Available free online at www.medjchem.com

Mediterranean Journal of Chemistry

Mediterranean Journal of Chemistry **2023**, 13(1), 59-63

Bioactive flavonoid from the *Commelina obliqua* Vahl. and its *In vitro* anti-inflammatory activity

Kesar Singh *, R N Yadava, and Ritu Yadav

Department of Chemistry, Dr. Harisingh Gour Vishwidyalaya (A Central University), Sagar, M.P. 470 003, India

Abstract: *Commelina obliqua* Vahl. This plant is a member of the Commelinaceae family, commonly referred to as the dayflower family. An entirely new bioactive compound recently discovered and identified 3, 5, 7, 3' 4′-pentahydroxy flavone-7-O-L-ribopyranosyl-3′-O-D-galactopyranoside having a melting point 320-22°C, molecular formula $C_{26}H_{28}O_{16}$, m/z $[595\text{-}H]^-$ ESI-MS negative mode by different chemical color reactions, chemical decomposition, and spectral analysis 1HNMR, 13CNMR, Electron spray ionization mass spectrometry, Fourier transform infrared spectroscopy. An *in vitro* anti-inflammatory test revealed significant anti-inflammatory effects besides the degradation of egg albumin. Compound M illustrated the more percentage inhibitory action (123.1974%) of protein denaturation at 500 µg/ml, and Diclofenac sodium demonstrated less inhibitory activity (120.0627%) at the equivalent combination. As a result, Compound M inhibits protein denaturation more effectively than Diclofenac sodium at the same combination. Therefore, compound M may utilize as an anti-inflammatory source.

Keywords: Flavonol glycoside; Commelinaceae; Commelina; anti-inflammatory; NMR; Mass Spectrometry.

1. Introduction

Commelina obliqua Vahl is a plant of the family Commelinaceae. It is usually identified as a day flower, in Hindi Kana, Kanjuna. The stem of this plant is 60-90cm. high, stout, branched, glabrous. The leaves measure 10-18 by 2.5-5 cm and are sessile, petiolate, lanceolate, or elliptical-lanceolate. Flowers are blue, about 17 mm across, in simple (not branched). Ovary 3-Celled, the cells l-ovulate. Seed 6 mm long, oblong or ellipsoid, smooth, puberulous, lead-colored. It is found all over India. Its root is used in vertigo and bilious affection [1]. A plant of the same genus, *Commelina benghalensis*, has previously been reported to contain compounds such as 1-Triacontanol, 1-Octacosanol, Dotriacontanol, Sterol, Campesterol, Campesterol, beta-Sitosterol, and Stigmasterol [2]. *Commelina nudiflora* treats intestinal obstruction, diarrhea, hemorrhoids, abnormal uterine bleeding, and vaginal discharge. In addition to that, it is also used to cure warts and erysipelas (deep red inflammation of the skin). In India, the plant is believed to be beneficial in curing leprosy, while in East Africa, this plant is consumed for sore throat. Phenol, Benzyl alcohol, Eugenol, Phenol 2,4-bis(1,1-dimethylethyl), Dodecanoic acid, Hexadecanoic acid ester, n-Hexadecanoic acid, phytol, and 9,12-Octadecadienoic acid(Z, Z) are previously reported compounds in the *Commelina nudiflora* [3–5]. In this work, we have described the isolation and structure elucidation of the compound M, which was identified as 3,5,7,3’4′-pentahydroxy flavonol-7-O-β-L-ribopyranosyl-3′-O-α-D galactopyranosyl and it's *In vitro* anti-inflammatory activity in contradiction of denaturation on egg albumin protein (Fig. 1).

2. Results and Discussions

The chemical compound was obtained after extraction (3.50 g) and isolation (1.50 g) having molecular formula $C_{26}H_{28}O_{16}$, melting point 320-22 m/z $[M\text{-}H]^-$ ESI-MS $[595\text{-}H]^-$ Exact mass 596.138 and calculated mass 596.136. It gave a confirmative Molisch and Shinoda [6–9] test revealing glycoside flavonoid structure. In the FT-IR spectra revealed absorption intensity at 3400cm^{-1}(-OH), 2960 cm^{-1} (-OR), 1660 (-C=O Carbonyl group, and 1570 cm^{-1} (C=C) bond. UV-Vis spectra, 2 maximum wavelengths of 329, 255 nm indicate its flavonoid structural unit. Their absorption intensity at 389, 364 nm with $AlCl_3$ and 270, 371 nm due to NaOAc and NaOMe revealed hydroxyl (-OH) at C-3, C-5, C-7, and C-4′ respectively [7]. The 1HNMR spectra, compound M, revealed 3 singlets at δ 12.61, 11.41, 8.2 and established -OH groups at the C-5, C-3, and C-4′ sites. 7 doublets at 7.63 (1H, d, J = 2.0 Hz, H-2’), 6.91 (1H, d, J = 9.2 Hz, H-5’), 6.77 (1H, d, J = 2.2 Hz,8-H), 6.43 (1H, d, J = 2.2 Hz,9-H), 5.24 (1H, d, J = 6.4 Hz, H-1’’), 4.74 (1H, d, J = 5.1 Hz, H-4’’), 3.62 (1H, d, J = 4.9 Hz, OH-4’’), were assigned to H-2’, H-5’,

**Corresponding author: Kesar Singh*
Email address: kesarsingh84@gmail.com
DOI: http://dx.doi.org/10.13171/mjc03303021681singh

Received January 7, 2023
Accepted February 23, 2023
Published March 3, 2023

8-H, 9-H, H-1'', H-4'', OH-4'' site correspondingly and a double doublet at δ 7.70 (1H, dd, J = 9.3, 2.0 Hz, H-6'), assigned for 7.70 (1H, dd, J = 9.3, 2.0 Hz, H-6'), ′ site. The anomeric proton signals at δ 5.24 (1H, d, J = 6.4 Hz, H-2''), 4.91 – 4.84 (1H, d, H-2'''), were assigned for H-2″, H-2‴, of L-ribose and D-galactose correspondingly. In the 1HNMR spectrum, Coupling constant J = 6.41 H_Z of H-2″ established the β-configuration of L-ribose while the coupling constant at *J* = 5.2 Hz for the anomeric proton of D-galactose confirmed the α-configuration of D-galactose [10].

Molecular weight is observed due to mass losses at m/z 595 $[M-H]^-$, 434[M^+-D-galactose], and 302 [M^+-L-ribose], which existed discovered by successive deficits into molecular ions of every moiety of galactose, ribose, indicating the existence of L-ribose at the carbon-7 location and D-gal. Carbon-3′.

Compound M was treated with 10% ethanolic H_2SO_4 to yield aglycone M-1, molecular formula $C_{15}H_{10}O_7$, melting point 318-20°C, $[M-H]^-$ m/z 301 (ESI-MS), and sugar units, which were separated and analyzed separately as a result, Aglycone M-1 is known as 3, 5, 7, 3′, 4′-Penta-hydroxy flavonol.

$BaCO_3$ is used to neutralize the liquid hydrolysate and then filtered. The remaining fraction is examined after concentration via Filter paper (Whatman No. 1) in PC (paper chromatography). Sugars are revealed to be *L*-ribose (R_f 0.69), *D*-galactose, and (R_f 0.386) (Co-PC) [11].

Measurable evaluation [12] of sugars showed that all two sugars existed in an equal mole ratio of 1:1. Periodate oxidation [13] of compound M proved that two sugars occurred as pyranose form. Permethylation of compound M [14] followed by acid hydrolysis helped to determine the position of sugar molecules, which produced methyl derivatives aglycone recognized as a 7,3'-dihydroxy-3,5,4'-trimethoxy flavonol. That indicates that glycosylation occurred at the C-7 and C-3' sites of aglycone. Methyl derivatives of sugar are associated as 3,4,5-tri-O-methyl-L-ribose [R_G 1.03], 3,4,5,6-tetra-O-methyl-D-gal. [R_G 0.74], indicating that C-2″ of L-ribose was linked to the −OH group at the C-7 location of the aglycone and C-2‴ of D-galactose was connected by the −OH group at C-3′ location of aglycone.

Takadiastase [15] enzymatic hydrolysis of compound M released L-ribose indicative of the occurrence of β-connection amongst L-ribose then 3, 5, 4′-trihydroxy flavone-3′-O-α-D-galactose as proaglycone. Proaglycone on additional hydration through Almond Emulsion enzyme released D-galactose; so the compound M is recognized as 3,5,7,3′,4′-penta-hydroxy flavonol-7-O-β-L-ribo-pyranosyl-3′-O-α-D-galactopyranosyl.

So confirms that the structure of Compound M was determined to be 3,5,7,3',4'-penta-hydroxyflavone-7-O-L-ribopyranosyl-3'-O-D-galactopyranosyl. The anti-inflammatory action of Compound M *in-vitro* was evaluated against egg albumin denaturation. The conclusion is presented in Tables 1 and 2. So that from the tables, it can be concluded Compound M illustrated the more percentage inhibitory action (123.1974%) of protein denaturation at 500 µg/ml, and Diclofenac sodium illustrated less inhibitory activity (120.0627%) at the equal combination. As a result, Compound M inhibits protein denaturation more effectively than Diclofenac sodium at the same combination. As a result, Compound M may utilize as an anti-inflammatory source.

3. Conclusion

A Phyto-constituents' examination of a methanol extract of the plant *Commelina oblique* Vahl. Illustrated the occurrence of a recent Bioactive Compound (M) recognized as 3, 5, 7, 3′, 4′-penta-hydroxy flavone 7-O-β- L-ribopyranosyl-3′-O-β-D-galactopyranosyl. *In vitro*, the separated chemical has a significant anti-inflammatory action upon protein denaturation. As a result, it can be employed as an anti-inflammatory agent source.

Acknowledgments

I want to thank the CDRI and SAIF Lucknow for recording different spectrum data and the head of the Chemistry Department at Dr. H. S. Gour Central University in Sagar for providing the required research laboratory amenities. Kesar Singh, the writer, is grateful to the University Grant Commision-Non NET scholarship for economic assistance.

4. Experimental Section

4.1 General Experimental Procdure

Thermoelectric melting point apparatus determined all melting points. Bruker alpha FT-IR and Lab India, UV-Visible double-beam spectrophotometer for Infrared and UV-Visible spectra, were measured, respectively. NMR spectra were taken by using Bruker NMR spectrometer 500 MHz for 1HNMR and 125 MHz for 13CNMR employing deuterated dimethyl sulphoxide-*d6* as the solvent. Mass spectral data were documented on water ZEVO-TQD mass spectrometer [16].

4.2. Plant material

The whole plant herb material was pleated nearby the Bhopal and Sagar local areas between July and September. It was studied by a taxonomist in the Botany Department, DHSG Central University, Sagar (MP.) India, taxonomically validated. A voucher copy BOT/H/07/174/03/17 is submitted to the Natural Products Laboratory in our chemistry department.

4.3. Extraction and Isolation

The plant was shade dry and powered. Solvent

extraction is carried out with the help of the Soxhlet apparatus. Following a series of additions of hexane,

chloroform, ethyl acetate, acetone, methanol, and ethanol. The methanol extract was distilled and separated. The methanol-soluble portion was concentrated using a rotatory vacuum evaporator, producing a brown sticky mass (3.50 g). The soluble methanol compound was used for TLC analysis with a silica gel-G plate via n-BAW (4:1:5) as a solvent system, and then TLC was subjected to iodine vapors. Three dots on TLC indicated it was a composition of 3 compounds. These compounds were separated using $CHCl_3$: MeOH (4:6) in column chromatography on silica-G as eluent and divided individually.

4.4. Study of Compound M

Acetone crystallization produced pale brown crystals (1.50 g). Its molecular formula is $C_{26}H_{28}O_{16,}$ melting point 320-22°C.

$[M-H]^-$ m/z $[596-H]^-$ (ESI-MS).

Analytical obtained: C, 52.35; H, 4.73; O, 42.91. Calculated for $C_{26}H_{28}O_{16}$: C, 52.40; H, 4.1; O, 42.91%.

UV-Vis: λmax MeOH (nm) 329, 255, ($AlCl_3$) 387, 365 (NaOMe), 371 (NaOAc) 270;

FT-IR: 3440, 1690, 1600, 1083 cm^{-1}.

1HNMR (DMSO-d_6, 500 MHz) δ 12.61 (1H, s, H-5), 11.41 (1H, s, H-3), 8.20 (1H, s, H-4'), 7.70 (1H, dd, J = 9.3, 2.0 Hz, H-6'), 7.63 (1H, d, J = 2.0 Hz, H-2'), 6.91 (1H, d, J = 9.2 Hz, H-5'), 6.77 (1H, d, J = 2.2 Hz, H-8), 6.43 (1H, d, J = 2.2 Hz, H-6), 5.24 (1H, d, J = 6.4 Hz, H-2''), 4.91–4.84 (1H, d, H-2'''), 4.74 (1H, d, J = 5.1 Hz, H-5''), 4.20–4.12 (2H, dd, *J* = 5.4, 2.9 Hz, OH-3'',OH-3'''), 4.12 – 4.06 (2H, m, H-5''', H-6'''), 3.95–3.86 (2H, m, OH-4''', H-6''), 3.76–3.65 (2H, m, H-3'', H-7'''), 3.62 (1H, d, J = 4.9 Hz, OH-4''), 3.57–3.41 (3H, m, H-6'', H-7''', H-5''), 3.39–3.27 (4H, m, 2H-6''', H-4''', H-3'''), 3.25–3.16 (1H, m, H-5''').

13CNMR (DMSO-d_6, 125 MHz) δ 177.20 (C-4), 160.32 (C-5), 155.52 (C-9), 150.33 (C-4'), 147.02 (C-2), 145.91 (C-3'), 143.19 (C-7), 136.29 (C-3), 123.98 (C-1'), 123.93 (C-6'), 117.36 (C-2'), 116.49 (C-5'), 112.36 (C-6), 107.70 (C-10), 106.79 (C-8), 102.52 (C-2'''), 82.54 (C-2''), 77.38 (C-4'''), 77.34 (C-6'''), 74.22 (C-4''), 74.08 (C-3'''), 72.81 (C-3''), 70.94 (C-5'''), 70.21 (C-5''), 69.22 (CH_2-6''), 62.05 (CH_2-C-7''').

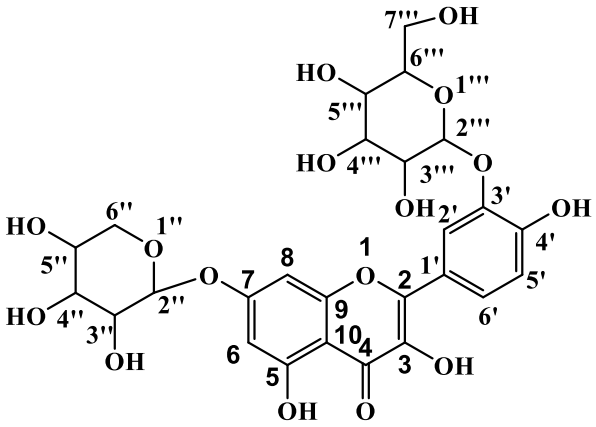

Figure 1. 3,5-dihydroxy-2-(4-hydroxy-3-((3,4,5-trihydroxy-6-(hydroxymethyl) tetrahydro-2*H*-pyran-2-yl)oxy) phenyl)-7-((3,4,5-trihydroxytetrahydro-2*H*-pyran-2-yl)oxy)-4*H*-chromen-4-one (Compound M)

4.4.1. Compound M (Acidic Hydrolysis) Compound M (400 mg) is solubilized in C_2H_5OH (30 mL) and heated on a reflux condenser with 20 mL of 10% sulphuric acid over the water bath for about 6 hours. The sample content is concentrated and kept to cool down. The residue obtained is extracted by Et_2O, with the help of an etheric water layer is washed, and the solvent is evaporated till dry. The residue which was obtained is applied to column chromatography on silica gel using $CHCl_3$: MeOH (3:6) which provides compound M-1 then it is recognized as 3,5,7,3', and 4'-pentahydroxyflavonol (Fig. 2). Neutralization with the help of $BaCO_3$ and the $BaSO_4$ was done of aqueous hydrolysate and filtered off, after the filtration the content is concentrated and applied on paper chromatography investigation using n-butanol:ethanol: water (4:1:5) solution system and aniline hydrogen phthalate as the visualizing agent. The sugars were recognized as L-ribose (R_f 0.69), D-galactose (R_f 0.38) [16].

4.4.2. Study of Compound M-1

The analysis of compound M-1 gives the molecular formula $C_{15}H_{10}O_7$ melting point 314-16°C.

$[M-H]^-$ Negative mode m/z 301 (ESI-MS).

The analysis found: C, 59.64; H, 3.39; O, 37.12. Calculated used for $C_{15}H_{10}O_7$: C, 59.61; H, 3.33; O, 37.06%.

UV-Vis: λmax MeOH (nm) 329, 255 ($AlCl_3$) 395, 374 (NaOMe) 375 (NaOAc) 274;

FT-IR: 3407, 1660, 1520 cm^{-1},1263 cm^{-1}, 1132 cm^{-1};

1HNMR (DMSO, 500 MHz) δ 12.51 (1H, s,5-OH), 10.80 (1H, s,3-OH), 9.62 (1H, s,3'-OH), 9.40 (1H, s,4'-OH), 9.34 (1H, s,7-OH), 7.69 (1H, d, *J* = 2.2 Hz,6'-H), 7.55 (1H, dd, *J* = 8.5, 2.2 Hz,2'-H), 6.89 (1H, d, *J* = 8.5 Hz,5'-H), 6.41 (1H, d, *J* = 2.0 Hz,8-H), 6.19 (1H, d, *J* = 2.1 Hz,6-H);

13CNMR (DMSO-d_6, 125 MHz) δ 177.23 (C-4), 164.97 (C-7), 160.80 (C-5), 157.58 (C-9), 148.46 (C-4'), 147.18 (C-2), 145.38 (C-3'), 136.31 (C-3), 122.53 (C-1'), 120.66 (C-6'), 115.79 (C-5'), 115.54 (C-2'), 103.17 (C-10), 99.53 (C-6), 94.38 (C-8).

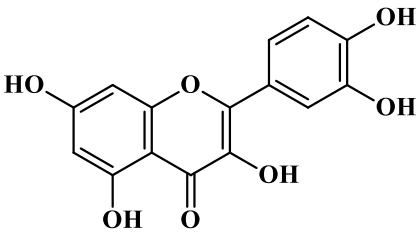

Figure 2. Compound M-1

4.4.3. Permethylation of Compound M

30 mg of the compound is mixed with dimethyl formamide of 25 ml and treated with MeI of 5 ml + 15 ml of Ag_2O in a round bottom flask which is fitted over an air condenser for about 24 hours, then rinsed and filtered. The filtrate obtained is concentrated with a rotatory evaporator and hydrolysis with the help of ethanolic H_2SO_4 to obtain the methylated derivative of aglycone that is determined as 7,3'-dihydroxy-3,5,4'-tri-methoxy flavone [17-19]. water hydrolysate is yielded after aglycone is neutralized with $BaCO_3$ and $BaSO_4$ filter off. The filtrate is concentrated for paper chromatography using n-butanol:ethanol: water (5:1:4) as a solvent system and aniline hydrogen phthalate as a visualizing agent. Methylated sugar was associated as 3,4,5-trihydroxy-o-methyle-L-ribose[R_G 0.69], 3,4,5- trihydroxy methyl-D-galactose [R_G 0.38] [16].

4.4.4. Enzymatic hydrolysis of Compound M: 30 mg of the compound is solubilized in 25 ml of the methanol and hydrolysis with the Takadiastase enzyme. The chemical mixture is separated after 2 days at room temperature. The hydrolyzed compound is concentrated and examined by paper chromatography with n-BuOH:C_2H_5OH: H_2O (5:1:4) solvent system and hydrogen phthalate as a derivatizing agent. The proaglycone is mixed with methanol 25 ml of hydrolysis with retained an equivalent amount of almond emulsion enzyme, which gives aglycone confirmed as 3,5,7,3', and 4' Penta-hydroxy flavonol (M-1), which reveals the presence of sugars L-ribose (R_f 0.69) and D-galactose (R_f 0.38).

5. Anti-inflammatory activity (*In-vitro*)

EGG albumin denaturation inhibition method

"Compound M strengths are 50-500μM/ml was prepared. Separately, 2 mL of 1% albumin solution (Hen's egg), 3 mL of phosphate buffer saline (pH 6.4), and 4 mL of various quantities of chemical M 50-500μM/ml was produced. Likewise, the sample solution (II) was made using 2 mL of 1% albumin solution (Hen's egg), 3 mL of phosphate buffer saline (pH 6.4), and 4 mL of Diclofenac sodium (reference drug) at various doses (50-500μM/ml) As a control, a similar amount of dd water was used. The reaction mixtures I and II were then incubated at 37°C for 15 minutes before being heated at 70°C for 5 minutes. After cooling, their absorbance was measured at 660 nm using a Lab India UV-Visible double beam spectrophotometer" [20,21].

The below-mentioned formula is used to calculate the Percentage inhibition of protein degradation:

% Inhibition = (Vt/Vc-1)*100

Where,

Sample absorption Vt

Control absorption Vc

Table 1. Activity of Diclofenac sodium on protein denaturation

Absorbance Control = 0.319		
The concentration of diclofenac sodium (μM/ml)	Absorbance of Diclofenac	Inhibitory percentage of Sodium Diclofenac
50	0.363	13.79310
100	0.405	26.95924
200	0.441	38.24451
300	0.452	41.69279
400	0.526	64.89028
500	0.702	120.06269

Table 2. Activity Compound M on protein denaturation

Absorbance Control = 0.319

The concentration of Compound M in the reaction combination (μM/mL)	Absorbance of Compound M	Inhibitory percentage of Compound M
50	0.414	29.78056
100	0.450	41.06583
200	0.514	61.12852
300	0.614	92.47648
400	0.685	114.73354
500	0.712	123.19749

References

1. K.R. Kirtikar, B.D. Basu, Indian-Medicinal-Plants, Periodical Experts Book Agency, Delhi, **1991**, 2534-2536.
2. J.A. Duke, Database of phytochemical constituents of GRAS herbs and other economic plants, CRC Press, Boca Raton, FL, **1992**.
3. Alikbangon / Commelina diffusa / climibing dayflower: Philippine Medicinal Herbs / Philippine Herbal Medicine. http://www.stuartxchange.org/Alikbangon.html (accessed 2023-02-27).
4. MD. Shah, U.J.A. Souza, M. Iqbal, The Potential Protective Effect of Commelina Nudiflora L. against Carbon Tetrachloride (CCl4)-Induced Hepatotoxicity in Rats, Mediated by Suppression of Oxidative Stress and Inflammation, Environ. Health Prev. Med., **2017**, 22 (1), 1-19.
5. R.A. Suganya, G.J. Jothi Preliminary Phytochemical Screening, Antibacterial and Antioxidant Activities of Commelina Nudiflora (Commelinaceae). *Int. Res. J. Pharm.* **2014**, *5* (11), 851–855.
6. F.G. Mann, B.C. Saunders, Practical Orgnanic Chemistry, London, Longmans, **1960**.
7. J. Shinoda, Color reaction of flavone and flavonol derivatives and the like, J. pharm. Soc. Japan, **1928**, 48, 214-220.
8. R.Y.M. Agarwal, Phytochemical Analysis of Some Medicinal Plants, Liaquat Med. Res. J., **2021**, 3, 1–5.
9. O. Otshudi, A. Longanga, A. Vercruysse, A. Foriers, Contribution to the Ethnobotanical, Phytochemical and Pharmacological Studies of Traditionally Used Medicinal Plants in the Treatment of Dysentery and Diarrhoea in Lomela Area, Democratic Republic of Congo (DRC), J. Ethnopharmacol., **2000**, 71, 411–423.
10. JB Harborne, T.J. Marby, The Flavonoids, Advances in Research, Chapman and Hall, London, New York, **1982**.
11. S.B. Mishra, V.K. Mohan, R.B. Arora, Some observations on hypoglycaemic activity of Momordica charantia, Indian Journal of Medical Research, **1960**, 48, 471-477.
12. E. Lederer, M. Ledrer, Chromatography, Elsevier Publishing Company, New York, **1957**.
13. E.L. Hirst, J.K.N. Jones, J. Chem. Soc., **1999**, 127, 628.
14. S.I.A. Hakomori, Rapid Permethylation of Glycolipid, and Polysaccharide Catalyzed by Methylsulfinyl Carbanion in Dimethyl Sulfoxide, J. Biochem., **1964**, 55, 205–208.
15. J.B. Horborne, Plant polyphenols—XIV, Phytochemistry, **1965**, 4, 107-120.
16. A. Chakravarty, In Vitro Anti-Inflammatory Activity of a New Allelochemical from the Bark of Pithecellobium Dulce (Roxb.) Benth. R, Indian J. Chem., **2017**, 56B, 447-452.
17. NPJ Price, Permethylation Linkage Analysis Techniques for Residual Carbohydrates, Appl. Biochem. Biotechnol., **2008**, 148 (1–3), 271–276.
18. M.A. Hossain, M.S. Rahman, Isolation and Characterisation of Flavonoids from the Leaves of Medicinal Plant Orthosiphon Stamineus, Arab. J. Chem., **2015**, 8, 218–221.
19. E. Bombardelli, A. Bonati, B. Gabetta, G. Mustich, Fitoterapia, **1972**, 35.
20. P. Chatterjee, S. Chandra, P. Dey, S. Bhattacharya, Evaluation of Anti-Inflammatory Effects of Green Tea and Black Tea: A Comparative in Vitro Study, J. Adv. Pharm. Technol. Res., **2012**, 3, 136-138.
21. S. Chandra, P. Chatterjee, P. Dey, S. Bhattacharya, Evaluation of in Vitro Anti-Inflammatory Activity of Coffee against the Denaturation of Protein, Asian Pac. J. Trop. Biomed., **2012**, 2, S178–S180.