GAVIN PUBLISHERS

Archives of Natural and Medicinal Chemistry

Shajib SM, et al. Arch Nat Med Chem: ANMC-117.

Research Article

DOI: 10.29011/2577-0195.000017

Antioxidant Flavonoids from *Nicotiana plumbaginifolia* Viv. Leaves

Mohammad Shafiullah Shajib[1], Bidyut Kanti Datta[1], Rasheduzzaman Chowdhury[2*], Mohammad Abdur Rashid[2*]

[1]Department of Pharmacy, Stamford University Bangladesh, Bangladesh

[2]Department of Pharmaceutical Chemistry, University of Dhaka, Bangladesh

[*]**Corresponding authors:** Rasheduzzaman Chowdhury, Department of Pharmaceutical Chemistry, University of Dhaka, Bangladesh. Tel: +16502506510; Email: chowdhury.rzaman@gmail.com

Mohammad Abdur Rashid, Department of Pharmaceutical Chemistry, University of Dhaka, Bangladesh. Tel: +88029661900 Extn: 8137; Email: rashidma@du.ac.bd

Citation: Shajib MS, Datta BK, Chowdhury R, Rashid MA (2018) Antioxidant Flavonoids from *Nicotiana plumbaginifolia* Viv. Leaves. Arch Nat Med Chem: ANMC-117. DOI: 10.29011/2577-0195.000017

Received Date: 30 April, 2018; **Accepted Date:** 18 May, 2018; **Published Date:** 24 May, 2018

Abstract

Plant derived phenolics and flavonoids exert profound antioxidant effects due to their ability to scavenge free radicals. *Nicotiana plubagnifolia* Viv. leaves are known to produce large quantities of phenolics and flavonoids. Here we report antioxidant activities of a methanol extract of *N. plubagnifolia* leaves and its isolates, 3,3',5,6,7,8-hexamethoxy-4',5'-methylenedioxyflavone (**1**), 3,3',4',5',5,6,7,8-octamethoxyflavone (exoticin) (**2**), 6,7,4',5'-dimethylenedioxy-3,5,3'-trimethoxyflavone (**3**) 3,3',4',5,5',8-hexamethoxy-6,7-methylenedioxy-flavone (**4**) and 5-hydroxy-3,3',6,7,8-pentamethoxy-4',5'-methylenedioxyflavone (**5**) against 2,2-diphenyl-1-picrylhydrazyl (DPPH) and NO radicals. This is the first report of **5** from *N. plumbaginifolia*. The structure of **5** was determined by using high-field ^{1}H and ^{13}C-NMR and by comparison with previously reported values. Amongst the tested materials, the extract and compound **5** produced considerable radical scavenging activity against DPPH and NO radicals, which were comparable to the reference standard, ascorbic acid. All extractives were then evaluated for their toxicity profiles by means of brine shrimp lethality assay. While the methanol extract was lethal against brine shrimp nauplii, the isolated compounds were relatively non-toxic. Thus, our findings suggest that the functionalized flavonoids could be suitable candidates for developing antioxidant drugs with minimal or no cytotoxicity.

Keywords: Antioxidant; Cytotoxicity; *Nicotiana plubagnifolia*; Polymethoxyflavone; Solanaceae

Introduction

Reactive free radicals that are frequently generated in cells cause oxidative damages to biological macromolecules and form various toxins. In severe cases, supplementary antioxidants are required to maintain the free radical contents in the body as part of the innate defense mechanism [1]. Aberrant regulation of free radicals has been linked to pathogenesis of many diseases including cancer and inflammation [1,2]. However, the therapeutic implications of current synthetic antioxidants are somehow limited [3]. Natural product derived phenolics and flavonoids can potentially scavenge free radicals via H- or electron-transfer with concomitant production of stable radicals bearing an odd electron [4,5]. This newly generated radical is far more stable as the odd electron can delocalize over the aromatic system containing an extended conjugation [6]. In addition, there is evidence that the antioxidant properties of phenolics and flavonoids often contribute to anticancer effects [7,8]. As part of our continuous impetus in identifying potential antioxidant/ anticancer compounds from natural sources, we investigated *Nicotiana plumbaginifolia* Viv. (Fam. Solanaceae), a plant that is known to contain phenolics and flavonoids.

N. plumbaginifolia Viv. is an herb found in weedy habitats of Bangladesh and is used in the traditional medicine for the treatment of toothache [9], piles [10], wounds [11], parasitic infection [12], pyrexia [13] and rheumatic swelling (amongst others). Leaves are used in the management of nausea, travel sickness [14], insecticide [15] and vermicide [16]. In addition to phenolics and flavonoids, the plant contains a wide range of secondary metabolites including alkaloids, saponins, tannins, glycosides, steroids and terpenoids [14,17-19].

Materials and Methods

Chemicals and Reagents

Except for sodium chloride (Merck Co., Darmstadt, Germany), ammonium molybdate (Merck, Mumbai, India) and sodium nitroprusside (Loba Chemie, Mumbai, India), all solvents and reagents including *n*-Hexane, chloroform, methanol, toluene, ethyl acetate, sulfuric acid, phosphoric acid, DPPH (2,2-Diphenyl-1-picrylhydrazyl), vanillin, dimethyl sulfoxide (DMSO), ascorbic acid, sulfanilamide, N-(1-Naphthyl)ethylenediamine dihydrochloride were from BDH (U.K.) or Sigma (St. Louis, MO, USA) Chemicals. Vincristine sulfate was a gift from Beacon Pharmaceuticals Limited, Bangladesh.

Extraction and Isolation of Compounds 1-5

Plant collection and extraction of *N. plumbaginifolia* Viv. leaves were conducted as described [17]. Successive chromatographic purification yielded twelve fractions (F1- F12) of which fraction 12 (4.58 g) lead to the isolation of **1-5**. Compounds (**1-4**), which we have reported previously were identified as 3,3',5,6,7,8-hexamethoxy-4',5'-methylenedioxyflavone (**1**), 3,3',4',5',5,6,7,8-octamethoxyflavone (exoticin) (**2**), 6,7,4',5'-dimethylenedioxy-3,5,3'-trimethoxyflavone (**3**) and 3,3',4',5,5',8-hexamethoxy-6,7-methylenedioxyflavone (**4**) [17]. Compound **5** (17.4 mg), which is a new report from *N. plumbaginifolia*, was isolated from the same fraction (F12) using preparative TLC (mobile phase, toluene: ethyl acetate, 4:1, R_f = 0.91) on repeated development. The compound was re-crystalized from $CHCl_3$-MeOH (98:2) as pale-yellow needles.

5-Hydroxy-3,3',6,7,8-pentamethoxy-4',5'-methylenedioxyflavone (5)

Pale-yellow needles; m.p. 186-187 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 12.36 (1H, s, 5-OH), 7.54 (1H, d, J = 1.2 Hz, H-2'), 7.42 (1H, d, J = 1.6 Hz, H-6'), 6.09 (2H, s, 4', 5'-O-CH_2-O-); 4.13 (3H, s, 7-OMe), 4.01 (3H, s, 8-OMe), 3.99 (6H, s, 6-OMe, 3'-OMe), 3.91 (3H, s, 3-OMe); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 179.3 (C-4), 155.4 (C-2), 153.0 (C-7), 149.2 (C-5), 149.1 (C-5'), 147.0 (C-9), 144.8 (C-6), 143.6 (C-3'), 141.6 (C-3), 139.3 (C-4'), 138.0 (C-8), 124.6 (C-1'), 114.1 (C-10), 109.2 (C-2'), 102.9 (C-6'), 102.2 (4', 5'-O-CH_2-O-), 62.1 (8-OMe), 61.7 (6-OMe), 61.2 (7-OMe), 60.2 (3-OMe), 56.7 (3'-OMe).

NMR Spectroscopy and Melting Point Determination

^{1}H and ^{13}C NMR of compound **5** were recorded in $CDCl_3$ with respect to the residual solvent at 400 and 100 MHz on AVANCE DRX 400 and ASCENDTM 400 (Avance III HD NanoBay), Bruker, Germany, respectively. Melting point of **5** was recorded on a Stuart SMP30 melting point apparatus. The plateau temperature was set at 300°C and ramp rate was set at 0.5°C/min.

DPPH Free Radical Scavenging Assay

The scavenging of DPPH free radicals by the crude extract or compounds (**1-5**) was evaluated as described [24]. Briefly, 1.6 mg of standard (ascorbic acid), plant extract or isolates was dissolved in methanol and diluted to obtain the concentrations of 1.5625 - 400 µg/ml. An 0.1mM DPPH solution was prepared in methanol. 2 ml of DPPH solution was then added to 2 ml of tested materials. The mixture was mixed well and left for 30 min in a dark area at the room temperature. The final absorbance of the mixture was measured at 517 nm using a spectrophotometer (HACH, DR 5000™, Shanghai, China) with respect to DPPH blank solution. Each experiment was carried out in triplicate. The inhibition of DPPH free radicals was measured as percent (%) using the following equation:

$$\text{Inhibition of DPPH free radicals (\%)} = 1 - \frac{\text{Absrobance of test sample or standard}}{\text{Absorbance of blank}}$$

The concentration required for scavenging 50% of DPPH free radicals (IC_{50}) was determined from the % of inhibition.

NO Scavenging Assay

The assay was conducted as described [25]. Briefly, 1.6 mg of plant extract, isolates (**1-5**) or standard (ascorbic acid) was dissolved in methanol to obtain solutions ranging from 1.5625-400 µg/ml. 1 ml of sodium nitroprusside (5 mM) was then thoroughly mixed with 4 ml of each solution and incubated for 2 h at 30°C. The final absorbance of the mixture was measured at 550 nm followed by the addition of 1.2 ml of Griess reagent (1% sulfanilamide, 0.1% naphthylene diamine dihydrochloride in 2% H_3PO_4). IC_{50} values were calculated from % scavenging of NO radicals. The assay was done in triplicates.

Brine Shrimp Lethality (BSL) Assay

The assay was conducted as described [23]. Briefly, 1 mg of the methanol extract, its isolates (**1-5**) or standard (vincristine sulfate) was dissolved in 60 µl of DMSO. For each of standard, control and experimental groups, ten brine shrimp nauplii were taken in a graduated test tube containing 5 mL simulated sea water. 30 µL of the test solution was then added to the test tubes leading to final concentrations ranging from 100 - 0.195 µg/ml. 30 µL DMSO served as a control. Following 24h of the transfer, each test tube was observed for the number of survived brine shrimp nauplii. The concentration of each sample responsible for the 50% lethality of the brine shrimp nauplii (LC_{50}) was determined by Probit analysis. A mean LC_{50} value was obtained by performing the experiment in triplicate.

Statistical Analysis

Data are presented as mean ± SEM (n = 3). IC_{50} values

were determined using GraphPad Prism 6.05, USA. The multiple comparison between the groups were determined from Tukey's post-hoc test using Statistical Package for Social Sciences (SPSS) software (Version 22, IBM Corporation, USA), where $p < 0.05$ was considered as statistically significant.

Results and Discussion

Repetitive chromatographic purification led to the isolation of **5** from the leaves of *N. plumbaginifolia*. Its flavonoid nature was evident from a characteristic dark blue and fluorescent blue colored spot on TLC plate under UV_{254nm} and UV_{366nm} light, respectively and a yellow color spot upon vanillin-H_2SO_4 treatment followed by heating at 11˚C. The ^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **5** revealed 21 carbon signals that are characteristic of a polymethoxyflavone: five methoxyl (-OCH_3) at δ_C 56.7, 60.2, 61.2, 61.7, 62.1, one methylenedioxy (O-CH_2-O) at δ_C 102.2, two methines (-CH) at δ_C 102.9, 109.2 and thirteen quaternary carbons, including a carbonyl group (C=O) at δ_C 179.3. The ^1H NMR spectrum (400 MHz, $CDCl_3$) of **5** displayed signals for five methoxyl groups at δ 3.91 (3H), 3.97 (6H), 4.01 (3H), 4.13 (3H) and a chelated -OH group at 12.36 (1H). The ^{13}C and DEPT spectra revealed that three out of the five -OCH_3 were downfield between δ_C 61.2 - 62.1, suggesting their attachment to quaternary carbons. Comparative analysis of ^1H and ^{13}C-NMR of **5** with similar polymethoxy flavones for the substituted ring A enabled assignment of three -OCH_3 groups [17,20]. The ^1H NMR spectrum further showed two aromatic signals at δ 7.54 (1H) and 7.42 (1H) for two meta-coupled protons, which were attributed to H-2' and H-6', respectively. The ^1H NMR signal at δ 6.09 integrated for two protons, revealed the presence of a methylenedioxy (O-CH_2-O) group which was further supported by the oxygenated methylene carbon at δ_C 102.0. The remaining two -OCH_3 at δ 3.91 (δ_C 60.2) and δ 3.99 (δ_C 56.7) were ascribed to C-3 (δ_C 141.6) and C-3' (δ_C 143.6) respectively.

Based on the above analyses, the structure of compound **5** was determined to be 5-hydroxy-3,3',6,7,8-pentamethoxy-4',5'-methylenedioxyflavone, which was previously reported from *Polygonum orientale* [21]. All the ^1H and ^{13}C NMR resonances of the compound were unambiguously assigned by comparison with the previously reported low resolution data (60 MHz) [21]. The melting point of **5** (186-187˚C) was almost identical to that published for 5-hydroxy-3,3',6,7,8-pentamethoxy-4',5'-methylenedioxyflavone [21], which further supported our structure determination. To the best of our knowledge, this is the first report of a complete assignment of high-resolution ^1H and ^{13}C-NMR of 5-hydroxy-3,3',6,7,8-pentamethoxy-4',5'-methylenedioxyflavone (**5**) (Figure 1), and the first report from *N. plumbaginifolia*.

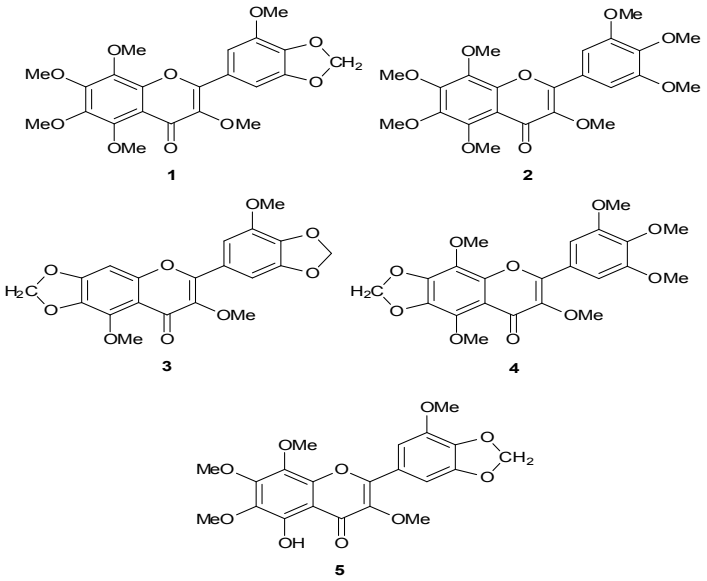


Figure 1: Chemical structures of compounds **1**-**5**.

1 = 3,3',5,6,7,8-hexamethoxy-4',5'-methylenedioxyflavone,

2 = 3,3',4',5',5,6,7,8-octamethoxyflavone (exoticin),

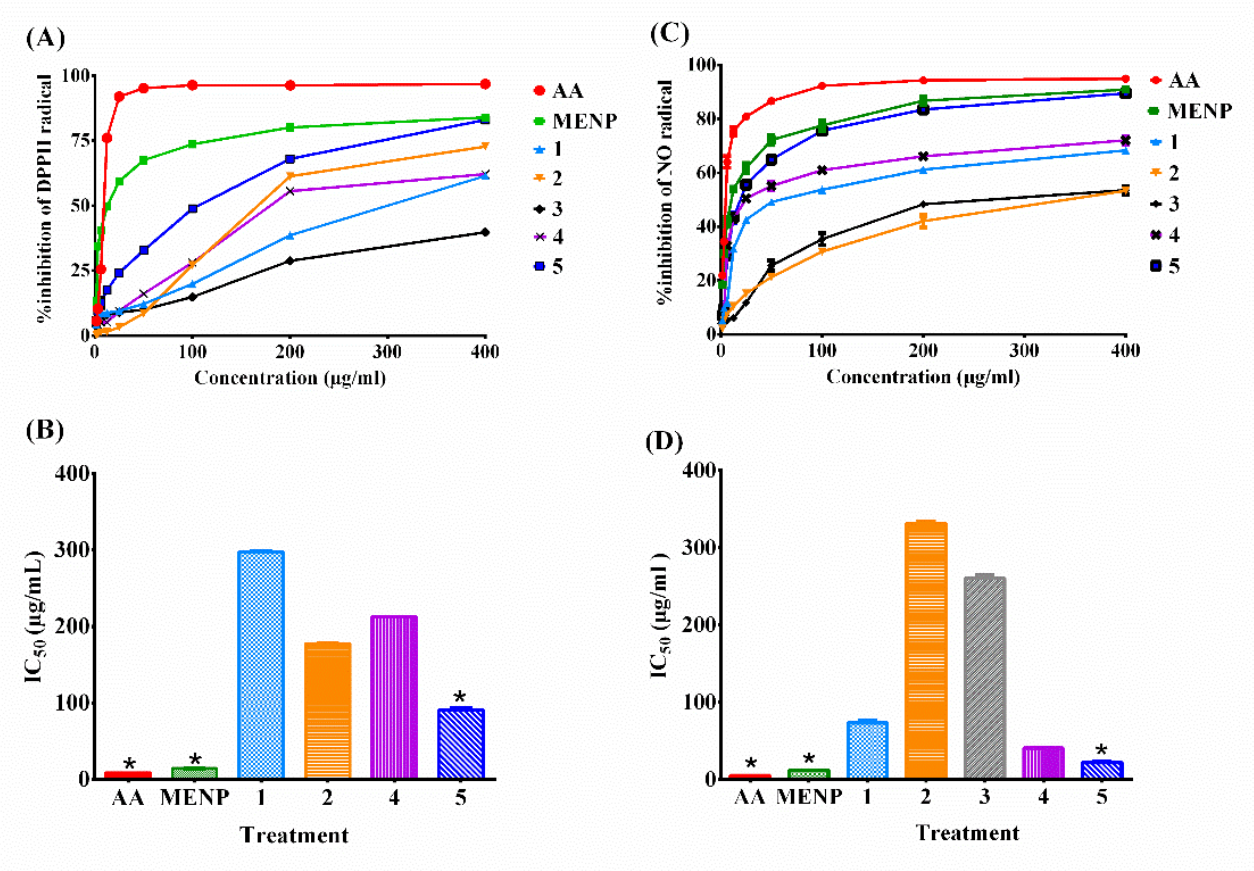
3 = 6,7,4',5'-dimethylenedioxy-3,5,3'-trimethoxyflavone,

4 = 3,3',4',5,5',8-hexamethoxy-6,7-methylenedioxyflavone,

5 = 5-hydroxy-3,3',6,7,8-pentamethoxy-4',5'-methylenedioxyflavone.

The plant extract and the isolated compounds (**1**-**5**) were then tested for their ability to scavenge the DPPH and NO radicals. The methanol extract and compound **5** produced statistically significant ($p < 0.05$) DPPH and NO radical scavenging activity compared to the control. In both assays, they produced concentration dependent effects (Figure 2). In DPPH radical assay, the extract and compound **5** exhibited 84 and 83% scavenging at 400 µg/ml with the IC_{50} values of 14.3 and 90.7 µg/ml, respectively. As compared to **5**, compounds **1**, **2**, **3** and **4** produced 61, 73, 40% and 62% scavenging effect at 400 µg/ml (Figure 2A) with substantially higher IC_{50} values of 297.0 (**1**), 177.6 (**2**), 212.5 (**4**) µg/ml (Figure 2B), respectively. IC_{50} for **3** could not be measured as it produced <50 % inhibition of DPPH radical at the maximum tested dose. The standard (ascorbic acid) produced >95% scavenging effect at 50-400 µg/ml (Figure 2A) with an IC_{50} value of 8.7 µg/ml, which was comparable to that of the methanol extract (Figure 2B). As shown in figure 2C, the extract and compound **5** showed strong NO scavenging activity of 91 and 90%, respectively which was comparable to the standard, ascorbic acid (97% at 400 µg/ml).

Compounds **1**, **2**, **3** and **4** exhibited maximum NO scavenging effect of 68, 53, 54 and 72%, respectively. The lowest IC_{50} value of NO scavenging was again shown by the extract (11.6 µg/ml), which was comparable to the ascorbic acid (4.8 µg/ml). Compound **5** revealed significantly ($p < 0.05$) lower IC_{50} value (22 µg/ml) in NO scavenging assay compared to other isolated compounds (Figure 2D). It has been precedented that -OH groups significantly increase the anti-radical potentiality of flavonoids possibly via enhancing H-transfer to free radicals whereas -OCH_3 groups reduce radical scavenging ability [22]. However, care should be taken to interpret the in vitro data as one of the caveats using the flavonoids is cell permeability. In fact, it is likely that -OCH_3 may facilitate cell permeability, which may undergo metabolic deprotection to produce free -OH in cells. Thus, although the presence of an additional -OH group in compound **5** apparently contributes to greater activity in our in vitro assays compared to other isolated flavonoids, further studies are required to validate the results in cells.



Figures 2(A-D): Antioxidant activity of the methanol extract of *N. plumbaginifolia* (MENP) and its isolated compounds (**1-5**). Panels (A) and (B) show % scavenging effect and IC_{50} values using DPPH free radical scavenging assay as indicated. Note, IC_{50} value for **3** could not be measured as it displayed <50 % inhibition of DPPH radical at the maximum tested dose. Panels (C) and (D) depict % scavenging effect and IC_{50} values using NO scavenging assay. Data are presented as mean ± SEM (n = 3). *$p < 0.05$ compared to control for the compound **1, 2**, **3**, **4** and **5** treatments. AA = ascorbic acid,

1 = 3,3',5,6,7,8-hexamethoxy-4',5'-methylenedioxyflavone,

2 = 3,3',4',5',5,6,7,8-octamethoxyflavone (exoticin),

3 = 6,7,4',5'-dimethylenedioxy-3,5,3'-trimethoxyflavone,

4 = 3,3',4',5,5',8-hexamethoxy-6,7-methylenedioxyflavone,
5 = 5-hydroxy-3,3',6,7,8-pentamethoxy-4',5'-methylenedioxyflavone.

Because antioxidant compounds are often toxic to cells, we evaluated the extract and the isolates for their cytotoxicity using a simple Brine Shrimp Lethality (BSL) assay [23]. The plant extract was substantially lethal to brine shrimp nauplii with an LC_{50} value of 8.6 µg/ml (Table 1), as compared to the standard drug, vincristine sulfate (1.4 µg/ml). The isolated compounds (**1-5**) were relatively non-toxic against the organism as evident by significantly higher LC_{50} values ranging from 66.2 to 75.5 µg/ml (Table 1). The observation that the methanol extract was substantially more toxic compared to the isolated compounds imply that there are potentially more cytotoxic principles in the *N. plumbaginifolia* leaves that we were unable to isolate.

Treatment	LC_{50} (µg/ml)
Vincristine sulfate	1.42 ± 0.48*
MENP	8.55 ± 1.65*
1	74.92 ± 0.95
2	69.29 ± 3.09
3	72.78 ± 1.43
4	75.50 ± 2.22
5	66.23 ± 3.09

Data are presented as mean ± SEM (n = 3). *p < 0.05, compared to **1**, **2**, **3**, **4** and **5** treatments. MENP = methanol extract of *N. plumbaginifolia* leaves.

1 = 3,3',5,6,7,8-hexamethoxy-4',5'-methylenedioxyflavone,
2 = 3,3',4',5',5,6,7,8-octamethoxyflavone (exoticin),
3 = 6,7,4',5'-dimethylenedioxy-3,5,3'-trimethoxyflavone,
4 = 3,3',4',5,5',8-hexamethoxy-6,7-methylenedioxyflavone,
5 = 5-hydroxy-3,3',6,7,8-pentamethoxy-4',5'-methylenedioxyflavone.

Table 1: Cytotoxicity of the methanol extract and its isolated compound (**1-5**) in brine shrimp lethality assay.

Conclusion

Our results suggest the occurrences of considerable antioxidant principles in the *N. plumbaginifolia* leaves including the polymethoxoy flavones. In particular, compound **5** demonstrated promising antioxidant properties presumably due to its ability for transferring hydrogen(s) efficiently. The polymethoxoy flavones are unusually rare amongst plants, occurring only in a few genera including *Polygonum* and *Murraya*, suggesting a unique biosynthetic pathway. Thus, the observation that *N. plumbaginifolia* leaves are rich in polymethoxyflaovens warrants further investigations for their potential chemotaxonomic relationships with *Polygonum* and *Murraya*. Because these compounds have the potential to scavenge radicals, further research can be directed towards comprehensive Structure-Activity Relationship (SAR) studies aimed at developing polymethoxyflavone-based antioxidants. While we primarily focused on identifying antioxidant flavonoids, the *N. plumbaginifolia* leaves may also serve as a potential source of cytotoxic compounds that will likely find ways forward to anticancer drug development.

Acknowledgements

We acknowledge Higher Education Quality Enhancement Project (HEQEP), AIF, Round-III, Window-2, CP-3258, University Grants Commission (UGC) of Bangladesh for funding.

References

1. Droge W (2002) Free radicals in the physiological control of cell function. Physiol Rev 82: 47-95.
2. Maeda H and Akaike T (1998) Nitric oxide and oxygen radicals in infection, inflammation, and cancer. Biochemistry 63: 854-865.
3. Moure A, Cruz JM, Franco D, Domínguez JM, Sineiro J, et al. (2001) Natural antioxidants from residual sources. Food Chem 72: 145-171.
4. Leopoldini M, Russo N, Toscano M (2011) The molecular basis of working mechanisms of natural polyphenolic antioxidants. Food Chem 125: 288-306.
5. Rice-Evans C (2001) Flavonoid antioxidants. Curr Med Chem 8: 797-807.
6. Wright JS, Johnson ER, DiLabio GA (2001) Predicting the activity of phenolic antioxidants: Theoretical method, analysis of substituent effects, and application to major families of antioxidants. J Am Chem Soc 123: 1173-1183.
7. Ren W, Qiao Z, Wang H, Zhu L, Zhang L (2003) Flavonoids: promising anticancer agents. Med Res Rev 23: 519-534.
8. Huang WY, Cai YZ, Zhang Y (2009) Natural phenolic compounds from medicinal herbs and dietary plants: potential use for cancer prevention. Nutr Cancer 62: 1-20.
9. Devi AD, Devi OI, Singh TC, Singh EJ (2014) A study of aromatic plant species especially in Thoubal district, Manipur, Northeast India. Int J Sci Res Pub 4: 1-12.
10. Bhowmik R, Saha MR, Rahman MA, Islam MAU (2015) Ethnomedicinal survey of plants in the Southern District Noakhali, Bangladesh. Bangladesh Pharm J 17: 205-214.
11. Dangwal LR, Sharma A, Rana CS (2010) Ethnomedicinal plants of the Garhwal Himalaya used to cure various diseases: a case study. New York Sci J 3: 28-31.
12. Rao PK, Hasan SS, Bhellum BL, Manhas RK (2015) Ethnomedicinal plants of Kathua district, J&K, India. J Ethnopharmacol 171: 12-27.

13. Sharma R, Manhas RK, Magotra R (2012) Ethnoveterinary remedies of diseases among milk yielding animals in Kathua, Jammu and Kashmir, India. J Ethnopharmacol 141: 265-272.

14. Singh KP, Daboriya V, Kumar S, Singh S (2010) Antibacterial activity and phytochemical investigations on *Nicotiana plumbaginifolia* viv. (wild tobacco). Romanian J Biol Plant Biol 55: 135-142.

15. Rothe SP (2012) Exotic medicinal plants from West Vidarbha region of Maharashtra. Adv Res Pharm Bio 2: 33-40.

16. Kulkarni S, Kulkarni DK, Deo AD, Pande AB, Bhagat RL (2014) Use of ethno-veterinary medicines (EVM) from Vidarbha region (MS) India. Biosci Discov 5: 180-186.

17. Shajib MS, Datta BK, Sohrab MH, Rashid MA, Nahar L, et al. (2017) highly oxygenated flavonoids from the leaves of *Nicotiana plumbaginifolia* (Solanaceae). Rec Nat Prod 11: 568-572.

18. Shajib MS, Rashid RB, Ming LC, Islam S, Sarker MMR, et al. (2018) Polymethoxyflavones from *Nicotiana plumbaginifolia* (Solanaceae) exert antinociceptive and neuropharmacological effects in mice. Front Pharmacol 9: 85.

19. Singh S, Khanna NM, Dhar MM (1974) Solaplumbin, a new anticancer glycoside from *Nicotiana plumbaginifolia*. Phytochemistry 13: 2020-2022.

20. Machida K, Osawa K (1989) On the flavonoid constituents from the peels of *Citrus hassaku* Hort. ex Tanaka. Chem Pharm Bull 37: 1092-1094.

21. Kuroyanagi M, Fukushima S (1982) Highly oxygenated flavonoids from *Polygonum orientale*. Chem Pharm Bull 30: 1163-1168.

22. Heim KE, Tagliaferro AR, Bobilya DJ (2002) Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. J Nutr Biochem 13: 572-584.

23. Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DJ, et al. (1982) Brine shrimp: a convenient general bioassay for active plant constituents. Planta Med 45: 31-34.

24. Islam S, Shajib MS, Ahmed T (2016) Antinociceptive effect of methanol extract of *Celosia cristata* Linn. in mice. BMC Complement. Altern Med 16: 400.

25. Alisi CS, Onyeze GOC (2008) Nitric oxide scavenging ability of ethyl acetate fraction of methanolic leaf extracts of *Chromolaena odorata* (Linn.). African J Biochem Res 2: 145-150.