DOI 10.1007/s10600-018-2257-7
Chemistry of Natural Compounds, Vol. 54, No. 1, January, 2018

PHYTOCHEMICAL SCREENING AND ANTIOXIDANT PROPERTIES OF *Phyllanthus emblica* FROM MAURITIUS

S. Jhaumeer Laulloo,[1*] M. G. Bhowon,[1]
L. S. Chua,[2] and H. Gaungoo[1]

The phytochemical screening showed the presence of phenols, flavonoids, non-flavonoid, tannins, alkaloids, saponins, and phytosterols in the different extracts (diethyl ether, ethyl acetate, butanol, and aqueous extracts) of dried fruits of Phyllanthus emblica *(Amla). Low-molecular-weight aliphatic acids, phenolic acids, methyl/ethyl gallate, phytosterols, and tannins were identified in the fruits using UPLC-MS/MS. The scavenging activity of Amla was assessed using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2′-azinobis-3-ethylbenzothiazoline)-6-sulfonic acid (ABTS) assays. The ethyl acetate extract showed the highest total phenolic and flavonoid contents, which was also found to have the highest antioxidant potential with* SC_{50}, *1.33 ± 0.77 and 4.13 ± 0.99 μg/mL for the DPPH and ABTS assays, respectively. The high phenolic, flavonoid, and the antioxidant activity of the extracts indicated that the local Amla could be exploited as an antioxidant supplement.*

Keywords: *Phyllanthus emblica*, phytochemical screening, phenolic compounds, antioxidant, UPLC-MS/MS, DPPH, ABTS.

Phyllanthus emblica, which is commonly known as Amla, is a plant of the family Euphorbiaceae. It is widely distributed in the subtropical and tropical areas of Asia [1]. The various parts of the plant have been used in both Indian Ayurveda as well as Chinese traditional medicine. The fruit is consumed as fresh fruit, juice, or in the form of preserve food products [2]. Various parts of the plant have been reported to contain different phytochemicals with various pharmacological properties, including antioxidant, anticarcinogenic, antitumor, and anti-inflammatory activities [3–5]. The phytochemicals include different classes of metabolites such as tannins, phenolics, simple lactones, terpenoids, and alkaloids. Several phenolic compounds such as pyrogallol, gallic acid, methyl/ethyl gallates, malic acid gallates and ellagic acids, chlorogenic acid, caffeic acid, syringic acid, *p*-coumaric acid, ferulic acid, and sinapinic acid were isolated and identified from Amla by numerous investigators [6–16]. A number of hydrolyzable and condensed tannins have been identified and isolated from the different parts of the *Phyllanthus emblica* plant [17, 18]. Moreover, flavonoids such as quercetin, kaempferol, and apigenin were also reported from the *Phyllanthus emblica* fruits [8–10, 14]. The other classes of phytochemicals identified from the plant include diterpenes, monosaccharides, and fatty acids.

In recent years, there has been an increased interest in the possible use of natural products for improving human health and preventing diseases. In Mauritius, the local population is not fully aware of the benefits of consuming Amla fruits. Therefore, in this study we investigated the phytochemicals of *Phyllanthus emblica* extracted by different solvents and evaluated their antioxidant capacity in terms of their scavenging activities. Phenolic compounds are well known for their antioxidant activities, and usually they have inhibitory effects on mutagenesis and carcinogenesis in humans.

The crude extract of the local Amla fruit was screened for the presence of different phytochemicals using a test-tube method [19–21] and was found to contain a number of secondary metabolites including phenolics, flavonoids, alkaloids, saponins, tannins, and phytosterols.

1) Department of Chemistry, Faculty of Science, University of Mauritius, Reduit, e-mail: sabina@uom.ac.mu; 2) Institute of Bioproduct Development, Universiti Teknologi Malaysia, 81310 UTM Skudai, Johor Bahru, Johor, Malaysia
Published in *Khimiya Prirodnykh Soedinenii*, No. 1, January–February, 2018, pp. 47–51. Original article submitted April 25, 2016.

TABLE 1. Quantification of Total Phenolic Content (TPC), Total Flavonoid Content (TFC), and Total Non-flavonoid Content (TNFC) in Different Extracts of *Phyllanthus emblica* Fruits

Extract	TPC/ mg GAE/g	TFC/ mg QE/g	TNFC/ mg GAE/g
Et_2O	508.73 ± 35.99	174.89 ± 7.86	55.53 ± 6.35
EtOAc	640.55 ± 109.3	215.45 ± 16.5	113.67 ± 6.43
Butanol	179.64 ± 77.14	20.45 ± 1.5G8	41.43 ± 0.24
Aqueous	346.91 ± 46.29	127.67 ± 8.64	51.54 ± 1.35

TABLE 2a. Tentative Identification of Secondary Metabolites from the Crude and Different Fractions of *Phyllanthus emblica* from Negative Ion Mode

Putative compound	$[M - H]^-$	Product ions	Crude	Et_2O	EtOAc	BuOH	Aqueous	References
Organic aliphatic acids								
Malic acid	133	115	+	+	+		+	[17]
Mucic acid	210	191		+			+	[27]
Monosaccharides								
Fructose or galactose	179						+	[16]
Phenol and phenolic acids								
Cinnamic acid	147	103			+			[7]
Sinapinic acid	223	223, 208, 179, 149	+	+	+		+	[16]
Gallic acid	169	125	+	+	+			[3,7, 11, 28, 29]
Chebulic acid	355	339, 249, 162			+			[8, 17]
Ellagic acid	301	301, 283, 270, 216, 201, 185		+				[17]
Ascorbic acid	175	116	+					[28, 30, 31]
Mucic acid lactone	191		+	+			+	[8]
Gallic acid esters								
Methyl gallate	183		+	+	+			[17, 32]
Ethyl gallate	197	169, 124		+				[7, 16, 22]
Mucic acid lactone gallate	343	191			+		+	[3, 23]
Mucic acid lactone methyl ester gallate	357	169			+		+	[8]
Mucic acid lactone ethyl ester gallate	371				+			[8]
Gallic acid *O*-glucoside	331		+		+		+	[8]
Mucic acid gallate	361	209, 191	+		+		+	[8, 15]
Mucic acid methyl-*O*-gallate	375	223, 191	+				+	[17]
Mucic acid dimethyl ester gallate	389	241, 169	+	+				[8, 17]
Tannins								
Resveratrol	227	185, 159		+				[22]
Vitamine								
Vitamin B1	299	237, 115, 113, 99				+		[33]
Flavonoids								
Quercetin	302	257, 201, 158				+		[34]
Others								
Phyllanthins	417	363, 319, 113				+		[25, 26]
Tributylphosphate	265	85, 83				+		[33]

The total phenolic (TPC), flavonoid (TFC), and non-flavonoid (TNFC) contents of the different *Phyllanthus emblica* extracts were determined quantitatively, and the data are summarized in Table 1. The ethyl acetate fraction had the highest concentration of TPC and TNFC, followed by the ether, aqueous, and butanol extracts.

In order to achieve a more detailed characterization of the metabolites, the different extracts (methanol (crude), ether, ethyl acetate, butanol, and aqueous extracts) were analyzed by UPLC-MS/MS. Based on the mass screening, a number of secondary metabolites were identified from both the positive and negative ion modes of the mass spectra of the different extracts. The identification was carried out by matching the fragment ion with those already reported in the literature. The known compounds were identified by comparison with reference compounds or literature data or their mass spectral fragments.

TABLE 2b. Tentative Identification of Secondary Metabolites from the Crude and Different Fractions of *Phyllanthus emblica* from Positive Ion Mode Scan

Putative compound	$[M + H]^+$	Product ions	Crude	Et_2O	EtOAc	BuOH	Aqueous	References
Organic acids								
Linolenic acid	279	221, 149, 95				+	+	[33]
Linoleic acid	281	263, 253, 223, 123, 109, 97, 95					+	[33]
Mucic acid	211	206, 154, 136, 134, 118, 116, 100, 98					+	[27]
Phenolic acids								
Gallic acid	171	153.0, 134.9, 127.0, 125.0, 109.0, 107.0, 97.0	+				+	[3, 7, 11, 28, 29]
Simple gallate esters								
Methyl gallate	185	153, 126, 107, 95		+				[17]
3-Ethyl gallic acid	201		+					[13]
Gallate ester lactones								
Mucic acid 1,4 lactone-*O*-gallate	345	153.0				+		[17]
Mucic acid 1,4 lactone 6-methyl ester 5-*O*-gallate	359	250, 243, 207, 191, 153,127, 95	+		+		+	[17]
Mucic acid 1,4 lactone ethyl ester-*O*-gallate	373	243, 153,127	+		+			[17]
Phytosterols								
Campesterol	401	189, 167,153, 143, 127				+		[31]
β-Sitosterol	415	407, 331, 171, 143, 153, 125, 116				+		[7, 31, 35]

TABLE 3. Scavenging Activity (SC_{50}) of Different *Phyllanthus emblica* Fruit Extracts

Standard	DPPH, µg/mL	ABTS, µg/mL	Fruit extract	DPPH, µg/mL	ABTS, µg/mL
Ascorbic acid	210.4 ± 139.4	–	Ether extract	3.51 ± 0.71	13.51 ± 0.67
Gallic acid	0.33 ± 0.209	–	Ethyl acetate extract	1.33 ± 0.77	3.91 ± 2.10
Quercetin	0.77 ± 0.031	–	Butanol extract	6.31 ± 2.44	4.13 ± 0.99
Trolox	–	20.58 ± 2.24	Aqueous extract	5.68 ± 3.06	5.22 ± 0.01

Table 2 represents a summary of different phytochemicals tentatively identified in different extracts. The aliphatic organic acids such as malic acid, linolenic acid, and linoleic acid were identified mainly in the aqueous and butanol extracts. Gallic acid identified in the crude, ether, ethyl acetate, and aqueous extracts was based on the fragmentation ions; *m/z* 169/125 in the negative ion mass spectrum and *m/z* 171/125 for positive ion mode spectrum [17, 18]. Phenolic acid and cinnamic acid derivatives such as cinnamic, caffeic, syringic, sinapinic, gallic, and ellagic acids were identified mainly in the ether and ethyl acetate extracts [7, 16, 22]. A number of ester and lactone derivatives including the methyl/ethyl gallate and mucic acid gallate and mucic acid lactone gallates were identified in the crude, ether, and/or the ethyl acetate extract based on their molecular ions. The local Amla fruit was found to be a rich source of lactones, including several isomers of mucic lactone gallates [8, 23, 24], and were identified in the ethyl acetate and ether extracts. Ascorbic acid was only found in the methanolic crude extract.

The tannins such as resveratrol and chebulic acids with the molecular ions of *m/z* 227 and 355 were present in the ether and ethyl acetate fractions, respectively. The alkaloid such as vitamin B1 and the lignan phyllanthin were detected only in the butanol extract based on their mass fragments [25, 26]. Quercetin was identified in the ether extract, while tributylphosphate with the molecular ion at *m/z* 265 was identified in the butanol extract. From the positive ion mode, the phytosterols such as campesterol and β-sitosterol were identified in the butanol fraction. Interestingly, the ether extract and ethyl acetate extract were found to containe a greater number of phenolics and lactones.

Several compounds with *m/z* values 133, 143, 161, 184, 215, 225, 237, 241, 293, 311, 318, 319, 333, 335 corresponding to $[M - H]^-$ and compounds with *m/z* 153, 200, 205, 211, 258, 266, 290, 297, 305, and 321 corresponding to $[M + H]^+$ ion in the positive and negative ion mode have not been reported earlier in the *Phyllanthus emblica* to the best of our knowledge.

Antioxidant assays such as DPPH and ABTS scavenging assays were carried out on different Amla extracts. The radical scavenging activities of the different extracts are compared with several standards such as ascorbic acid, gallic acid, quercetin, and Trolox, and the results are summarized in Table 3. The 50% scavenging concentration (SC_{50}) was based on the amount of compound required to decrease the initial DPPH radical concentration by 50%.

From previous reports it is known that phenolic compounds, especially hydrolyzable tannins and flavonoids, together with vitamin C, are important compounds that show strong antioxidant properties [24]. The presence of the phenols and phenolic acids explains the antioxidant property of the different extracts. The ethyl acetate and ether extracts contain a number of phenolic compounds such as gallic acid, sinapinic acid, chebulic acid, alkyl gallates, and mucic acid gallates, and also hydrolyzable tannin such as resveratrol, which may be responsible for their high DPPH antioxidant properties. The aqueous extract also contained phenolic acids such as caffeic acid, sinapinic acid, and gallates, which contribute to its antioxidant properties. The presence of the flavonoids campesterol and β-sitosterol in the BuOH extract may be responsible for the good antioxidant properties in terms of the ABTS assay.

The SC_{50} values for the DPPH radical scavenging activity were in the order of ethyl acetate > ether > aqueous > butanol extracts while for the ABTS assay the order was ethyl acetate > butanol > aqueous > ether extracts.

The content of bioactive compounds as well as the antioxidant properties of Amla fruits from Mauritius was analyzed. The fruit was found to have high levels of phenolic acids, mucic acid, malic acid, simple gallate esters, and lactones. The ether and ethyl acetate extracts having highest phenolic and flavonoid contents showed highest antioxidant activity, indicating that phenolics are the major contributor to the antioxidant activity. Amla from Mauritius also showed better scavenging activity than those reported from other parts of the world such as India and China. According to these findings, the consumption of Amla fruits can contribute to health improvement, and therefore an increase awareness of this fruit as a potential antioxidant supplement among the local population is desired.

EXPERIMENTAL

Chemicals and Plant Material. 2,2′-Azino-bis(3-ethylbenthiazoline-6-sulfonic acid) (ABTS) (98%), 1,1-diphenyl-2-picrylhydrazyl (DPPH) (97%), gallic acid (ACS > 98%), quercetin (98%), and phosphate buffer saline tablet (0.01 M, 1 tablet in 200 mL of deionized water) were obtained from Sigma Aldrich Laboratories, Canada. Aluminum chloride, butanol, and Folin–Ciocalteu reagent (99%) were purchased from BDH laboratories, England. Ascorbic acid and hydrochloric acid were obtained from S D Fine-Chem Ltd., Mumbai. All other reagents were of analytical grade.

The fruits of *Phyllanthus emblica* were bought from the local market in Mauritius. The fruits ripen during the dry season from September to December. The fruits were washed, cut into small pieces, and oven-dried at 40°C and then finely ground using an electric blender. The ground powder (40 g) was extracted with methanol in a Soxhlet extractor. The crude methanolic extract was then partitioned with solvents of increasing polarity, diethyl ether, ethyl acetate, butanol, and water.

Total Phenolic Content (TPC). The total Phenolic content (TPC) of the crude and the four extracts was estimated using a modified Folin–Ciocalteu method [36]. An aliquot (0.2 mL) of the extracts (100 μg/mL in methanol) diluted with 1 mL of water was mixed with 0.2 mL of Folin Ciocalteu reagent and 0.8 mL of 2% sodium carbonate solution. The tubes were vortexed for 10 s and allowed to stand for 5 min at 60°C for color development. Absorbance was recorded against reagent blank at 650 nm using a UV-Vis spectrophotometer (Biochrome (Libra) S22, Cambridge, UK). Gallic acid (0–120 μg/mL) was used as standard chemical for calibration curve preparation. The total phenolic content was expressed as mg of gallic acid equivalent per gram of extract (mg GAE/g extract).

Total Flavonoid Content (TFC). The total flavonoid content (TFC) of the different extracts was determined by the method described by [6]. An aliquot (0.25 mL) of the extracts (100 μg/mL in methanol) was mixed with 1.25 mL distilled water and 0.075 mL of 5% sodium nitrite solution and allowed to stand for 5 min. Aluminum chloride solution (0.15 mL, 10%) followed by 0.5 mL of 1 M sodium hydroxide were added to the solution, which was then diluted up to 2.5 mL with distilled water. The absorbance was measured at 510 nm. Quercetin (0–100 μg/mL) was used as standard chemical for calibration curve construction. The total flavonoid content was expressed in mg QE/g of extract.

Total Non-flavonoid Content (TNFC). The total nonflavonoid content (TNFC) was determined using the method described by [37]. An aliquot (1 mL) of the extracts (100 μg/mL in methanol) was mixed with 1 mL of hydrochloric acid (20%) and 0.5 mL of 37% formaldehyde, and the mixture was vortexed, left in the dark, and filtered after 24 h. An aliquot of the filtrate (0.25 mL) was mixed with 3.5 mL of distilled water and 0.25 mL of Folin–Ciocalteu reagent followed by 1 mL of

20% sodium carbonate. The mixture was vortexed and heated at 40°C for 40 min. Absorbance of the resulting blue solution was measured at 685 nm. Gallic acid (0–120 μg/mL) was used as standard for the reaction, and the total non-flavonoid content was expressed in mg GAE/g of extract.

Metabolite Screening by UPLC-MS/MS. The analytical UPLC, Waters Acquity (Milford, MA) system was coupled with a triple quadrupole-linear ion trap tandem mass spectrometer (Applied Biosystems 4000 Q TRAP; Life Technologies Corporation, Carlsbad, CA) with an electrospray ionization (ESI) source. A C18 reversed phase Acquity column (150 × 4.6 mm, 1.7 μm) protected by a guard column was used throughout this study.

The mobile phase was a binary solvent system consisting of solvent A (water with 0.1% formic acid) and solvent B (CH_3CN). The UPLC gradient was 0–5 min, 10% B; 5–15 min, 10–90% B; 15–20 min, 90% B; 20–25 min, 90–10% B; 25–30 min, 10% B for final washing and equilibration of the column for the next run. The flow rate was 0.15 mL/min, and the injection volume was 5 μL. All samples were filtered with 0.2 μm nylon membrane filter prior to injection.

The mass spectra were acquired from *m/z* 100–1000 with a 20-ms ion accumulation time. All mass spectrometric data were acquired in both positive and negative ionization modes. The capillary and voltage of the ESI source were maintained at 400°C, and 5.5 kV and –4.5 kV for positive and negative ion modes, respectively. All other parameters were as follows: nitrogen was used as ion source gas for nebulization, 40 psi; for drying solvent, 40 psi; curtain gas, 10 psi; collision gas, high; declustering potential, –40 V, and collision exit energy, –10 V. The scan rate was 1000 amu/s. Data acquisition and data processing were performed using Analyst 1.4.2. The scan mode of enhanced mass spectra (EMS) was used to screen the sample profile. Enhanced product ion (EPI) scan was used to determine the characteristic ions and to confirm the presence of aglycone peaks. Three parallel EPI runs with different collision energies ranging from –10 to –50 V were carried out to obtain the most information-rich fragmentation pattern.

DPPH Radical Scavenging Activity. The DPPH free radical-scavenging activity of the *Phyllanthus emblica* fruit extracts were determined by the method described by [38]. The absorbance of the solutions was measured at a wavelength of 492 nm using a Labsystems Multiskan Ms. EIA Reader (Thermo Fisher Scientific/Lab System, California, USA). Ascorbic acid, gallic acid, and quercetin were used as standard chemicals. The percentage scavenging activity of the samples was then calculated using the equation

$$\%\text{Activity} = [(A_0 - A_S)/A_0] \times 100, \qquad (1)$$

where A_0 is absorbance of control and A_S is absorbance of tested sample.

ABTS Scavenging Assay. The ABTS assay was carried out according to the reported procedure [39]. A standard curve was obtained using Trolox (0–35 μg/mL) in methanol. The percentage antioxidant activity was calculated using Eq. (1), where A_0 is absorbance of blank (ABTS only) and A_S is absorbance of tested sample.

For the antioxidant assays, all the tests were done in triplicate and expressed as a mean ± standard deviation (SD).

REFERENCES

1. X. Liu, M. Zhao, J. Wang, B. Yang, and Y. Jiang, *J. Food Compos. Anal.*, **21**, 219 (2008).
2. A. K. Meena, A. Singh, and M. M. Rao, *Asian J. Pharm. Clin. Res.,* **3**, 242 (2010).
3. W. Luo, M. Zhao, B. Yang, J. Ren, G. Shen, and G. Rao, *Food Chem.*, **126**, 277 (2011).
4. M. S. Baliga and J. J. Dsouza, *Eur. J. Cancer Prev.*, **20**, 225 (2011).
5. X. Liu, M. Zhao, K. Wu, X. Chai, H. Yu, Z. Tao, and J. Wang, *Food Chem.*, **131**, 685 (2012).
6. X. Liu, C. Cui, M. Zhao, J. Wang, W. Luo, B. Yang, and J. Yueming, *Food Chem.*, **109**, 909 (2008).
7. C-B. Yang, F. Zhang, M-C. Deng, G-Y. He, J-M. Yue, and R-H. Lu, *J. Chin. Chem. Soc.*, **54**, 1615 (2007).
8. Y-J. Zhang, T. Tanaka, C. R. Yang, and I. Kouno, *Chem. Pharm. Bull.,* **49**, 537 (2001).
9. Y-J. Zhang, T. Tanaka, C. R. Yang, T. Abe, and I. Kouno, *J. Nat. Prod.*, **64**, 1527 (2001).
10. Y. J. Zhang, T. Tanaka, Y. Iwamoto, C. R. Yang, and I. Kouno, *J. Nat. Prod.*, **64**, 870 (2001).
11. S. K. El-Desouky, S. Y. Ryu, and Y-K. Kim, *Nat. Prod. Res.*, **22**, 91 (2008).
12. A. Kumaran and R. J. Karunakaran, *Plant Foods Human Nutr.*, **61**, 1 (2006).
13. L-Z. Zhang, W-H. Zhao, Y-J. Gua, G-Z. Tu, S. Lin, and L-G. Xin, *Zhongguo Zhong Yao Za Zhi*, **28**, 940 (2003).
14. Y. Zhang, T. Abe, T. Tanaka, C. Yang, and I. Kouno, *Chem. Pharm. Bull.*, **50**, 841 (2002).
15. Y. J. Zhang, T. Nagao, T. Tanaka, C. R. Yang, H. Okabe, and I. Kouno, *Biol. Pharm. Bull.*, **27**, 251 (2004).

16. J. Kubolaa, S. Siriamornpun, and N. Meeso, *Food Chem.*, **126**, 972 (2011).
17. B. Yang, M. Kortesniemi, P. Liu, M. Karonen, and J-P. Salminen, *J. Agric. Food Chem.*, **60**, 8672 (2012).
18. F. Pellati, R. Bruni, D. Righi, A. Grandini, M. Tognnolini, F. P. Prencipe, F. Poli, S. Benvenuti, D. Del Rio, and D. Rossi, *J. Ethnopharmacol.*, **147**, 277 (2013).
19. K. N. Sachan, S. S. Gangwar, and R. Sharma, *Internat. J. Mod. Pharm. Res.*, **2**, 1 (2013).
20. D. Krishnaiah, T. Devi, A. S. R. Bono, and R. Sarbatly, *J. Med. Plants Res.*, **3**, 67 (2009).
21. R. Saxena, P. Patil, and S. Khan, *Nanobiotechnica Universale*, **1**, 103 (2010).
22. J. Sun, F. Liang, Y. Bin, P. Li, and C. Duan, *Molecules*, **12**, 679 (2007).
23. W. Luo, L. Wen, M. Zhao, B. Yang, J. Ren, G. Shen, and G. Rao, *Food Chem.*, **132**, 1527 (2012).
24. Y. Yang, X-Q. Li, C-P. Tang, and Y. Sheng, *Chin. J. Nat. Med.*, **11**, 97 (2013).
25. P. Khanna and R. Bansal, *Ind. J. Exp. Biol.*, **13**, 82 (1975).
26. V. Rajakannan, M. S. Sripathi, S. Selvanayagam, D. Velmurugan, U. D. Murthy, M. Vishwas, S. P. Thyagarajan, S. S. Sundara Raj, and H-K. Fun, *Acta Crystallogr.,* **E59**, o203 (2003).
27. B. R. Premi, V. Sethi, and D. B. Saxena, *Food Chem.*, **61**, 9 (1998).
28. P. Mayachiew and S. Devahastin, *Food Sci. Technol.*, **41**, 1153 (2007).
29. W. Luo, M. Zhao, B. Yang, G. Shen, and G. Rao, *Food Chem.*, **114**, 499 (2009).
30. V. Raghu, K. Patel, and K. Srinivasan, *J. Food Compos. Anal.*, **20**, 529 (2007).
31. K. Judprasong, S. Charoenkiatkul, P. Thiyajai, and M. Sukprasansap, *Food Chem.*, **140**, 507 (2013).
32. K. H. Khan, *Bot. Res. Int.*, **2**, 218 (2009).
33. G. X. Wu, Y. X. Li, M. Y. Chen, and Y. F. Hong, *Zhong Yi Yao Xue Bao*, **31**, 21 (2003).
34. C. D. Ventatapura, G. K. Burdipad, and L. V. Gollapalle, *J. Chin. Integr. Med.,* **9** (9), 1022 (2011).
35. W. Y. Qi, Y. Li, K. Wang, and K. Gao, *Fitoterapia*, **84**, 252 (2013).
36. R. K. Sharma, S. Chatterji, D. K. Rai, S. Mehta, P. K. Rai, R. K. Singh, G. Watal, and B. Sharma, *J. Med. Plant Res.*, **3**, 944 (2009).
37. V. Katalinic, M. Milos, D. Modun, C. Music, and M. Boban, *Food Chem.*, **86**, 593 (2004).
38. S. B. Moosun, S. Jhaumeer-Laulloo, E. C. Hosten, R. Betzb, and M. G. Bhowon, *Inorg. Chim. Acta*, **430**, 8 (2015).