0.5 mM DPPH in methanol was prepared. A stock solution (1 mg/ml) sample in methanol was prepared. Different concentrations (100-1000 ug/ml) were added to 1 ml (0.5 mM DPPH) and final volume was made to 3 ml with methanol. Then the mixture was shaken vigorously and kept standing at room temperature for 10 min. The absorbance of the mixture was measured at 517 nm on UV-spectrophotometer. The decrease in the absorbance means increase in DPPH-radical scavenging activity. The inhibition was calculated as percentage 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.

2.4. Animals

Animals used were adult male albino mice weighing 20-25g. 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.

2.5. Determination of LD_{50}:

The LD_{50} of *C. notabilis* Randell. 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 [11]. 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.

2.6. Analgesic activity:

The writhing test

Pain was induced by injection of irritants into the peritoneal cavity of mice. The animal reacts with a characteristic stretching behaviour which is called writhing [10]. The extract was administered subcutaneously in the three dose levels (125, 250 and 500 mg/kg). After 30 min.; 0.6% acetic acid in distilled water (10 ml/kg) was injected intraperitoneally and the stretching reaction was evaluated [12]. 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.

2.7. Anti-inflammatory activity:

Carrageenan- induced rat paw edema:

The method developed by Winter et al [13] 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 plethysmo meter (Harvard Apparatus Co. Model No.LE7500, USA). The paw volume was measured after 1, 2and 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.

2.7. 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 hr blood samples were withdrawn from the retro-orbital venous plexus from18 h food-deprived rats under lightether anesthesia and the plasma was separated by centrifugation for the determination of glucose level. Only ratswith plasma glucose levels more than 250 mg/dl were selected and considered as hyperglycemic animals thathave 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 the 70 % methanol extract of *Cassia notabilis* leaves 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.

2.8. 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.

2.9. Chemical characterization of the isolated compounds

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:

6-Methoxyapigenin-4`-methyl ether 7- O-α-L-rhamnopyranoside-8-C-β-D-glucopyranoside (1)

A dark purple spot under UV light, gave bright yellow with ammonia vapor and orange yellow with $AlCl_3$ reagent. UV λ_{max} 364 (I) and 275 (II), IR (KBr) $\nu_{max}(cm^{-1})$: 3433.64, 2925.48, 1455.99, 1275.68. ESIMS m/z [M+H] +: 623.22 calculated for $C_{29}H_{34}O_{15}$. ^{1}H-NMR (400 MHz) and^{13}C-NMR (100 MHz) (Table1).

Apigenin 7-O- β-D-glucpyranosyl-(1→6)-β-D-glucopyranoside (2)

A dark purple under UV light, gave bright yellow with ammonia vapor and orange yellow with $AlCl_3$ reagent. ^{1}H-NMR (CD_3OD, δ ppm, *J* in Hz): Aglycone: 7.81 (2H, d, *J* = 8.4 Hz, H-2'/6'), 6.85 (2H, d, *J* = 8.4 Hz, H-3'/5'), 6.74 (1H, s, H-3), 6.57 (1H, s, H-8), 6.42 (1H, s, H-6), Sugar moiety: 5.18 (1H, d, *J* = 7.0 Hz, H-1"), 4.58 (1H, d, *J* = 7.0 Hz, H-1'"), 3.81 to 3.20 (rest of sugars' protons). ^{13}C- NMR(CD_3OD, 100 MHz): 184.41 (C-4), 176.30 (C-2), 163.00 (C-7), 160.69 (C-5, C-4'), 155.50 (C-9), 127.63 (C-2'/6'), 124.82 (C-1'), 115.67 (C-3'/5'), 103.82 (C-10), 102.71 (C-3), 101.26 (C-1"), 100.25 (C-1'"), 97.87 (C-6), 96.38 (C-8), 76.54 (C-5",5'"), 76.40 (C-3"), 76.30 (C-3'"), 73.90 (C-2"), 73.30 (C-2'"), 69.87 (C-4"), 69.60 (C-4'"), 68.77 (C-6"), 62.06 (C-6'") [14].

Acacetin 7-O-α-L- rhamopyranoside (3)

A dark purple under UV light, gave bright yellow with ammonia vapor and orange yellow with $AlCl_3$ reagent. ^{1}H-NMR (CD_3OD, δ ppm, *J* in Hz). Aglycone: 7.79 (2H, d, *J* = 6.8 Hz, H-2'/6'), 6.84 (2H, d, *J* = 7.6 Hz, H-3'/5'), 6.72 (1H, s, H-3), 6.55 (1H, s, H-8), 6.39 (1H, s, H-6), 3.82 (3H, s, OCH_3). Sugar moiety: 4.97 (1H, s, H-1"), 3.85 to 3.39 (rest of sugars' protons), 1.18 (1H, s, CH_3). ^{13}C-NMR(CD_3OD, 100 MHz): 182.69 (C-4), 165.40 (C-2), 163.41 (C-7), 161.57 (C-5), 161.46 (C-4'), 157.56 (C-9), 128.24 (C-2'/6'), 121.65 (C-1'), 115.67 (C-3'/5'), 105.68 (C-10), 102.71 (C-3), 100.22 (C-1"), 99.79 (C-6), 94.69 (C-8), 72.87 (C-4"),70.99 (C-5"), 70.45 (C-3"), 70.33 (C-2"), 18.00 (C-6"), 56.92 (OCH_3) [15].

Luteolin 7-O- β-D-glucpyranosyl-(1→6)-β-D-glucopyranoside (4).

A dark purple under UV light, gave bright yellow with ammonia vapor and orange yellow with $AlCl_3$ reagent. ^{1}H-NMR (CD_3OD, δ ppm, J in Hz):

Aglycone: 7.34 (2H, m, overlapped H-2'/6'), 6.81 (1H, d, *J* = 8.4 Hz, H-5'), 6.71(1H, s, H-8), 6.50 (1H, s, H-3), 6.41 (1H, s, H-6). Sugar moiety: 5.18 (1H, d, *J* = 6.9 Hz, H-1''), 4.58 (1H, d, *J* = 7.8 Hz, H-1'''), 3.94 to 3.28 (rest of sugars' protons).^{13}C-NMR(CD_3OD, 100 MHz): 182.41 (C-4), 164.92 (C-2), 163.41 (C-7), 162.57 (C-4'), 161.40 (C-5), 155.90 (C-9), 147.81 (C-4'), 143.81 (C-3'), 122.75 (C-1'), 119.19 (C-6'), 115.70 (C-5'), 114.60 (C-2'), 103.82 (C-10), 102.71 (C-3), 101.26 (C-1''), 100.25 (C-1'''), 98.87 (C-6), 96.38 (C-8), 76.54 (C-5'',5'''), 76.40 (C-3'', 3'''), 73.90 (C-2''), 73.30 (C-2'''), 69.87 (C-4''), 69.60 (C-4'''), 68.77 (C-6''), 61.33 (C-6''') [16].

Luteolin 7-O-β-D-glucopyranoside (5)

A dark purple spot under UV light, gave bright yellow with ammonia vapor and orange yellow with $AlCl_3$ reagent. ^{1}H-NMR (CD_3OD, δ ppm, *J* in Hz). Aglycone: 8.45 (1H, s, OH-5), 7.39 (1H, d, *J* = 8 Hz, H-6'), 7.35 (1H, br s, H-2'), 6.82 (1H, d, *J* = 8 Hz, H-5'), 6.78 (1H, br s, H-8), 6.69 (1H, s, H-3), 6.45 (1H, br s, H-6). Sugar moiety: 5.06 (1H, d, *J* = 6.9 Hz, H-1''), 3.9-3.0 (m, rest of sugar protons).^{13}C-NMR(CD_3OD, 100 MHz): 181.90 (C-4), 164.60 (C-2), 163.00 (C-7), 161.20 (C-9), 156.50 (C-5), 150.40 (C-4'), 145.9 (C-3'), 121.1 (C-1'), 119.3 (C-6'), 116.0 (C-5') , 113.4 (C-2'), 105.4 (C-10), 102.71 (C-3), 99.9 (C-1''), 99.6 (C-6), 94.8 (C-8), 77.2 (C-5''), 76.4 (C-3''), 73.2 (C-2''), 69.6 (C-4''), 60.7 (C-6'') [17].

Diosmetin 7-O-α-L-rhamopyranoside (6)

A dark purple under UV light, gave bright yellow with ammonia vapor and orange yellow with $AlCl_3$ reagent. ^{1}H-NMR (CD_3OD, δ ppm, *J* in Hz): Aglycone: 7.33 (2H, m, overlapped H-2'/6'), 6.81 (1H, d, *J* = 8.1 Hz, H-5'), 6.70 (1H, br s, H-8), 6.50 (1H, s, H-3), 6.40 (1H, br s, H-6), 3.64 (3H, s, OCH_3), Sugar moiety: 4.96 (1H, s, H-1''), 3.85 to 3.31 (rest of sugars' protons), 1.18 (1H, s, CH_3).^{13}C- NMR (CD_3OD, 100 MHz, δppm): 182.50 (C-4), 165.55 (C-2), 163.41 (C-7), 161.57 (C-5), 161.40 (C-9), 150.55 (C-4'), 145.79 (C-3'), 122.55 (C-1'), 119.3 (C-6'), 116.0 (C-5') , 113.4 (C-2'), 103.05 (C-10), 102.55 (C-3), 100.26 (C-1''), 99.76 (C-8), 94.67 (C-6), 72.87 (C-4''), 70.99 (C-5''), 70.45 (C-3''), 70.33 (C-2''), 56.93 (OCH_3), 16.95 (CH_3-6'') [18].

1,7,8-trihydroxyanthraquinone3-O-β-D-glucopyranoside (7)

An orange spot under UV light, doesn't change with ammonia vapor and $AlCl_3$ reagent. ESIMS m/z $[M+H]^+$: 435.78 calculated for $C_{20}H_{18}O_{11}$, ^{1}H-NMR (400 MHz) and ^{13}CNMR (100 MHz) data (Table 2).

3. Results and Discussion

Fractionation of the 70 % aqueous methanolic extract of *C. notabilis* Randell. resulted in the isolation and identification of seven compounds, two of them **1** and **7** (Fig 1) were new. The structures of the isolated compounds were established through chromatography as well as conventional chemical and spectroscopic methods of analysis: UV, ^{1}H and ^{13}C NMR [19, 20].

Compound **1** was isolated as an amorphous yellow powder, a dark purple color under UV unchanged by ammonia vapor indicating substitution of 4`-OH by methoxy group [20].

UV spectral data in MeOH gave the characteristic two bands at λmax 364 (I) and 275 (II) for a flavone nucleus [20]. Absence of bathochromic shift in band II upon addition of NaOAc reagent indicated a substituted 7-OH group. IR spectrum showed a broad peak at ν_{max}(cm^{-1}) 3433.64 for OH group. A C-O bond appeared as a peak at 1275.68. It showed pseudo-molecular ion peaks $[M+H]^+$at *m/z*623.22 in the positive ESI-MS; corresponding to the molecular formula $C_{29}H_{34}O_{15}$.

^{1}H-NMR spectrum of compound **1** showed two doublets of B ring at δ 7.98 with *J* = 8.4 Hz and 6.95 with *J* = 8.4 assigned to H-2'/6' and H-3'/5' respectively. Also, a singlet signal at δ 6.63 assigned to H-3 with the absence of both protons of H-6 and H-8, suggested the structure of compound **1** to be apigenin aglycone with the substitution on carbons 6 and 8. Two signals for two methoxy groups assigned at δ 3.67 and 3.65 were attributed to OCH_3-4' (terminal) and OCH_3-6, respectively. A proton at δ 1.08 for a methyl group, together with an anomeric singlet proton at 5.49 assigned for rhamnose sugar. While, another anomeric proton of glucose moiety appears as doublet at δ 5.05 as a broad doublet for a C-glucosyl moiety at position 8.

^{13}C-NMR spectrum of compound **1** revealed the presence of twenty-seven carbon resonances, where fifteen carbons in the aromatic region were characteristic for the apigenin glycone [21]. The upfield shift of C-5''' at δ 81.45 indicates the substitution of the glucose moiety at C-8 as a C-glucose [22]. The two methoxy moieties appeared at δ 56.92 as substituents on C-4` and C-6. The carbons of rhamnose moiety appeared from δ 102.05-69.98 together with the methyl group at δ 16.95 assigned for the glycosidation of C-7 with *O*-rhamnose.

These spectral data were compared with what was reported by [23]. HMBC experiment showed the two protons of 3'/5' at δ 6.95 cross peak with the two carbons of 2'/6' at δ 128.70 and carbon 4' at δ 155.84. The two protons of 2'/6' at δ 7.98 also showed cross peak with carbon 2 at δ 155.84. The anomeric proton of rhamnose in position 7 at δ 5.49 showed cross peak with carbon 8 at δ 109.68. The anomeric proton of *C*-glucose moiety at position 8 at δ 5.05 showed cross peak with carbon 9 at δ 153.87. Compound **1** was identified as 6-methoxy-apigenin-4`-methyl ether 7-*O*-α-L-rhamnopyranoside-8-*C*-β-D-glucopyranoside. According to the literature survey

using Reaxys, the compound 1 was previously undescribed.

^{1}H-NMR spectrum compound **7** showed in the aromatic region two doublets at δ 7.90 with *J* = 8.6 Hz and 7.17 with *J* = 8.6 assigned to H-5/6 along with other two doublets at δ 6.63, *J* = 1.6 for H-4 and at δ 6.42, *J* = 1.6 for H-2. The spectrum showed a doublet at 4.95 with *J* = 6.8 Hz assigned to an anomeric proton of glucopyranoside moiety in addition to signals at δ 3.91 to 3.20 attributed to the remaining sugar protons. ^{13}C-NMR spectrum of compound **7** revealed the presence of twenty carbon resonances, fourteen of them belong to anthraquinone moiety including two carbonyl resonances at δ 194.83 (C-10), 185.54 (C-9), and twelve aromatic carbon resonances, four of them are angular quaternary. Additionaly six carbon resonances in the aliphatic region were attributed to glucose moiety. The downfield shift of carbon 3 to δ 163.06 indicates the location of the glucose moiety to position 3. From these data, Compound **7** was identified as 1, 7, 8-trihydroxyanthraquinone 3-*O*-β-D-glucopyranoside. Reaxys' literature search revealed that compound **7** had never been described before.

Table 1: ^{1}H and ^{13}C NMR data of Compound 1 in CD_3OD

No.	δC	δH
2	161.43	
3	102.04	6.63, s
4	181.80	
5	153.87	
6	133.41	
7	155.84	
8	104.48	
9	153.87	
10	104.07	
1'	122.07	
2'/6'	128.70	7.98, d, *J* = 8.4
3'/5'	115.61	6.95, d, *J* = 8.4
4'	161.43	
1''	102.05	5.49, br s
2''	73.90	sugar protons 3.91-3.20
3''	72.16	
4''	69.98	
5''	78.05	
6''	16.95	1.08, d, *J* = 6.8
1'''	71.65	5.05, d, *J* = 9.1
2'''	71.51	sugar protons 3.91-3.20
3'''	71.51	
4'''	70.42	
5'''	81.45	
6'''	61.72	
6-OCH_3	60.50	3.65, s
4'-OCH_3	56.92	3.67 , s

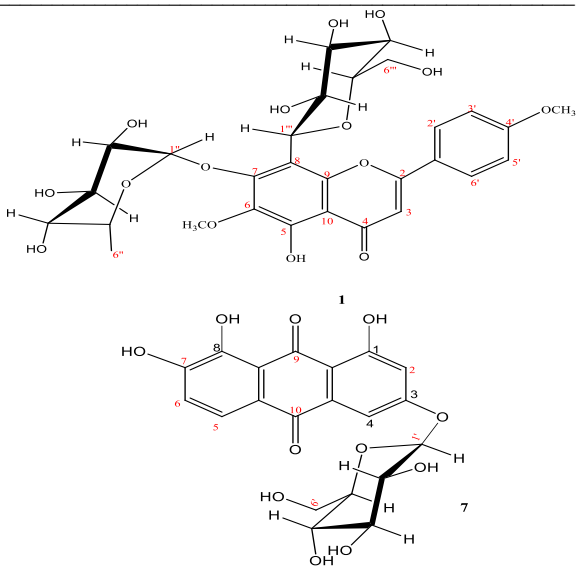

Fig. 1 Structure of the metabolites 1 & 7

Table 2: ^{1}H and ^{13}C NMR data of Compound 7 in CD_3OD

Position	δ_C	δ_H
1	159.04	
2	103.82	6.63 d, *J* = 1.6
3	163.06	
4	106.88	6.42 d, *J* = 1.6
5	127.79	7.90, d, *J* = 8.6
6	119.53	7.17, d, *J* = 8.6
7	150.20	
8	149.04	
9	189.54	
10	194.83	
1a	116.88	
4a	135.07	
5a	123.50	
8a	118.70	
1'	100.19	4.95, d, *J* = 6.8
2'	74.73	sugar protons 3.91-3.20
3'	75.11	
4'	74.31	
5'	79.71	
6'	60.18	

Table 3: Antioxidant activity of *Cassia notabilis* Randell.

Tested sample	Conc. µg	% scavenging
Cassia notabilis	100	12.90024
	250	14.62259
	500	33.32563
	750	53.93596
	1000	82.34886
Vitamin C	5	36.15511
	10	43.49172
	15	44.1744
	20	50.7282
	25	64.2363

In-vitro Antioxidant 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 colour fading can be quantitatively measured from the changes in absorbance using spectrophotometric technique. The extract was subjected to screening for the possible antioxidant activity. IC_{50} value was calculated as the concentration ofsample required to scavenge 50% of DPPH free radicals. *C.notabilis* Randell. extract showed mild antioxidant activity where IC 50% was (654.6 µg). Vitamin C was used as reference standard and its IC 50 % was (16.77 µg). (Table 3). This finding is in rhythm with previous investigations on *Cassia* genus; The antioxidant activity of *C.spectabilis* was evaluated by Sangetha *et al.* [24] 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 fromwhole plants of *C. tora* L. showed strong antioxidant activity [25].

In-vivo investigations

Our study revealed the presence of analgesic, anti-inflammatory and anti-hyperglycemic potentials for the extract.

Analgesic activity

Irritating agent (acetic acid) resulted in severe writhes in mice.Aspirin administration inhibited writhes by 73-77.5 %. Oral administration of *C. notabilis* 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 (4).

Anti-inflammatory activity

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. notabilis* Randell. caused a dose dependent anti-inflammatory effect as the higher dose significantly inhibited paw edema % to (60.24%, 49.06% and 57.24% in 1, 2 and 3 hrs, respectively). These results were comparable to those of indomethacin as shown in table (5).

Table 4: Analgesic activity of *Cassia notabilis* Randell.

Groups	C +ve	Aspirin (100 mg/kg)	*C. notabilis* (125 mg/kg)	*C.notabilis* (250 mg/kg)	*C. notabilis* (500 mg/kg)
Count Mean±S.E.	85.75 ± 1.7#	19.25 ± 0.5 *	43.00 ± 0.7 #	40.88 ± 0.4#	36.25 ± 0.5 *
%Inhibition		77.55%	49.85%	52.33%	57.73%

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.*

Table 5: Anti-inflammatory activity of *Cassia notabilis* Randell.

Time(h) / Groups	% Edema			% Inhibition		
	1st	2nd	3rd	1st	2nd	3rd
Control	38.52 ± 1.2 #	73.48 ± 2.2 #	89.86 ± 1.6 #			
Indomethacin	14.37 ± 1.1 *	35.13 ± 2.2 *	25.62 ±1.8 *	62.7	52.19	71.49
***C. notabilis* 125**	37.48 ± 1.0 #	51.16 ± 1.9 *#	76.46 ± 2.9 *#	2.71	30.37	14.91
250	37.11 ± 2.2 #	50.30 ± 1.5 *#	41.88 ± 3.0*#	3.68	31.55	53.39
500	15.32 ± 0.6 *	37.43 ± 1.4 *	38.42± 2.1 *#	60.24	49.06	57.24

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.*

Antihyperglycemic activity.

Finally, anti-hyperglycemic property was elucidated; the effect of *C. notabilis* Randell. 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. notabilis* (92.32 ± 6.04, 89.99 ± 3.4 and 88.45 ± 2.04mg/dl respectively). Table 6 also shows the effect of the 70 % methanol extract of *C. notabilis* 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 (100.4 ± 2.5vs. 60.35 ± 3.5 and 94.95 ± 3.96 vs. 61.73 ± 1.6 mg/dl, respectively). Administration of the 70 % methanol extract at a doses of 125, 250 and 500mg/kg significantly reduced triglycerides to (97.7 ± 2.3, 89.98 ± 5.5 and 79.38 ± 4.7) mg/dl and cholesterol to(64.6 ± 2.6 , 64.95 ± 1.5 and 66.36 ± 3.2) mg/dl respectively.

Table 6: Antihyperglycemic activity of *Cassia notabilis* Randell.

Parameters Groups	Glucose µg/dL	Triglycerides µg/dL	Cholesterol (µg/dL)
Control	72.4 ± 2.6 #	60.35 ± 3.5 #	61.73 ± 1.6 #
Diabetic	243.2 ± 5.3 *	100.4 ± 2.5 *	94.95 ± 3.96 *
Gliclazide (20 mg/kg)	84.92 ±2.3 #	60.63 ± 3.9 #	62.88 ± 1.2 #
***C. notabilis* (125 mg/kg)**	92.32 ± 6.04 *#	97.7 ± 2.3 *	64.6 ± 2.6 #
***C. notabilis* (250 mg/kg)**	89.99 ± 3.4 *#	89.98 ± 5.5 *	64.95 ± 1.5 #
***C. notabilis* (500 mg/kg)**	88.45 ± 2.04 #	79.38 ± 4.7 *#	66.36 ± 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.*

4. Conclusions

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

5. Conflicts of interest

The authors declared no potential conflicts of interest.

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