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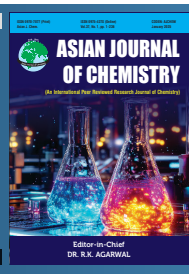

Hypoglycemic Evaluation, Isolation and Characterization of Four Major Compounds from the Ethanolic Extract of *Crataeva religiosa* Hook Leaves

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Crateva religiosa, belonging to the Capparaceae family, is a medicinal plant with a history of treating numerous diseases. Silica gel column chromatography of aqueous ethanol extract of the plant afforded three cinnamic acid derivatives *viz*. *p*-methoxy cinnamic acid, *p*-coumaric acid, ferulic acid and a flavonoid glycoside; quercetin 3-O-β-glucopyranoside and characterized by analyzing different spectroscopic data (UV, IR, 1D/2D NMR) and comparing with the previously reported data. Among the isolated compounds, *p*-methoxy cinnamic acid and *p*-coumaric acid were new findings from this leaves extract. An aqueous 80% ethanol extract of the leaves was tested for hypoglycemic studies for the first time on streptozotocin-induced Type-2 diabetic rats. Fasting serum glucose level significantly decreased after 21 days of chronic feeding of the extract. This extract did not change the body weight of the rats. Glibenclamide, as a standard drug showed a similar hypoglycemic effect to the aqueous ethanol extract of *C. religiosa* leaves. Fasting serum insulin increased in both *C. religiosa* and glibenclamide treated groups, but the difference with control remained just outside the significance level. Liver glycogen content increased significantly and serum cholesterol level decreased by 24.14% in the *C. religiosa* treated group. This study establishes the therapeutic potentiality of *C. religiosa.*

Keywords: *C. religiosa*, Hypoglycemic, *p*-Methoxycinnamic acid, *p*-Coumaric acid, Quercetin 3-O-β-glucopyranoside.

INTRODUCTION

Diabetes mellitus is a range of metabolic disorders caused by problems with insulin, leading to impaired protein, fat and carbohydrate metabolism [1]. Hyperglycemia is a global health concern that can result in several consequences of Diabetes mellitus. Numerous major health issues, including high blood pressure, neuropathy, nephropathy, retinopathy, foot injury and skin difficulties are all made more likely by diabetes [2]. Diabetes mellitus, with its increasing prevalence worldwide, is a major global cause of mortality, contributing to nearly 150 million deaths each year [3].

It is projected that by 2030, 552 million will be affected by the threat, up from 422 million in 2011 [4,5]. About 90% of cases worldwide are of Type-2 diabetes, which is the most common type of the disease and results from the body's inability to use insulin or make enough of it [6]. This non-insulin dependent kind was formerly more common in middle-aged or older adults; but, due to childhood obesity rates rising, it is now being seen in young people [7]. Type-2 diabetes is mostly managed by using oral hypoglycemic medications, such as biguanides, α-glucosidase inhibitors and sulphonylureas; however, their usage is frequently restricted due to these drugs' high cost, poor tolerability, development of therapeutic resistance and a variety of adverse effects [8].

Plants are the major source of bioactive compounds [9]. The use of therapeutic plants for healing is as old as humanity itself. As ancient civilizations faced different diseases, they learned to look for medicines in bark, seeds, fruits and plant parts. Thus, herbal medicines are one of the other approaches

used in traditional medicine to treat this kind of illness [10]. Many millions of individuals in developing nations still utilize herbal medications [11]. About 25-33% of currently available modern medicines in the United States have their roots in plant, animal or mineral systems [11,12].

Globally, *C. religiosa* Hook. (Syn: *C. nurvala*; Family: *Capparidaceae*) (Fig. 1) is found in India, Nepal, Myanmar, Sri Lanka, Malaysia, Pakistan, Bangladesh and the sub-Himalayan tract and outer Himalayan valleys. The plant is also called Barun in Hindi, three-leaved caper in English and both Assmarighna and Pashuganda in Sanskrit [13]. With fragrant white blooms and rectangular to rounded firm fruits, it is a leafy small to medium sized soft wooded tree [14]. In terms of ethnopharmacology, the herb is used as a rubefacient, counterirritant, bitter tonic, diuretic, laxative, lithotriptic, antirheumatic and antiperiodic [15-18]. The bark is used to treat kidney and bladder stones, antiemetic and calculous diseases, snakebite antidotes, fever vomiting and gastrointestinal irritation [17,19]. In addition to being lithotriptic and laxative, roots and bark also stimulate biliary secretion and promote hunger [20]. Leaves are given as internal febrifuge and tonic [21,22]. The parts have also been reported to have several other medicinal effects including anti-inflammatory [23], antioxidant [24], anti-arthritic [25], antimycotic [26], antidiabetic [27], antimicrobial [28] and antidiarrhoeal [29]. The fruits are eaten as a tonic and febrifuge in Northeastern Brazil [30].

Sugars and saponins are also found in the bark of *C. religiosa*. From the root bark of *C. religiosa*, Lakshmi & Chuhan extracted lupeol [31]. The bark of *C. religiosa* has been shown to contain diosgenin, a triterpene and cadabicine and cadabicine diacetate two different alkaloids [32]. Using repeated chromatography, Huque *et al.* [33] identified two triterpenoids, lupeol and phragmalin triacetate from the ethyl acetate fractions of the stem bark of *C. religiosa*. According to a chemical analysis, four compounds were found in the leaves of *C. nurvala*: dodecanoic anhydride, kaemferol-3-O-α-D glucoside, methyl pentacosanoate and querscitin-3-O-α-D-glucoside [19]. Glucocaparin was isolated by Sethi *et al.* [34] in the fruits of *C. religiosa.* Pentadecane, octanamide, 12-tricosanonoe and friedelin were four chemical compounds that Khadilkar *et al*. [35] identified from the fruits of *C. religiosa*.

Several pharmacognostic and phytochemical studies have been conducted previously on the bark and leaf of *C. religiosa*, but very little has been done on the ethanolic extract of the plant. Thus, in the current investigation, we examined the hypoglycemic activity of an aqueous 80% ethanol extract of *C. religiosa* leaves in streptozotocin-induced diabetic rats and isolated four major chemical components from the tested extract. The hypoglycemic activity of an aqueous 80% ethanol extract of *C. religiosa* leaves in streptozotocin-induced diabetic rats was studied for the first time and it showed significant results. Based on this study, it can be said that this plant may be a possible source of therapeutic medicine for Type-2 diabetics and researcher should emphasize this plant for future research.

EXPERIMENTAL

Research-grade solvents and chemicals were obtained from established suppliers like E. Merck (Germany), BDH (England) and RDH (Sweden). Common commercial solvents like ethanol, methanol, chloroform and dichloromethane were purified by distillation in a glass apparatus prior to their use. All solvent removals were performed using a rotary evaporator under

Fig. 1. *C. religiosa* (a) plant, (b) flowers, (c) fruits and (d) leaves

vacuum at a controlled temperature below 40 ºC. Freeze-drying was conducted using a Hetosic CD 52 freeze dryer (Hetolab Equipments, Denmark). Prior to this, samples were frozen in round-bottom flasks within a Hetofrig CB 5 methanol freezer (Heto Birkero, Denmark), reaching temperatures between -30 ºC and -40 ºC. Thin-layer chromatography (TLC) was performed using pre-coated aluminum sheets with a 0.2 mm silica-gel layer. Samples were applied to the plates with capillary tubes and TLC separation was achieved through the ascending technique in glass jars or tanks. The resulting TLC plates were then visualized under ultraviolet (UV) light at 254 nm and 350 nm wavelengths. The study utilized glass columns in a range of sizes. Large columns (90 cm × 10 cm) were fitted with rotaflow valves, while smaller, burette-style columns (30 cm × 1 cm) had Teflon flow control. The stationary phase consisted of column-grade silica gel (230-400 mesh, ASTM).

Plant collection: The leaves of *C. religiosa* were collected from Kathmandu, Nepal. A taxonomist at the Department of Botany, University of Dhaka confirmed the plant's taxonomy.

Characterization: The isolated compounds were purified and then analyzed using 400 MHz ^{13}C and ^{1}H NMR spectroscopy (Bruker) in CD_3OD and $CDCl_3$. Furthermore, 2D NMR (HMBC, HSQC, DEPT) was also carried out for the confirmation of the structure of the compounds. The FT-IR analysis was carried out with FT-IR spectroscope (Spectrum-100, PerkinElmer, Singapore) in the 4000-500 cm^{-1} region. The UV-1280 UV-VIS spectrophotometer was used for monitoring for the presence of phytochemicals by determining the type of electronic transition of compounds in the wavelength range of 200–800 nm.

General procedure

Extraction of plant materials: After cleaning with water to make them dirt free, the leaves were dried at 40 ºC in oven. Then the dried sample was crushed using a blender to coarse powder. The dried powdered leaves (980 g) were extracted with aqueous 80% ethanol at room temperature for overnight and the solvent was eliminated. The residue was further extracted with 80% ethanol two more times mixed with all of the extracts and dried by rotavapor at 40 ºC and part of it was used for biological activity. The rest of the aqueous ethanol extract (100 g) was partitioned [36] between dichloromethane and water in a separatory funnel and the dichloromethane part was separated. The aqueous fraction was further partitioned between ethyl acetate and water and ethyl acetate soluble fraction was collected. Again, the water fraction was partitioned between 1-butanol and water and two parts were separated. After the evaporation of solvent under reduced pressure and freeze-drying 1-butanol soluble part (20.1 g) and water-soluble part (50.0 g) were obtained [37,38].

Isolation of compounds: 1-Butanol extract (20.1 g) was passed through a Sephadex LH-20 gel (Amersham Bioscience, Sweden) column using the different solvent polarity of water, methanol and acetone to remove chlorophyll and six fractions were obtained. Fraction four (2.9 g) was partitioned between 10% methanol in ethyl acetate and water. The methanol-ethyl acetate soluble fraction (2.24 g) was applied to column chromatography. At first, hexane was passed through the column and the polarity was increased gradually by adding DCM followed by MeOH from which eight different sub-fractions were obtained. Compounds **1** (8.0 mg ≈ 0.36%) and **2** (10.0 mg ≈ 0.45%) were isolated from subfractions 3 and 7, respectively. Subfraction 6 (23.4 mg) was further purified using silica gel column chromatography, employing a gradient of increasing polarity from dichloromethane to ethyl acetate, resulting in the isolation of compound **3** (6.3 mg ≈ 0.28%) and compound **4** (9.2 mg ≈ 0.41%).

Compound 1 (4-hydroxy cinnamic acid or *p*-coumaric acid): White crystalline solid (m.p.: 209-211 ºC); The UV spectrum of compound **1** in methanol showed characteristic absorption maxima at 305.0 and 227.0 nm. IR (KBr, cm^{-1}): 3400, 2800,1640, 1440, 1250, 820; ^{1}H NMR (CD_3OD, 400 MHz, δ ppm): 7.60 (1H, d, *J* = 16.0 Hz, H-7), 7.45 (2H, d, *J* = 8.4 Hz, H-2, 6), 6.77 (2H, d, *J* = 8.4 Hz, H-3, 5), 6.28 (1H, d, *J* = 16.0 Hz, H-8); ^{13}C NMR (CD_3OD, 400 MHz, δ ppm): 171.1 (s, C-9), 161.1 (s, C-4), 146.6 (d, C-7), 131.1 (s, C-2,6), 127.3 (s, C-1), 116.8 (d, C-3,5), 115.7 (d, C-8) [39].

Compound 2 (*p*-methoxy cinnamic acid or *trans*-4-methoxycinnamic acid): White crystalline solid (m.p.: 170-172 ºC); The UV spectrum of compound **2** in methanol showed characteristic absorption maxima at 311.0 and 229.0 nm. IR (KBr, cm^{-1}): 3400, 2900, 2500, 1740, 1540, 1500, 1440, 1250, 1140; ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): 7.63 (1H, d, *J* = 16.0 Hz, H-7), 7.42 (2H, d, *J* = 8.4 Hz, H-2, 6), 6.84 (2H, d, *J* = 8.4 Hz, H-3, 5), 6.29 (1H, d, *J* = 16.0 Hz, H-8), 3.79 (3H, s, H-10); ^{13}C NMR ($CDCl_3$, 400 MHz, δ ppm): 168.1 (s, C-9), 157.8 (s, C-4), 144.8 (d, C-7), 130.0 (s, C-2,6), 127.2 (s, C-1), 115.9 (d, C-3,5), 115.2 (d, C-8) [40].

Compound 3 (4-hydroxy-3-methoxycinnamic acid or ferulic acid): White crystalline solid (m.p.: 167-169 ºC); The UV spectrum of compound **3** in methanol showed the characteristic absorption maxima at 219.0, 242.0 and 326.0 nm. IR (KBr, cm^{-1}): 3400, 2300, 2000, 1800, 1150, 1000; ^{1}H NMR (CD_3OD, 400 MHz, δ ppm): 7.54 (1H, d, *J* = 15.9 Hz, H-7), 7.04 (1H, d, *J* = 2.0 Hz, H-2), 6.94 (1H, dd, *J* = 8.2, 2.0 Hz, H-6), 6.79 (1H, d, *J* = 8.2 Hz, H-5), 6.26 (1H, d, *J* = 15.9 Hz, H-8) 3.76 (3H, s, H-10); ^{13}C NMR (CD_3OD, 400 MHz, δ ppm): 169.8 (s, C-9), 158.1 (s, C-3), 149.4 (d, C-4), 146.7 (s, C-7), 127.7 (s, C-1), 122.6 (d, C-6), 116.2 (d, C-5), 114.8 (d, C-8). 114.6 (d, C-2). 51.7 (d, C-10) [41].

Compound 4 (quercetin 3-O-β-D-glucopyranoside or isoquercetin): Yellow solid (m.p.: 223-225 ºC); The UV spectrum of compound **4** in methanol showed the characteristic absorption maxima at 212.0, 256.0 and 357.0 nm. IR (KBr, cm^{-1}): 3400, 1660, 1540, 1490, 1200, 1050, 820, 760; ^{1}H NMR (CD_3OD, 400 MHz, δ ppm): 7.71 (brS, H-2′), 7.56 (d, *J* = 7.2 Hz, H-6′), δ 6.86 (d, *J* = 8.4 Hz, H-5′), 6.33 (s, H-8), 6.12 (s, H-6), 5.21 (d, *J* = 7.2 Hz, H-1″), 3.82-3.36 (H-2″, H-3″, H-4″, H-5″, H-6″); ^{13}C NMR (CD_3OD, 400 MHz, δ ppm): 179.3 (C-4), 165.9 (C-7), 162.8 (C-5), 159.0 (C-2), 158.3 (C-9), 149.8 (C-4′), 145.8 (C-3′), 135.6 (C-3), 123.2 (C-6′), 123.0 (C-1′), 117.6 (C-2′), 116.0 (C-5′), 105.6 (C-10), 104.4 (C-1″), 99.9 (C-6), 94.7 (C-8), 78.2(C-5″), 78.0 (C-3″), 75.7 (C-2″), 71.1 (C-4″), 62.5 (C-6″) [42].

Biological assays

Experimental animals and induction of diabetes: Male (180-200 g) Long-Evans rats used in this research were grown at the BIRDEM (Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders) animal house. Using the Boner-Weir method, a single (90 mg/kg body weight) dose of streptozotocin was injected intraperitoneally into 48 h old pups to induce Type-2 diabetes [43].

Animal ethics: The animal study was approved by the Ethical Review Committee (ERC) of Bangladesh University of Health Science (BUHS), as per memo BUHS/BIO/EA/18/164/1.

Experimental groups: 90 Rats with Type-2 diabetes were employed in the investigation. In the acute experiment, rats were divided into three groups and trials were conducted in three different postprandial conditions (fasting, postprandial glucose load and glucose load 30 min prior).

For the chronic study, the Type-2 diabetic rats were separated into the following groups:

Group 1: Control rats (received only water; n = 6).

Group 2: Treated with standard drug (glibenclamide at a dose of 5 mg/kg. body wt. for 21 consecutive days; n = 6).

Group 3: Treated with 80% ethanol extract of *Crataeva religiosa* leaves (at a dose of 1.25 g/kg body weight twice daily for 21 days; n = 6) [44,45].

Blood collection and biochemical analysis: For the acute investigation, blood was collected from the tail tip at different time intervals under mild ether anesthesia. Type-2 diabetic rats were given two meals every day for 21 days for the chronic study. The body weight was monitored every week during the long-term trial. Throughout the 21-day study, blood was taken from tail tips at the beginning, on days 7 and 14. At the end of the study, the animals were decapitated and blood was collected. Centrifugation was used to separate the serum and the biochemical tests (except insulin) were done on the same day. The liver and pancreas were taken out, blotted dried and processed for further study. The parameters measured were blood sugar (serum glucose) using the GOD-PAP method [46], Insulin levels in the blood (serum insulin) and the total amount of insulin in the pancreas, both measured using ELISA method [47], blood fat levels (lipid profile) using an enzymatic colorimetric assay [48,49] and stored glucose in the liver (liver glycogen) using the Antrone reagent method [50].

Statistical analysis: Statistical analysis was done by using SPSS-16 (Statistical Package for the Social Sciences). The results are shown as mean ± standard deviation (SD). To determine the significant differences in the data, a one-way ANOVA test was used, followed by Duncan's multiple range test to pinpoint, which specific data differed [51]. A *p*-value below 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

The silica gel column chromatography of 1-butanol soluble part obtained from aqueous 80% ethanol extract of *C. religiosa* afforded three cinnamic acid derivatives (Fig. 2); *p*-coumaric acid (**1**), *p*-methoxy cinnamic acid (**2**), ferulic acid (**3**) and a flavonoid glycoside; quercetin 3-O-β-D-glucopyranoside (or isoquercetrin, **4**). The structures of the compounds **1-4** were determined using a combination of spectroscopic techniques, including UV, IR, ^{1}H and ^{13}C NMR and 2D NMR analyses (COSY, HSQC, HMBC, DEPT-90 and DEPT-135).

Compound **1** was obtained as a colourless to white crystalline solid and is soluble in EtOAc. The ^{1}H NMR spectrum of compound **1** revealed that the compound has a 1,4-disubstituted benzene ring structure by signals at 7.45 ppm (2H, d, J = 8.4 Hz) and 6.81 ppm (2H, d, J = 8.4 Hz). The ^{1}H NMR spectral data also indicated the presence of two olefinic protons (δ 7.60, d, J = 16.0 Hz and δ 6.28, d, J = 16.0 Hz). The higher coupling constant (J = 16.0 Hz) between olefinic protons indicated the presence of *trans* configuration [52]. The ^{13}C NMR spectrum of compound **1** (Fig. 3) indicated that the compound has seven types of carbon atom. A peak at 171.1 ppm signifies a carboxy-

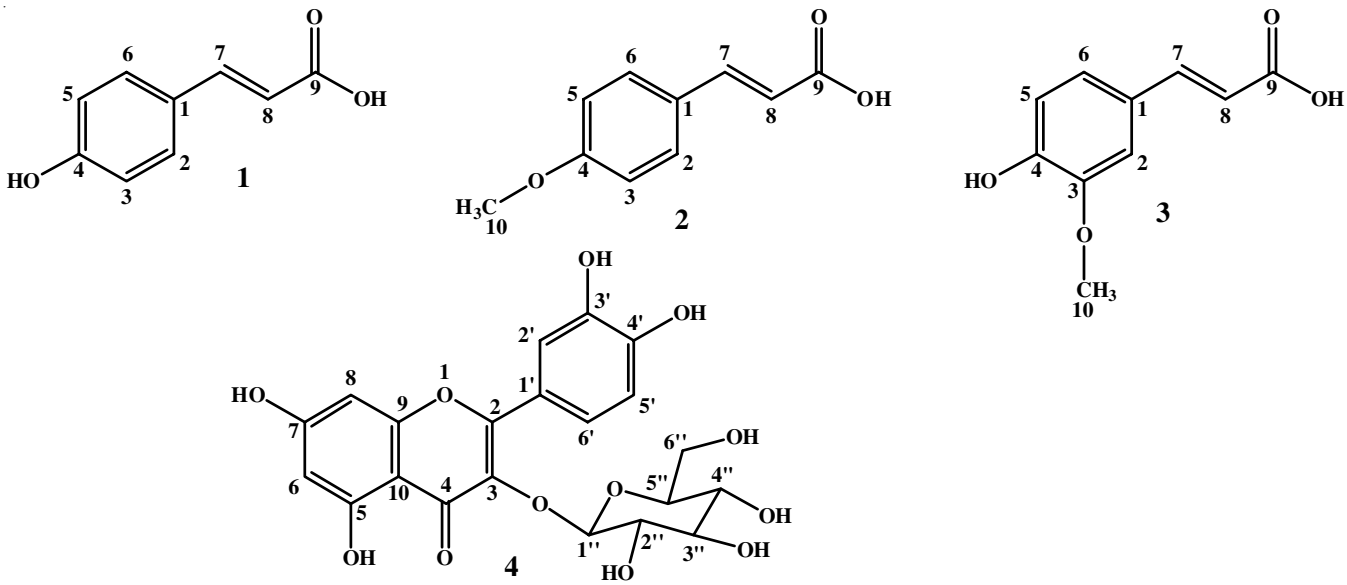

Fig. 2. Cinnamic acid derivatives (**1, 2, 3**) and a flavonoid glycoside (**4**) isolated from the ethanolic extract of *C. religiosa* leaves

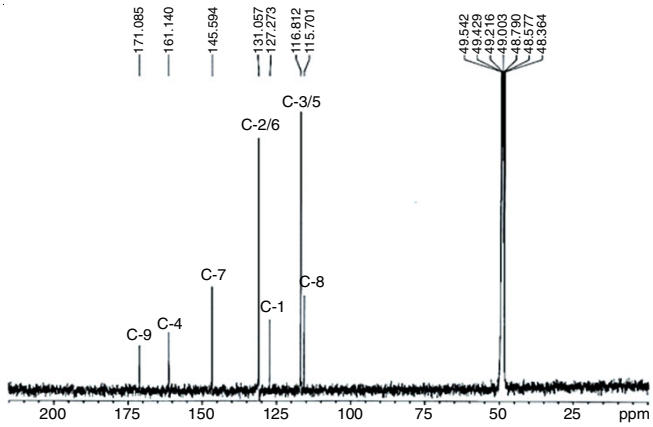

Fig. 3. ^{13}C NMR spectrum of compound **1** showing the presence of seven types of carbon atom in the compound

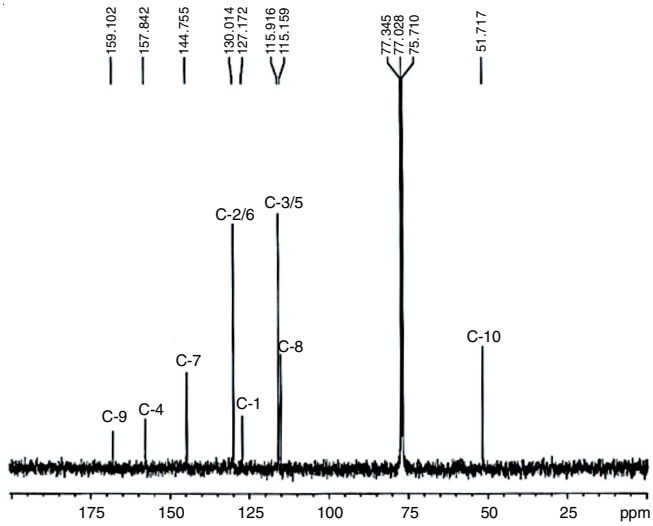

Fig. 4. ^{13}C NMR spectrum of compound **2** showing the presence of eight types of carbon atom in the compound

lic acid carbon at the C-9 position. The peaks at 146.6 ppm and 115.7 ppm correspond to the olefinic carbons located at C-7 and C-8, respectively. The aromatic carbons at positions C-2/6 and C-3/5 produced signals at 131.1 ppm and 116.8 ppm, respectively. The DEPT 135 and ^{13}C NMR revealed the presence of three quaternary carbons, C-1 at 127.3 ppm, C-4 at 161.1 ppm and C-9 at 171.1 ppm and four types of methine carbons (146.6, 131.1, 116.8 and 115.7 ppm).

Based on UV, IR, ^{1}H NMR and ^{13}C NMR spectral data and comparing with the literature [39], it was concluded that compound **1** was 4-hydroxycinnamic acid or 3-(4-hydroxyphenyl)-2-propenoic acid or *p*-coumaric acid. Although *p*-coumaric acid is present in many edible plants, including peanuts, carrots, tomatoes and garlic but it is the first-time reported compound from *C. religiosa*. The structure of the compound was further confirmed from the reported HPLC-MS and MS chromatogram of the compound from the literature [53].

Compound **2** was white crystalline solid and soluble in DCM. The ^{1}H NMR spectrum of compound **2** revealed that the compound has a 1,4-disubstituted benzene ring structure by signals at 7.42 ppm (2H, d, J = 8.4 Hz) and 6.84 ppm (2H, d, J = 8.4 Hz). Moreover, the ^{1}H NMR spectra showed that there was a single methoxy group present at 3.79 ppm and two olefinic protons (7.63 ppm, d, J = 16.0 Hz and 6.29 ppm, d, J = 16.0 Hz). The higher coupling constant (J = 16.0 Hz) between olefinic protons suggested the presence of *trans*-configuration [52]. The ^{13}C NMR spectrum of compound **2** (Fig. 4) revealed that the compound has ten carbons. The presence of a carboxylic carbon at C-9 was suggested by a signal at 168.1 ppm and olefinic carbons at C-7 and C-8 were identified by signals at 144.8 ppm and 115.2 ppm, respectively. The aromatic carbons at positions C-2/6 and C-3/5 produced signals at 130.0 ppm and 115.9 ppm, respectively. The ^{13}C NMR, DEPT 135 and HSQC revealed the presence of three quaternary carbons, C-1 at 127.2 ppm, C-4 at 157.8 ppm and C-9 at 168.1 ppm and four types of methine carbons (144.8, 130.0, 115.9 and 115.2 ppm) and one methyl carbon, C-10 at 51.7 ppm.

The results from ^{1}H NMR, ^{13}C NMR and DEPT 135 data suggested that the compound has cinnamic acid moiety like compound **1**. The cinnamic acid moiety was further confirmed by HSQC and long-range HMBC correlations. The HMBC spectrum (Fig. 5) showed correlations between H-7 (6.29) to C-1 (127.2) and C-9 (168.1), H-8 (7.63) to C-1(127.2) and C-9, H-2 to C-4 (157.8) and H-3 to C-2 (130.0) and C-4 (157.8). A ^{3}J correlation was also observed between δ 3.79 (H-10) to 157.8 (C-4) in the HMBC spectrum and confirmed that the methoxy group was joined to C-4 of the benzene ring.

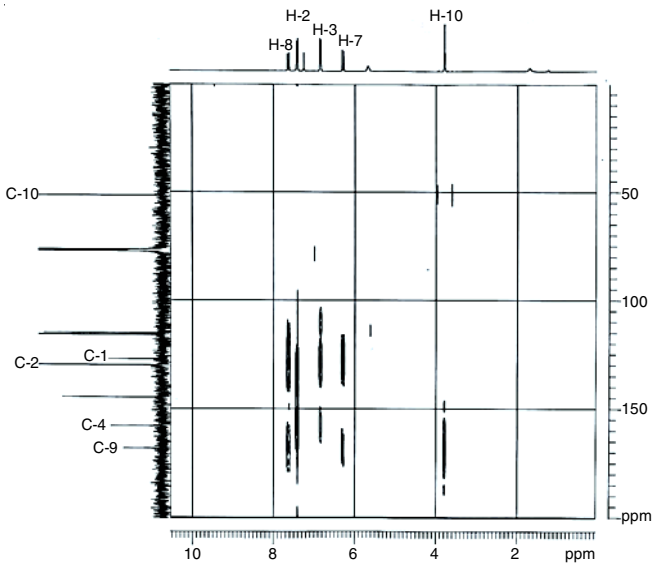

Fig. 5. HMBC spectrum of compound **2** showing the correlations of protons and carbons in the compound

Similarly, from UV, IR, ^{1}H NMR, ^{13}C NMR, DEPT-135, HSQC and HMBC data, it was concluded that compound **2** was *trans*-4-methoxy cinnamic acid or *p*-methoxy cinnamic acid or (*E*)-3-(4-methoxyphenyl)-2-propenoic acid. Although this compound has been previously reported [40], it was isolated from the *C. religiosa* plant for the first time. The structure of compound **2** was further confirmed from the reported HPLC-MS chromatogram of the compound from the literature [54].

Compounds **3** was previously reported from this plant. Compound **3** was characterized from its ^{1}H NMR, ^{13}C NMR and DEPT-135 data and comparing them with literature data

[41]. In the same manner, compound **4** was also previously reported from this plant. The ^{13}C NMR spectrum of compound **4** indicated that the compound has 21 carbons in its structure. DEPT 90 (Fig. 6) of the compound shows that it has ten methine carbons at 123.2 (C-6′), 117.6 (C-2′), 116.0 (C-5′), 104.4 (C-1″), 99.9 (C-6), 94.7 (C-8), 78.2(C-5″), 78.0 (C-3″), 75.7 (C-2″) and 71.1 (C-4″) ppm. It was further characterized by analyzing its ^{1}H NMR, ^{13}C NMR, DEPT-135, H-H COSY, HMBC, HSQC data. The structure was confirmed by comparing literature data of it [42].

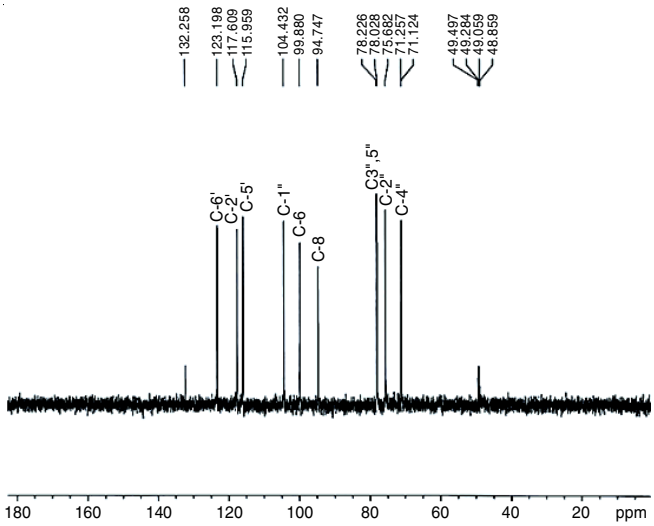

Fig. 6. DEPT 90 spectrum of compound **4** showing all the methine carbons in the compound

Hypoglycemic studies: The aqueous 80% ethanol extract of *C. religiosa* was assessed for the hypoglycemic effect on streptozotocin-induced diabetic rats. The acute effect of *C. religiosa* on the fasting blood glucose level of Type-2 model rats is shown in Table-1. No effect was found in the fasting states of this model rats. However, the extract significantly lowered the serum glucose levels when it was fed simultaneously with oral glucose (serum glucose M ± SD in mmol/L: 17.72 ± 1.33 at 75 min in the control group *vs.* 14.43 ± 3.25, $p = 0.023$). By feeding the extract 30 min before oral glucose, the serum glucose level dropped considerably ($p = 0.039$). The hypoglycemic effect of the extract was comparable with glibenclamide (standard drug) in this prandial state (Table-1).

Table-2 demonstrates that rat body weights did not significantly fluctuate within any group during the 21-day chronic study. This impact was quite comparable to that of glibenclamide, a common drug. The findings indicate *C. religiosa* has no impact on depot fat degradation, thus potentially maintain stable body weight in Type-2 diabetes. Serum glucose level was 7.95 ± 1.73 mmol/L on the 0 day and it decreased significantly ($p = 0.001$) on day 7 to 5.36 ± 1.23 and finally significantly ($p = 0.05$) changed on day 21 to 6.81 ± 1.36 with aq. 80% ethanol extract of *C. religiosa.* This effect was comparable with the standard drug glibenclamide (M ± SD in mmol/L 7.02 ± 1.97 *vs.* 5.13 ± 1.20 on the 7th day, 4.30 ± 0.75 on the 14th day and 5.21 ± 0.64 on the 21st day, $p = 0.03$; $p = 0.02$ and p = not significant, respectively). This finding imply that *C. religiosa* leaf extract has potential for controlling high blood sugar in Type-2 diabetes.

TABLE-1
ACUTE EFFECT OF *C. religiosa* EXTRACT ON FASTING AND DIFFERENT PRANDIAL STATES OF BLOOD GLUCOSE LEVELS IN TYPE-2 DIABETIC MODEL RATS

Group	Min 0	Min 60	Min 120	Iobv
	Fasting			
Water control (n = 6)	7.38 ± 0.56	7.08 ± 1.59	6.20 ± 1.49	-1.46 ± 1.95
Glibenclamide (n = 6)	7.27 ± 0.39	6.23 ± 1.28	5.74 ± 1.47*	-2.57 ± 2.36
C. religiosa extract (n = 6)	5.75 ± 1.10	6.69 ± 1.70	6.34 ± 1.62	1.53 ± 3.25
	Simultaneously with the glucose load			
Water control (n = 6)	7.19 ± 1.338	15.34 ± 2.25	17.72 ± 1.33	18.67 ± 2.91
Glibenclamide (n = 6)	7.69 ± 1.63	16.49 ± 2.96	13.64 ± 3.72**	14.65 ± 6.37
C. religiosa extract (n = 6)	7.62 ± 2.19	14.06 ± 3.33	14.43 ± 3.25	13.25 ± 4.08
	30 min before glucose load			
Water control (n = 6)	6.40 ± 0.56	15.90 ± 2.31	17.72 ± 3.18	20.82 ± 4.93
Glibenclamide (n = 6)	7.06 ± 1.60	15.12 ± 3.01	14.60 ± 3.40	15.60 ± 6.10
C. religiosa extract (n = 6)	7.42 ± 1.28	18.83 ± 3.53	14.98 ± 5.15	18.96 ± 9.23

Values are in Mean ± SD; n = number of rats, ANOVA (Bonferroni test) was done as the test of significance, *p < 0.05-0.03; **p < 0.001. Iobv = Sum of the increments over the basal value.

TABLE-2
CHRONIC EFFECT OF *C. religiosa* ON BODY WEIGHT AND BLOOD GLUCOSE LEVELS OF TYPE-2 DIABETIC MODEL RATS

Group	Body weight (BW)				Blood glucose levels (BGL)			
	0 day	7 days	14 days	21 days	0 day	7 days	14 days	21 days
Water control (n = 6)	198 ± 16	208 ± 13	210 ± 13	217 ± 10	6.85 ± 0.98	5.39 ± 1.04	5.24 ± 0.75	6.32 ± 0.98
Glibenclamide (n = 6)	215 ± 32	213 ± 27	210 ± 24	205 ± 21	7.02 ± 1.97	5.13 ± 1.20*	4.30 ± 0.79**	5.21 ± 0.64
C. religiosa extract (n = 6)	200 ± 23	200 ± 21	197 ± 22	195 ± 30	7.95 ± 1.73	5.36 ± 1.23**	7.23 ± 1.33	6.81 ± 1.36*

Values are in Mean ± SD; n = number of rats, ANOVA (Bonferroni test) was done as the test of significance, *p < 0.05-0.03; **p < 0.001. ns = non-significant.

It has been established that cinnamic acid and its derivatives exert its hypoglycemic action by improving insulin secretion [55], improving glucose tolerence [56] and increasing glucose uptake activity in 3T3-L1 adipocytes [57]. One analysis of structure-activity relationships found that ferulic acid, a cinnamic acid derivative with *para*-hydroxy and *meta*-methoxy groups, showed the most potent insulin-secreting activity [55]. Similarly, *p*-methoxycinnamic acid (10 μM) boosted the insulin secreting effect of glibenclamide in pancreatic β-cells. The insulin-secreting capabilities of *p*-methoxycinnamic acid have been extensively investigated in both normal and streptozotocin (STZ)-induced diabetic rats [56]. In both fasting and glucose-loaded states, *p*-methoxycinnamic acid (10-100 mg/kg) reduced plasma glucose and increased plasma insulin in both normal and diabetic rats [56]. Flavonoids mainly suppress carbohydrate metabolism and gluconeogenesis; facilitate glucose uptake, glycogenesis and insulin secretion; and mitigate insulin resistance [58]. Furthermore, an eight-week oral treatment with isoquercitrin significantly decreased fasting blood glucose levels in a Type-2 diabetic rat model [59]. Since, the extract is rich in these phytochemicals, its hypoglycemic activity probably is related with these mechanisms.

Similar to *C. religiosa*, other species within the *Crataeva* genus also possess blood sugar lowering (hypoglycemic) properties. For instance, an alcoholic extract from the stem bark of *C. nurvala* significantly reduced blood glucose levels in alloxan-induced diabetic rats [60]. Both petroleum ether and ethanol extracts of *C. nurvala* demonstrated substantial antidiabetic effects (with a statistical significance of $p < 0.001$) and also prevented weight loss typically seen in diabetic animals [61]. These findings from the literature support our own conclusion that the aqueous 80% ethanol extract of *C. religiosa* improves glucose tolerance. This suggests it could be beneficial for diabetic conditions where there are issues with insulin secretion in response to glucose.

Conclusion

As per the findings reported herein, the silica gel column chromatography of the *C. religiosa* leaves aqueous ethanol extract yielded four compounds: three cinnamic acid derivatives (*p*-methoxy cinnamic acid, *p*-coumaric acid and ferulic acid) and the flavonoid glycoside quercetin 3-O-β-glucopyranoside. Among these, *p*-coumaric acid and *p*-methoxy cinnamic acid were first time extracted from the leaf extract of the plant. The efficacy of aqueous 80% ethanol extract of *C. religiosa* leaves in controlling hyperglycemia was investigated. Results from the acute study showed that the extract was ineffective in reducing blood glucose in fasted Type-2 diabetic rats, yet it exhibited significant antihyperglycemic effects in prandial conditions. These results suggest that the extract may exert its effects by inhibiting intestinal glucose absorption and/or by enhancing insulin secretion. In the chronic study, when the extract was fed for 21 consecutive days significant antihyperglycemic activity was observed on the 7th day of feeding in Type-2 diabetic model rats. The hypoglycemic effect of extract persisted throughout the 21-day study, indicating sustained bioactivity. Therefore, the extract has probably insulin mimetic or insulin action enhances activity. The plant deserves continued chemical and biological research to clarify its functional mechanisms.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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