Research Journal of
Phytochemistry

ISSN 1819-3471

Academic
Journals Inc.

www.academicjournals.com

Research Journal of Phytochemistry 7 (1): 1-9, 2013
ISSN 1819-3471 / DOI: 10.3923/rjphyto.2013.1.9

Two Cinnamoyl Triterpenes and Steroids from *Crotalaria incana* (Fabaceae)

A.T.M. Zafrul Azam, Fatema Moni, Md. Hamiduzzaman, Mohammad Mehedi Masud and Choudhury Mahmood Hasan

Phytochemical Research Laboratory, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka 1000, Bangladesh

Corresponding Author: Dr. A.T.M. Zafrul Azam, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka 1000, Bangladesh

ABSTRACT

Crotalaria incana (L.) is an important medicinal plant in Bangladesh. Its traditional uses have a great appeal locally and specially to the tribal people in the hilly area of country. Its folk medicinal uses reflect the presence of medicinally important secondary metabolites of great interest. In order to explore the possibilities of developing new drug molecules from the investigated plant, it was subjected to phytochemical screening. So the present study has reported the isolation and characterization of two steroids and two cinnamoyl triterpenes from the leaves of *C. incana* (family-Fabaceae) based on different separation techniques and ^{1}H-NMR data. Reported compounds (1) β-stigmasterol, (2) β-sitosterol, (3) (3β)-(4-hydroxy-*E*-cinnamoyl)olean-5,12-dien-28-ol and (4) (3β)-(2,3,4-trihydroxy-*Z*-cinnamoyl)olean-5-ene-12,28-diol were isolated from the petroleum ether soluble fraction of crude methanol extract of leaves of *C. incana* by combination of column chromatography and preparative thin layer chromatography over silica gel (60H). The structures of these compounds were determined by ^{1}H-NMR spectroscopic method and comparing with published data. This is the first report of isolation of compound 3 and 4 from this plant.

Key words: *Crotalaria incana*, phytochemical screening, β-stigmasterol, β-sitosterol, cinnamoyl triterpenes, ^{1}H-NMR analysis

INTRODUCTION

Medicinal plants extracts can be used directly or indirectly for the treatment of different ailments. Therefore, the use of traditional medicine and medicinal plants in most developing countries, as a basis for the maintenance of good health, has been widely observed (Edward, 2001). Scientists throughout the world are trying to explore the precious assets of medicinal plants to help the suffering humanity. Furthermore, in the world more than 30% of the pharmaceutical preparations is based on plants (Shinwari and Khan, 1998). Bangladesh is a good source of the medicinal plants belonging to various families including Fabaceae. In Bangladesh there are about 17 plants belonging to the family Fabaceae revealing important secondary metabolites (Rahmatullah *et al*., 2010). Therefore an attempt has been taken to study the chemical constituents specially secondary metabolites of *C. incana*, a member of the Fabaceae family growing in Bangladesh.

Crotalaria incana (L.) (Family: Fabaceae, common name woolly rattlepod, fuzzy rattlebox) is a yellow flower bearing plant, 2.5-13.3 dm long and evergreen shrub native to tropical America, Africa, Madagascar; found in hilly areas and Mymensing in Bangladesh (Polhill, 1994). Fabaceae

family is a large family containing three subfamilies, 650 genera and 18000 species available throughout the world (McKey, 1994; Sprent, 2001). The fabaceous plants contain a wide range chemical and unique pharmacologically active compounds, including anti-inflammatory, anti-rheumatic, anti-diarrhoea and anti-emetic activities. *C. incana* which is included in Fabaceae family has been used for various traditional medicinal practices such as astringent, jaundice and palpitation, inflammation, skin diseases and purgative (Wagner *et al.*, 1999).

On the other hand, phytochemical investigations of a large number of Fabaceae plants have shown to contain a wide range of secondary metabolites such as flavonoids, alkaloids, glycosides, naphthaquinones and anthraquinones. Previous phytochemical screening showed that *C. incana* synthesizes alkaloids like monocrotaline (Asres *et al.*, 2004), integerrimine (Adams and van Duuren, 1953) and toxic compounds other than alkaloids and those are ninhydrin reacting compound which occupied the same position on 2D chromatograms as α-amino-ß-oxalyl-aminopropionic acid. It is a neurotoxic amino-acid common to those species of *Lathyrus* responsible for classical lathyrism in man and domestic animals (Bell, 1968).

Methanol extracts of *C. incana* revealed the antiproliferative activity against the HEp-2 (laryngeal cancer) and NCI-H292 (lung cancer) cell lines using the (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazole) (MTT) method. In addition, it possesses significant antioxidant activity which was evaluated with the DPPH (2,2-diphenyl-2-picrylhydrazyl) assay and the tannin content was determined by the radial diffusion method (De Melo *et al.*, 2010).

For the first time the present study reports the isolation of (3) (3β)-(4-hydroxy-*E*-cinnamoyl)olean-5,12-dien-28-ol and (4) (3β)-(2,3,4-trihydroxy-*Z*-cinnamoyl)olean-5-ene-12,28-diol from the petroleum ether soluble fraction of crude methanolic extract of *C. incana*.

MATERIALS AND METHODS

General experimental procedure: ^{1}H-NMR spectra were recorded using a Bruker AMX 500 (500 MHz) instrument and the spectra were referenced to the residual nondeuterated solvent ($CDCl_3$) signal. Gel permeation chromatography was conducted over Sephadex (LH-20). Preparative TLC was carried out by using normal phase Si-gel 60 F254 (Merck) on glass plates (20×20 cm) of 0.5 mm thickness. Spots on TLC and preparative TLC plates were visualized after spraying the developed plates with 1% vanillin in sulfuric acid, followed by heating at 110°C for few minutes.

Collection of plant material: Plant sample of *Crotalaria incana* (L.) was collected from Mymensing in September 2010 from Bangladesh. This plant was identified by the taxonomist of the Botany Department of the University of Dhaka. The reference sample for the plant was DUSH Accession Number 3015 and Call no 01 has been deposited in University of Dhaka Herbarium for further reference.

Extraction and isolation: The powdered leaf of *C. incana* (600 g) was macerated with 2.5 L of methanol in 4 L round bottom flask at room temperature for 15 days with occasional shaking. The extraction was performed at room temperature and the solvent was evaporated by rotary evaporator. It provided 19.54 g crude extract. Solvent-solvent partitioning was done using the standard protocol designed by Kupchan and modified by VanWagenen *et al.* (1993). The crude extract (5 g) was dissolved in 10% aqueous methanol. It was extracted with petroleum ether, then

with carbon tetrachloride and finally with chloroform. Subsequent evaporation of solvents afforded petroleum ether (1.5 g), carbon tetrachloride (0.25 g), chloroform (0.15 g) and aqueous soluble (2.2 g) materials.

An aliquot of petroleum ether soluble materials was fractionated by Column Chromatography (CC) over lipophilic Sephadex (LH-20) using n-hexane-dichloromethane-methanol (2:5:1) followed by mixtures of dichloromethane and methanol at different ratio to increase the polarity of the mixture and at the end with methanol. By this way we collected a number of column fractions. Preparative thin layer chromatography of the column fractions yielded four compounds. The R_f value for β-stigmasterol was 0.33, β-sitosterol was 0.44 in toluene-ethyl acetate (95:5), (3β)-(4-hydroxy-*E*-cinnamoyl)olean-5,12-dien-28-ol was 0.76 in toluene-ethyl acetate (96:4) and (3β)-(2,3,4-trihydroxy-*Z*-cinnamoyl)olean-5-ene-12,28-diol was 0.73 in toluene-ethyl acetate (95:5), respectively.

RESULTS

Repeated chromatographic separation and purification of the methanolic crude extract from powdered leaf of the *C. incana* yielded four compounds and the structures of those were revealed by extensive analysis of ^{1}H-NMR data as well as by comparing with published values and co-TLC with authentic samples.

Characterization of compound 1 as β-stigmasterol: ^{1}H-NMR (500 MHz, $CDCl_3$): δ 0.67 (3H, s, Me-13), 0.81 (3H, d, J = 7.4 Hz, Me-20), 0.83 (3H, d, J = 7.6 Hz, Me-26), 0.85 (3H, t, J = 7.6 Hz, Me-27), 0.92 (3H, d, J = 6.4 Hz, Me-28), 1.00 (3H, s, Me-10), 3.51 (1 H, m, H-3), 5.03 (1 H, dd, J = 15.0, 9.0 Hz, H-23), 5.16 (1H, dd, J = 15.0, 6.5 Hz, H-22), 5.34 (1H, br. s, H-6).

It was needle shaped off white crystals appeared as a purple spot on TLC (silica gel PF_{254}) when the developed plate was sprayed with vanillin sulphuric acid followed by heating at 110°C for 5-10 min. The ^{1}H-NMR spectrum (500 MHz, $CDCl_3$) revealed a one proton multiplet at δ 3.51, the position and multiplicity of which was indicative of H-3 of the steroidal nucleus. The typical signal for the olefinic H-6 of the steroidal skeleton was evident from a multiplet at δ 5.34 integrating one proton. The olefinic protons (H-22 and H-23) appeared as characteristic downfield signals at δ 5.16 and δ 5.03, respectively in the ^{1}H-NMR spectrum. Each of the signal was observed as double of doublets (J = 15.0 Hz, 6.5 Hz) which indicated couplings with the neighboring olefinic and methine protons. The spectrum further revealed signals at δ 0.67 and δ 1.00 (3H each) assignable to two tertiary methyl groups at C-13 and C-10, respectively. The ^{1}H-NMR spectrum showed two doublets centered at δ 0.83 (J = 6.0 Hz) and 0.85 (J = 6.0 Hz) which could be attributed to the methyl groups at C-25. The doublet at δ 0.91 (J = 6.4 Hz) was demonstrative of a methyl group at C-20. On the other hand, the triplet (J = 6.5 Hz) of three-proton intensity at δ 0.83 could be assigned to the primary methyl group attached to C-28. The above spectral features are in close agreement to those observed for β-stigmasterol (Ikan, 1991). On this basis compound 1 was characterized as β- stigmasterol. The identity of 1 was further confirmed by comparing its spectral data with previously reported values as well as Co-TLC with an authentic sample of β-stigmasterol previously isolated in our laboratory (Fig. 1).

Characterization of compound 2 as β-sitosterol: ^{1}H-NMR (500 MHz, $CDCl_3$): δ 0.67 (s, Me-13), 0.81 (1H, d, J= 7.0 Hz, H-27), 0.83 (1H, d, J = 7.0 Hz, H-26), 0.85 (1H, d, J = 7.0 Hz, H-29), 0.92 (1H, d, J = 6.4 Hz, H-21), 1.00 (s, Me-10), 3.51 (1H, m, H-3), 5.34 (1H, d, J = 5.2 Hz, H-6).

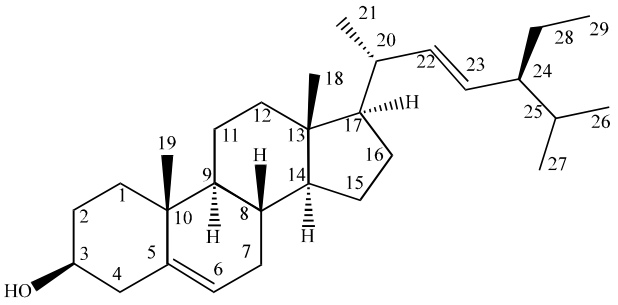

Fig. 1: Chemical structure of β-stigmasterol

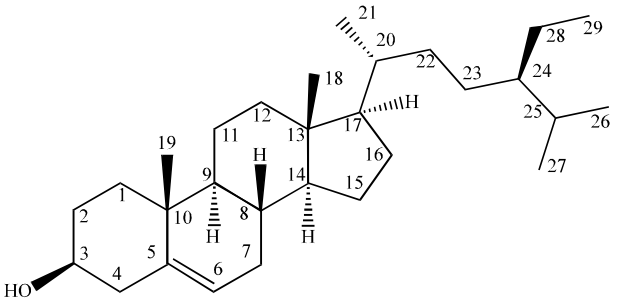

Fig. 2: Chemical structure of β-sitosterol

It was appeared as colorless precipitate and a purple spot on TLC (silica gel PF_{254}) when the developed plate was sprayed with vanillin sulphuric acid followed by heating at 110°C for 5-10 min. The ^{1}H-NMR (500 MHz, $CDCl_3$) data displayed a one proton multiplet at δ 3.51, the position and multiplicity of which was indicative to H-3 of a steroid nucleus. The typical olefinic H-6 of the steroidal skeleton was evident as a doublet (J = 5.2 Hz) at δ 5.34 that integrated for one proton. The spectrum also revealed signals at δ 0.67 and δ 1.00 (3H each) assignable to two tertiary methyl groups at C-13 (H_3-18) and C-10 (H_3-19), respectively. The ^{1}H-NMR spectrum showed two doublets (J = 7.0 Hz) centered at δ 0.83 and δ 0.85 which could be attributed to the methyl groups at C-25. The doublets (J = 6.4 Hz) at δ 0.92 was assignable to the methyl group at C-20. On the other hand, the triplet (J = 6.5 Hz) of three proton intensity at δ 0.81 could be ascribed to the primary methyl group attached to C-28. Two singlets of three proton intensity each at δ 0.67 and 1.00 could be assigned to the primary methyl group attached to C-18 and C-19, respectively. The above spectral features were in close agreement with those observed for β-sitosterol (Morales *et al.*, 2003). On this basis compound 2 was characterized as β- sitosterol. The identity of compound 2 was further confirmed by comparing its spectral data with previously reported values as well as Co-TLC with an authentic sample of β-sitosterol previously isolated in our laboratory (Fig. 2).

Characterization of compound 3 as (3β)-(4-hydroxy-*E*-cinnamoyl)olean-5,12-dien-28-ol: ^{1}H-NMR (500 MHz, $CDCl_3$): δ 0.87 (3H, m, H_3-29), 0.89 (3H, m, H_3-30), 0.91 (3H, m, H_3-26) , 0.93 (3H, m, H_3-24), 0.95 (3H, m, H_3-23), 0.98 (3H, m, H_3-27), 1.17 (3H, m, H_3-25), 3.22 (1 H, dd, J = 10.0, 1.50 Hz, H-28), 3.55 (1H, dd, J = 10.0, 1.50 Hz, H-28), 4.62 (1H, dd, J = 8.80, 7.60 Hz, H-3), 5.06 (1H, br. s, H-12), 5.19 (1H, br. s, H-6), 6.29 (1H, d, J = 16.0 Hz, H-8'), 6.82 (2H, d, J = 8.4 Hz, H-3', 5'), 7.43 (2H, d, J = 8.4 Hz, H-2', 6') and 7.59 (1H, J = 16.0 Hz, H-7').

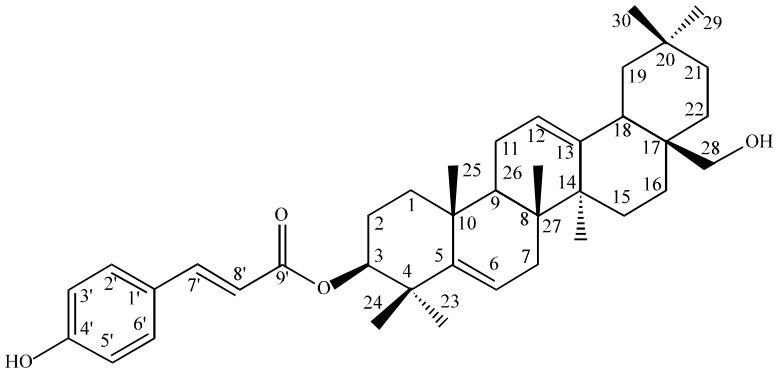

Fig. 3: Chemical structure of (3β)-(4-hydroxy-*E*-cinnamoyl)olean-5,12-dien-28-ol

It was pale colorless precipitate and appeared as yellowish spot on developed TLC (silica gel PF_{254}) when the developed plate was sprayed with vanillin sulphuric acid followed by heating at 110°C for 5-10 min. The ^{1}H-NMR (500 MHz, $CDCl_3$) spectrum represents features of a new cinnamoyl triterpene. The methyl group resonances at δ 0.87, 0.89, 0.91, 0.93, 0.95, 0.98 and 1.17 were attributed to H_3-29, H_3-30, H_3-26, H_3-24, H_3-23, H_3-27 and H_3-25, respectively.

Each proton showed two doublets of doublets at δ 3.22 (J = 10.0, 1.50 Hz), 3.55 (J = 10.0, 1.50 Hz) indicating H_2-28 and one proton doublet of doublets at δ 4.62 (J = 8.80, 7.60 Hz) suggests the presence of H-3. The typical signals for the olefinic H-6 and H-12 of the structure were evident from two broad singlets (or t-like) at δ 5.19 (1H) and 5.06 (1H), respectively. Two doublets (1H each) at δ 6.29 (J = 16.0 Hz) and 7.59 (J = 16.0 Hz) are evident of the presence of trans-coupled protons H-8' and H-7', respectively of the cinnamoyl moiety. Two doublets (2H each) at δ 6.82 (J = 8.4 Hz) and 7.43 (J = 8.4 Hz) are assignable to aromatic protons H-3', 5' and H-2', 6', respectively (Takahashi *et al.*, 1999; Kuo *et al.*, 1997). On this basis, compound 3 was characterized as (3β)-(4-Hydroxy-*E*-cinnamoyl) oleana-5, 12-dien-28-ol (Fig. 3).

Characterization of compound 4 as (3β)-(2,3,4-trihydroxy-*Z*-cinnamoyl)olean-5-ene-12,28-diol: ^{1}H-NMR (500 MHz, $CDCl_3$): δ 0.87 (3H, m, H_3-29), 0.89 (3H, m, H_3-30), 0.91 (3H, m, H_3-26) , 0.93 (3H, m, H_3-24), 0.95 (3H, m, H_3-23), 0.98 (3H, m, H_3-27), 1.17 (3H, m, H_3-25), 3.22 (1H, dd, J = 10.0, 1.50 Hz, H-28), 3.55 (1H, dd, J = 10.0, 1.50 Hz, H-28), 4.55 (1H, m, H-12), 4.62 (1H, dd, J = 8.80, 7.60 Hz, H-3), 5.19 (1H, br. s, H-6), 5.83 (1H, d, J = 12.4 Hz, H-8'), 6.78 (1 H, d, J = 8.4 Hz, H-5'), 6.81 (1H, d, J = 12.4 Hz, H-7'), 7.64 (1H, d, J = 8.4 Hz, H-6').

It was pale colorless precipitate and appeared as pale yellow spot on developed TLC (silica gel PF_{254}) when the developed plate was sprayed with vanillin sulphuric acid followed by heating at 110°C for 5-10 min. The ^{1}H-NMR (500 MHz, $CDCl_3$) spectrum represents the features for a new cinnamoyl triterpene. The methyl group resonances at δ 0.87, 0.89, 0.91, 0.93, 0.95, 0.98 and 1.17 were attributed to H_3-27, H_3-29, H_3-30, H_3-26, H_3-24, H_3-23 and H_3-25, respectively. The spectrum revealed two doublets of doublets (1H each) at δ 3.22 (J = 10.0, 1.50 Hz) and 3.55 (J = 10.0, 1.50 Hz) indicating H_2-28. A one proton doublet of doublets at δ 4.62 (J = 8.80, 7.60 Hz) suggests the presence of H-3. A multiplet at δ 4.55 (1H) is indicative of H-12 (Yoshikawa *et al.*, 1997). The typical signal of one proton intensity for the olefinic H-6 of the structure was evident from a broad singlet (or t-like) at δ 5.19. Two doublets (1H each) at δ 5.83 (J = 12.4 Hz) and 6.81 (J = 12.4 Hz) are evident of the presence of cis-coupled protons H-8' and H-7', respectively of the cinnamoyl

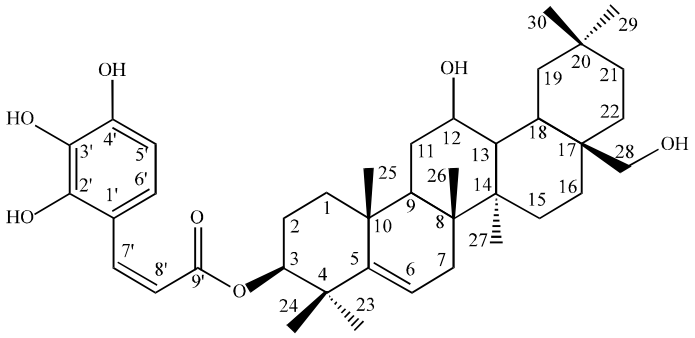

Fig. 4: Chemical structure of (3β)-(2,3,4-trihydroxy-*Z*-cinnamoyl)olean-5-ene-12,28-diol

moiety (Takahashi *et al.*, 1999). Two doublets (1H each) at δ 6.78 (J = 8.4 Hz) and 7.64 (J = 8.4 Hz) are assignable to aromatic protons H-5' and H-6', respectively (Hatano *et al.*, 1997). On this basis, compound 4 was characterized as (3β)-(2,3,4-Trihydroxy-*Z*-cinnamoyl)olean-5-ene-12,28-diol (Fig. 4).

DISCUSSION

Steroids and triterpenes are common secondary metabolites of plant origin. They take part in different functions in plant biology, especially in defence system and as precursors for new compound synthesis. Previous phytochemical studies support that steroids and triterpene derivatives are present in this species. So these reviews from the plant species of Fabaceae family make a correlation with the presence of cinnamoyl triterpenes ans steroids in this plant.

From the methanolic extract of the leaves of *Millettia versicolor* Bark, seven compounds (two sterols, one stanol and four triterpene alcohols) were isolated determined their structure by analyzing HPLC/MS, GC/MS, IR, ^{1}H-RNM and ^{13}C data (Ongoka *et al.*, 2008).

Naturally occurring triterpenes and its derivatives has been isolated from the various species of licorice (*Glycyrrhiza* L.) and choricarinal (*Meristotopis* Fisch et) by applying various chromatographic methods (Ammosov and Litvinenko, 2003).

The phytochemical investigation of the roots of *Piptadenia rigida* Benth., Fabaceae, known as "angico", afforded sitosterol, lupeol, betuline, the chalcone isoliquiritigenin, the flavonoids, 7,4'-dihydroxyflavone, 7,3',4'-trihydroxyflavone, 7,8,3',4'-tetrahydroxyflavanone, 4-hydroxy-3,5-dimethoxybenzaldehyde and methyl-3,4-dihydroxy-benzoate. The structures of compounds were identified by IR, NMR and mass spectral data analysis of natural compounds and some derivatives and by comparison with literature data (De Carvalho *et al.*, 2011).

One new phenylpropanoid, turformosin A and one new triterpene, turformosinic acid together with 16 known compounds, were isolated from the stems of *Turpinia formosana* Nakai. All structures were elucidated on the basis of spectroscopic analysis, including 1D- and 2D-NMR techniques and MS analysis (Huang *et al.*, 2012).

The roots of the plant *Medicago sativa* (family Fabaceae) have yielded two triterpene glycosides, caulosaponin B and the new glycoside medicoside C (Timbekova and Abubakirov, 1985).

The structure of a new cycloartane-type triterpene glycoside was determined as 24,25-O-β-d-diglucopyranosyl-6α-hydroxycycloart-3-one (SU3) by spectroscopic methods. This is the first cycloartane diglycoside reported from the genus *Sutherlandia*. It was isolated from a dwarf form of *S. frutescens*, currently known as *Sutherlandia humilis* (Olivier *et al.*, 2009).

The new pentacyclic triterpene, 11α-methoxy-β-amyrin was isolated from *Myroxylon balsamum* (L.) Harms (syn. *Myroxylon peruiferum* L.f.). Its structure was established on the basis of IR, MS, ^{1}H-NMR, ^{13}C-NMR. 2D-NMR experiments were also used to establish the structure and the hydrogen and carbon chemical shift assignments of the new triterpene (Mathias *et al.*, 2000).

Successive chromatographic separation and purification of crude methanolic extract of leaf of *C. incana* yielded four compounds (1, 2, 3 and 4). Among them compound 1 and 2 are commonly found in plants. Phytosterols which encompass plant sterols and stanols, are steroid compounds similar to cholesterol which occur in plants and vary only in carbon side chains and/or presence or absence of a double bond. Stanols are saturated sterols, having no double bonds in the sterol ring structure. More than 200 sterols and related compounds have been identified in plants and animals (Akhisa and Kokke, 1991). Brassinolide (BL) is the most bioactive form of the growth-promoting plant steroids termed brassinosteroids (BRs) (Grove *et al.*, 1979). Phytosterols play not only a role as essential hormones and animals but also enriched foods and dietary supplements for example oil and food stuff prepared from it. The ability of phytosterols to reduce cholesterol levels was first demonstrated in humans in 1953 (Pollak, 1953). Steroids are precursor for other steroidal compounds biosynthesis both in plants and animals.

On the other hand (3α)-(4-Hydroxy-E-cinnamoyl) oleana-5,12-dien-28-ol and (3α)-(2,3,4-Trihydroxy-*Z*-cinnamoyl)olean-5-ene-12,28 diol are the newly isolated cinnamoyl triterpene from *C. incana*. Similar types of triterpene derivatives also found in different plant species like five member ring containing cinnamoyl triterpenes (Vladimir *et al.*, 2008). They are secondary metabolites of plants. They take part in defense mechanism of plants. The holothuroid triterpene glycosides have strong membranolytic action against cellular and model membranes containing sterols as result of the formation of single-ion channels and more large pores that is the basis of hemolytic, antifungal, antitumor cytotoxic activities of these compounds (Ostlund Jr., 2002).

CONCLUSION

Chromatographic methods are important ways for separation of crude mixture of different plant samples. On the other hand ^{1}H-NMR data is obviously best method for structure determination of known and unknown compounds. Our investigation reveals the isolation and characterization of two common (steroid) and two new (cinnamoyl triterpenes) compounds by analyzing ^{1}H-NMR data and comparing with authentic published data from different research articles. Moreover isolated compounds correlate the functions of plant physiology which occur naturally in plant species. So the isolation of the reported compounds from our investigated plant is completely free from obscure.

ACKNOWLEDGMENTS

Authors like to be grateful and thankful to the Dept. of Botany, University of Dhaka for the identification of the investigated plant and Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka for proper instrumental and lab support.

REFERENCES

Adams, R. and B.L. van Duuren, 1953. Usaramoensine, the alkaloid in *Crotalaria usaramoensis* E.G. Baker. integerrimine from *Crotalaria incana* Linn and senecionine from *Senecio glabellus* D.C. stereochemical relationships. J. Am. Chem. Soc., 75: 4631-4635.

Akhisa, T. and W. Kokke, 1991. Naturally Occurring Sterols and Related Compounds from Plants. In: Physiology and Biochemistry of Sterols, Patterson, G.W. and W.D. Nes (Eds.). American Oil Chemists Society, Champaign, IL, USA., pp: 172-228.

Ammosov, A.S. and V.I. Litvinenko, 2003. Triterpenoids from plants of *Glycyrrhizia* L. and meristotopis fisch et mey genuses. Pharm. Chem. J., 37: 31-42.

Asres, K., F. Sporer and M. Wink, 2004. Patterns of pyrrolizidine alkaloids in 12 Ethiopian *Crotalaria* species. Biochem. Syst. Ecol., 32: 915-930.

Bell, E.A., 1968. Occurrence of the neurolathyrogen alpha-amino-beta-oxalylaminopropionic acid in two species of crotalaria. Nature, 218: 197-197.

De Carvalho, M.G., M.S.R. Gomes, M.C.C. de Oliveira, C.J. da Silva and A.G. de Carvalho, 2011. Chemical constituents from *Piptadenia rigida* Benth., Fabaceae, angico. Rev. Bras. Farmacogn., 21: 397-401.

De Melo, J.G., T.A.S. Araujo, V.T.N.A. Castro, D.L.V. Cabral and M.D. Rodrigues *et al*., 2010. Antiproliferative activity, antioxidant capacity and tannin content in plants of semi-arid northeastern Brazil. Molecules, 15: 8534-8542.

Edward, A., 2001. Pathogenesis *Justicia adhatoda*. New Old and Forgotten Remedies, pp: 210-220.

Grove, M.D., G.F. Spencer, W.K. Rohwedder, N.B. Mandava and J.F. Worley *et al*., 1979. Brassinolide, a plant growth promoting steroid isolated from *Brassica napus* pollen. Nature, 281: 216-217.

Hatano, K., D. Branch, E.J. Lentz, E. Baron, A.V. Filippenko and P.K. Garnavich, 1997. On the spectroscopic diversity of type ia supernovae. Astrophysical J., 543: 49-52.

Huang, H.C., C.T. Chiou, P.C. Hsiao, C.C. Liaw and L.J. Zhang *et al*., 2012. Cytotoxic phenylpropanoids and a new triterpene, turformosinic acid from Turpinia formosana Nakai. Molecules, 17: 1837-1851.

Ikan, R., 1991. Natural Product: A Laboratory Guide. 2nd Edn., Academic Press, New York, USA., ISBN-13: 978-0123705518, Pages: 316.

Kuo Y.H., C.I. Chang and Y.H. Kuo, 1997. Triterpenes from *Diospyros maritime*. Phytochemistry, 46: 1135-1137.

Mathias, L., I.J.C. Vieira, R. Braz-Filho and E.R. Filho, 2000. A new pentacyclic triterpene isolated from *Myroxylon balsamum* (syn. Myroxylon peruiferum). J. Braz. Chem. Soc., 11: 105-505.

McKey, D., 1994. Legumes and Nitrogen: The Evolutionary Ecology of a Nitrogen-Demanding Lifestyle. In: Advances in Legume Systematics, The Nitrogen Factor, Sprent, J.I. and D. McKey (Eds.). Royal Botanic Gardens, Kew, UK., pp: 211-228.

Morales, G., P. Sierra, A. Mancilla, A. Paredes, L.A. Loyola, O. Gallardo and J. Borquez, 2003. Secondary metabolites from four medicinal plants from northern Chile: Antimicrobial activity and biotoxicity against *Artemia salina*. J. Chil. Chem. Soc., 48: 13-18.

Olivier, D.K., C.F. Albrecht, B.E. van Wyk and F.R. van Heerden, 2009. SU3, an oxocycloartane diglucoside from Sutherlandia humilis. Phytochem. Lett., 2: 123-125.

Ongoka, P.R., J.T. Banzouzi, C. Poupat, A. Ekouya, J.M. Ouamba and M. Moudachirou, 2008. Steroids isolated from *Millettia versicolor* Baker (Fabaceae). Afr. J. Biotechnol., 7: 1727-1730.

Ostlund Jr., R.E., 2002. Phytosterols in human nutrition. Annu. Rev. Nutr., 22: 533-549.

Polhill, R.M., 1994. Classification of the Leguminosae. In: Phytochemical Dictionary of the Leguminosae, Bisby, F. (Ed.). Chapman and Hall, New York, USA.

Pollak, O.J., 1953. Reduction of blood cholesterol in man. Circulation, 7: 702-706.

Rahmatullah, M., M.A. Rahman, M.Z. Haque, M.A.H. Mollik and Z.U.M.E.U. Miajee *et al.*, 2010. A survey of medicinal plants used by folk medicinal practitioners of station Purbo Para village of Jamalpur Sadar Upazila in Jamalpur district, Bangladesh. Am. Eurasian J. Sustain. Agric., 4: 122-135.

Shinwari, M.I. and M.A. Khan, 1998. Indigenous use of medicinal trees and shrubs of Margalla hills National Park Islamabad. Pak. J. For., 48: 63-90.

Sprent, J.I., 2001. Nodulation in Legumes. Royal Botanic Gardens, Kew, UK.

Takahashi, H., M. Iuchi, Y. Fujita, H. Minami and Y. Fukuyama, 1999. Coumaroyl triterpenes from *Casuarina equisetifolia*. Phytochemistry, 51: 543-550.

Timbekova, A.E. and N.K. Abubakirov, 1985. Triterpene glycosides of alfalfa. II. Medicosides C. Chem. Nat. Compounds, 21: 763-766.

VanWagenen, B.C., R. Larsen, J.H. Cardellina II, D. Randazzo, Z.C. Lidert and C. Swithenbank 1993. Ulosantoin, a potent insecticide from the sponge Ulosaruetzleri. J. Org. Chem., 58: 335-337.

Vladimir, I.K., L.A. Dmitry, A. Sergey, S.S. Alexandra and A.S. Valentin, 2008. Triterpene Glycosides from sea Cucucmbers (Holothurioidea, Echinodermata). Biological Activities and Functions. In: Studies in Natural Products Chemistry, Atta-Ur-Rahman (Ed.). Elsevier, New York, pp: 135-196.

Wagner, W.L., D.R. Herbst and S.H. Sohmer, 1999. Manual of the Flowering Plants of Hawaii. Vol. 2. Revised Edn., University of Hawaii Press, Honolulu, HI.

Yoshikawa, M., T. Murakami, T. Ueno, K. Yashiro and N. Hirokawa *et al.*, 1997. Bioactive saponins and glycosides. VIII. Notoginseng (1): New dammarane-type triterpene oligoglycosides, notoginsenosides-A, -B, -C and -D, from the dried root of *Panax notoginseng* (Burk.) F.H. Chen. Chem. Pharm. Bull., 45: 1039-1045.