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Original Article

Studies on Antioxidant and Phytochemical Profiles of Leptochloa uniflora Hochst

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Grasses are natural homogenous group of plants and belong to the Gramineae family (Poaceae). The value of grasses to mankind has been recognized since the dawn of human civilization and culture of cereal grasses. In the present study, Leptochloa uniflora Hochst (Poaceae) was subjected to antioxidant and phytochemical profiles using UV-Vis, FTIR and HPTLC. Antioxidant assays were studied using DPPH, phosphomolybdenum and H2O2 scavenging activity. Antioxidant activities on L. uniflora showed efficient radical scavenging effects. Phytochemical profiles determined the presence of various bioactive compounds. It is hoped that the present work revealed the utility of L. uniflora plant extracts for field applications.

Keywords: Phytochemical, antioxidant, HPTLC, *Leptochloa uniflora*

Plants are potential sources of natural antioxidants. They may produce various anti-oxidative compounds to counteract reactive oxygen species (ROS) in order to survive. In recent years much attention has been devoted to natural antioxidant and their association with health benefits (1). Antioxidants act as a major defense against radical mediated toxicity by protecting the damages caused by free radicals. Although medicinal plants are used as antioxidants in traditional medicine, their therapeutic properties could be due to the presence of bioactive compounds present in them. The screening studies for antioxidant properties of medicinal plants have been performed increasingly during the last few decades in hope of finding an efficient remedy for several diseases (2, 3).

Despite of tremendous progress in human medicines, infectious diseases caused by bacteria, fungi, viruses and parasites are also still a major threat to public health. This impact is particularly large in developing countries due to relative unavailability of medicines and the emergence of widespread drug resistance (4-6). During the last two decades, the development of drug resistance as well as the appearance of undesirable side effects of certain antibiotics has led to the search of new anti-microbial agents mainly among plant extracts with the goal to discover new chemical structures (7-9).

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Phytochemical techniques are mainly applied to the quality control of herbal medicines. In most cases, biologically active components in herbal medicines have not been determined. Chromatographic and spectroscopic studies have become a routine analytical technique due to their advantages of reliability in quantification of analytes at micro and nanogram levels (10). They have been proved as very useful techniques because of their low operating cost, high sample throughput and need for minimum sample clean-up.

Grasses are natural homogenous group of plants belonging to the Gramineae family (Poaceae). They are undoubtedly one of the most fascinating families of flowering plants with a wide range of diversity and are playing a significant role in the lives of the human beings and animals. The value of grasses to mankind has been recognized since the dawn of human civilization and culture of cereal grasses. In India, the Gramineae is one of the most dominant families both on the basis of its number of genera and species (11). With this background, the present study was aimed to study the antioxidant, larvicidal properties and phytochemical analysis of *Leptochloa uniflora* Hochst (Poaceae).

Materials & methods

Plants collection

Healthy, disease free plant samples of *Leptochloa uniflora* Hochst (Poaceae) were harvested from Mukkudal, Tirunelveli district, Tamil Nadu, India. The collected samples were identified by Prof. Dr. P. Ravichandran, Sri Paramakalyani center for environmental studies, Manonmaniam Sundaranar University, Alwarkurichi, Tamil Nadu, India. They were brought to the laboratory and washed well with running tap water for 10 min to remove the soil particles and adhered debris. Then the samples were washed thoroughly with distilled water. For drying, washed plant samples were blotted on the blotting paper and spread out at room temperature under shade for a period of fifteen days. The shade dried whole plants were ground to fine powder using tissue blender. The powdered samples were stored in refrigerator at 4°C for further use.

The powdered materials (30 g) were extracted successively with 180 ml of petroleum ether, chloroform, acetone, methanol and whater by using soxhlet extractor for 8 h at a temperature not exceeding the following point of the solvent.

Determination of total phenolics content

The total phenolics content was determined according to the method described by Siddhuraju and Becker (12). The analysis was performed in triplicates and the results were expressed as gallic acid equivalents (GAE).

Determination of flavonoids content

The flavonoids content of all the extracts was quantified as it acts as a major antioxidant in plants, reducing oxidative stress, estimated as per described by Zhishen et al. (13). The amount of flavonoids was calculated in GAE.

DPPH radical scavenging activity

The antioxidant activity of the extract was determined in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH, according to the method of Braca et al. (14). Radical scavenging activity of the sample was expressed as IC_{50} which is the concentration of the sample required to inhibit 50% of DPPH concentration.

Phosphomolybdenum assay

The antioxidant activity was evaluated by the green phosphomolybdenum complex formation according to the method described by Prieto et al. (15). The results are mean values expressed as g of ascorbic acid (AA) equivalents/100 g extract.

H_2O_2 scavenging assay

The ability of extracts to scavenge hydrogen peroxide was determined according to the method of Ruch et al. (16). Absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. For each concentration, a separate blank sample was used for background subtraction. The percentage inhibition activity was calculated using the formula: % scavenging activity = [(Control OD - Sample OD) / Control OD] ×100

Phytochemical screening

The different extracts were tested for steroids, alkaloids, phenolic compounds, cardiac glycosides, flavonoids, tannins, saponins, amino acids, anthraquinones, coumarin glycosides and carbohydrates according to the standard methods described by Harborne (17). The different extracts were centrifuged at 3000 rpm for 10 min and filtered through Whatman No. 1 filter paper by using high pressure vacuum pump. The samples were diluted to 1:10 with the same solvent. The extracts were scanned in the wavelength ranging from 200-1000 nm using Shimazdu spectrophotometer. The prominent characteristic peaks were detected and their absorbance was recorded.

Fourier transform infrared spectroscopy and High performance thin layer chromatography analysis

Due to the results of phytochemical analysis, the methanolic extracts of *L. uniflora* were selected for Fourier transform infrared spectroscopy (FTIR) and HPTLC analysis. FTIR analysis was performed using Perkin Elmer Spectrophotometer system, to detect the characteristic peaks and their functional groups. The crude powders and methanolic extracts of *L. uniflora* were passed in to the FTIR and the peak values were recorded. 3 µl of test solutions and 2 µl of standard solution were loaded as 5 mm band length in the 7 x 10 Silica gel 60F254 TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument. The samples loaded plate was kept in TLC twin trough developing chamber (after saturation with solvent vapor) with respective mobile phases viz., toluene-acetone-formic acid (4.5: 4.5: 1) for phenolics, toluene-ethyl acetate-formic acid-methanol (3: 3: 0.8: 0.2) for tannins and ethyl acetate-methanol-water (10:1.35: 1) for alkaloids. The developed plate was dried by hot air to evaporate solvents from the plate. The plates were kept in photo-documentation chamber (CAMAG REPROSTAR 3) and images were captured at visible light, UV 254 nm and UV 366 nm. The developed plates were sprayed with respective spraying reagents viz., 20% sodium carbonate reagent followed by folin cio-calteu reagent for phenolics, 5% ferric chloride reagent for tannins and Dragendorff's reagent followed by 10% ethanolic sulphuric acid reagent for alkaloids. The plates were dried at 100 °C in hot air oven. The peak table, peak display and peak densitogram were noted. The software used was win CATS 1.3.4 version.

Results

Total phenolics contents

The total phenolics contents obtained from *L. uniflora* using different solvent systems are presented in Table 1. Maximum amount of total phenolics was observed in methanolic extracts (148.09 mg GAE/g) where as acetone and petroleum ether extracts demonstrated moderate levesl of phenolics. Chloroform extracts of *L. uniflora* displayed minimum amounst of phenolics (22.38 mg GAE/g).

Total flavonoids contents

Flavonoids contents of different extracts of *L. uniflora* varied considerably from 7855 to 616 mg GAE/g extract (Table 1). Highest amount of total flavonoids was observed in acetone extracts (7855

Table 1. Total phenolics and flavonoids contents of *L. uniflora*

Extracts	Phenolics mg GAE/g ± SD	Flavonoids mg GAE/g ± SD
Pet. ether	36.03 ± 1.92	643.34 ± 8.82
Chloroform	22.38 ± 2.18	616.67 ± 6.67
Acetone	79.36 ± 2.74	7855 ± 19.2
Methanol	148.09 ± 2.18	2848.89 ± 13.4

mg GAE/g) whereas the other extracts showed moderate levels of flavonoids.

DPPH radical scavenging activity

The DPPH radical scavenging activity of different extracts of *L. uniflora* are shown in Figure 1. DPPH is a stable free radical which is commonly used for assessing antioxidant activity in plant samples. The DPPH assay results were expressed in IC_{50} values. Concentration of the sample necessary to decrease initial concentration of DPPH by 50% (IC_{50}) under the experimental condition was determined. Therefore, the lower value of IC_{50} indicates a higher antioxidant activity. The free radical scavenging activity of *L. uniflora* extracts were exerted as follows: methanolic extract (IC_{50} 86.65µg/ml) > acetone> petroleum ether > chloroform extracts.

Phosphomolybdenum assay

The phosphomolybdenum method is based on the reduction of Mo (VI) to Mo (V) by the antioxidant compounds and the formation of green phosphate/Mo (V) complex with an absorption at 695 nm. The results of phosphomolybdenum assay

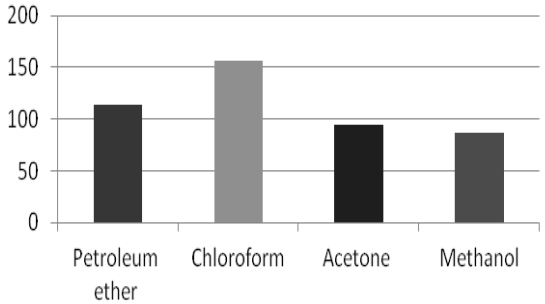

Figure 1. DPPH radical scavenging activity of *L. uniflora*.

of different extracts of *L. uniflora* are illustrated in Table 2. The phosphomolybdenum reduction power of *L. uniflora* extracts were exerted as follows: methanolic extract> acetone> petroleum ether> chloroform extracts. Methanolic extract displayed highest phosphomolybdenum reduction (801.7 g AA/100 g) followed by moderate reduction in acetone extract (366.5 g AA/100 g). Petroleum ether and chloroform extracts of *L. uniflora* illustrated the lowest level of reduction.

H_2O_2 scavenging activity

The extracts of *L. uniflora* showed an efficient H_2O_2 scavenging activity in concentration-dependent manner (Table 2). Among the tested extracts of *L. uniflora,* chloroform extract exhibited highest inhibition percentage (66.9%) followed by acetone and petroleum ether extracts (66.3% and 42.3%, respectively).

Phytochemical screening

Preliminary phytochemical screening of 12 different chemical compounds was performed in five different extracts of *L. uniflora*. Thus, out of 60 tests for the presence or absence of above compounds, 21 tests gave positive (presence) results. Methanolic extract of *L. uniflora* displayed the presence of maximum number of 6 metabolites out of 12 metabolites examined followed by

Table 2. Total phenolics and flavonoids contents of *L. uniflora*

Extracts	Phosphomolybdenum assay	H_2O_2 scavenging assay
Petroleum ether	151.4 ± 3.29	42.3±18.7
Chloroform	176.9 ± 2.02	66.9±9.81
Acetone	366.5 ± 1.29	66.3±15.1
Methanol	801.7 ± 3.13	40.6±40

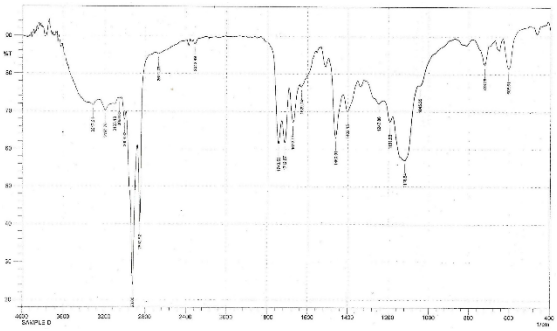

Figure 2. FT-IR spectrum for chloroform extract of *L. uniflora*.

petroleum ether and chloroform extracts with 5 metabolites. Next to that, acetone extract of *L. uniflora* displayed the presence of 3 metabolites. Aqueous extract of *L. uniflora* demonstrated the presence of 2 metabolites . The steroids and sterol showed their presence in petroleum ether, chorofo-rm, acetone and methanolic extracts. Saponins was present in chloroform, acetone and methanolic extracts (Table 3).

Spectroscopic profile

The qualitative UV-Vis spectroscopic profile of *L. uniflora* extracts was assessed at a wavelength of 300-1000 nm due to sharpness of peaks and proper baseline. The spectroscopic profile of petroleum ether extract of *L. uniflora* showed the presence of metabolites and functional groups at 928, 608, 609, 559, 533 and 412 nm with an absorption of 0.006, 1.505, 0.265, 0.152, 0.332 and 3.414, respectively. The chloroform extract demonstrated the presence of metabolites and functional groups at 666, 609 and 538 nm with an absorption of 3.923, 2.900 و and 2.683, respectively. Acetone extract displayed the presence of metabolites and functional groups at 664, 607,534 and 338 nm with an absorption of 1.279, 0.340, 0.382 and 3.763, respectively. The spectroscopic profile of methanolic extract illustrated the presence of metabolites and functional groups at 661 and 605 nm with an absorption of 1.173 and 0.434, respectively.

The FTIR spectrum was used to identify the functional group of the active metabolites based on the peak value in the region of infrared radiation. The methanolic extract of *L. uniflora* was passed

Table 3. Phytochemical screening of different extracts of *L. uniflora*

Compounds	Petroleum ether	Chloroform	Acetone	Methanol	Aqueous	Total
Steroids	+	+	+	+	-	4
Alkaloids	-	-	-	+	+	2
Phenolic groups	+	-	-	+	-	2
Cardiac glycosides	+	+	-	-	-	2
Flavanoids	+	+	-	-	-	2
Saponins	-	+	+	+	-	3
Tannins	-	-	-	+	+	2
Amino acids	-	-	-	-	-	0
Anthraquinone	-	-	-	-	-	0
Coumarin glycosides	-	-	-	-	-	0
Carbohydrate	-	-	-	-	-	0
Sterol	+	+	+	+	-	4
Total	5	5	3	6	2	**21**

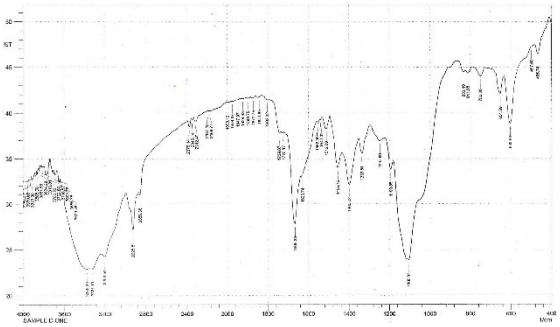

Figure 3. FT-IR spectrum for crude powder of *L. uniflora.*

into the FTIR and the functional groups of the components were separated based on their peak ratio. The results showed different peak values and confirmed the presence of functional groups such as alkynes, alkenes, alkanes, anhydrides, aliphatic amines, sulfur compounds, phosphorus compounds, silicon and boron compounds, etc… (Table 4; Fig. 2).

FTIR analysis of crude powder of *L. uniflora* showed more peaks when compared to methanolic extract. The results confirmed the presence of various functional groups such as alkynes, alkenes, sulfur compounds, phosphorous compound, sulfonic acids, alcohols, phenols, acyclic compound, amino acid hydrochlorides, isonitriles, acetonitrile, carbondisulfide, secondary amides, silicon and boron compounds, etc... (Table 5; Fig. 3).

Table 4. FTIR analysis for chloroform extract of *L. uniflora*

Peak values	Functional groups	Range (nm)
605.61	Alkynes and allenes	550-690
723.26	Alkanes	720-750
1045.35	Anhydrides	1045-1145
1118.64	Aliphatic amines C-N vib	1020-1220
1191.93	Sulfur compounds	1190-1240
1247.86	Phosphorus compounds	1150-1350
1402.15	Phosphorus compounds	1280-1450
1460.01	Silicon and boron compounds	1250-1500
1629.74	Sulfur compounds	1130-1675
1672.17	Oximes (C=N str)	1620-1690
1712.67	Ureas	1720-1790
1743.53	Ketones	1740-1750
2312.49	Carbondisulfide	2100-2360
2671.23	Deuterated R-OH	2400-2780
2852.52	Alkanes, C-H str sym	2500-3030
3006.82	Amino acid, Hydrochlorides	2995-3050
3060.82	Amino acid, Hydrochlorides.	3010-3060
3105.18	Amino acid, Hydrochlorides	3030-3130
3197.76	Amides, bonded NH str.	2650-3200
3317.34	Amides, N-H, trans str.	2950- 3400

Table 5. FTIR analysis in crude powder of *L. uniflora*

Peak values	Functional groups	Range (nm)
466.74	Unknown	
497.60	Unknown	
603.68	Alkynes and allenes	550-690
651.89	Sulfur compounds	550-690
750.26	Phosphorous compound	700-750
811.98	Phosphorous compound	740-830
833.19	Compounds containing nitrogen- oxygen bond, N-O str	810-850
1105.14	Sulfur compounds,	1100-1300
1193.85	Sulfur compounds	1100-1300
1247.86	Phosphorus compounds	1150-1350
1336.58	Alcohols and phenols	1310-1410
1400.22	Compounds containing nitrogen- oxygen bond, (R-N-N=O)	1350-1400
1456.16	Nitrosamines (R-N-N=O)	1130-1675
1512.09	Acyclic compound	1130-1675
1541.02	Pyrroles C=C str	1500-1550
1552.59	Purins C=N str	1500-1550
1629.74	Pyridines C=C str	1480-1660
1666.38	Acyclic saturated, C=N str	1640-1690
1726.17	R-OH, Ar- OH	1700-1730
1739.67	Ureas	1715-1740
1803.32	Aroyl peroxides esters,	1780-1805
1841.89	Alkenes	1800-1850
1872.75	Amido acids	1835-1945
1899.75	Amido acids	1835-1945
1924.83	Amido acids	1835-1945
1947.97	Charged amine derivatives	1800-2200
1974.97	Charged amine derivatives	1800-2200
1998.12	Amino acid hydrochlorides	1950-2000
2169.77	Isonitriles C=N str	2120-2185
2187.13	Charged amine derivatives	1800-2200
2312.49	Acetonitrile	2180-2340
2349.14	Carbondisulfide	2100-2360
2376.14	Charged amines (C=NH+)	2325-1500
2856.38	Alkanes	2845-2870
3203.54	R-COOH	2500-3300
3321.19	Secondary amides	3270-3330
3375.20	Charged amine derivatives	3095-3400
3629.78	Oximes	3496-3650
3656.78	Alcohols, Phenols	3584-3676
3683.78	Silicon and boron compounds	3200-3700

The results of the preliminary phytochemical studies confirmed the presence of alkaloids, tannins and phenolics in the methanolic extract of *L. uniflora*. Different compositions of the mobile phase for HPTLC analysis were tested in order to obtain high resolution and reproducible peaks. The alkaloid profile of *L. uniflora* showed the presence of 14 different type of compounds with different R_f values ranging from 0.04 to 0.82. Among these, seven are known alkaloids viz., 0.14, 0.16, 0.25, 0.32, 0.38, 0.46 and 0.56 (Table 6). The phenolic profile of *L. uniflora* revealed the presence of 7 various types of compounds with different R_f values rangeing from 0.12 to 0.92. Among these, four known compounds were present viz., 0.63, 0.70, 0.77 and 0.84 (Table 7).

The chloroform extract of *L. uniflora* revealed the presence of 5 different types of tannins with different R_f values ranges from 0.37 to 0.93. Among these, four known compounds (0.67, 0.75, 0.82 and 0.93) and one unknown compound was identified (Table 8).

Discussion

As a result, more degree of alkaloids diversity was found in *L. uniflora* extracts.

Phytochemicals are chemical compounds formed during the plants normal metabolic processes. Phenolics are one of the most ubiquitous groups of secondary metabolites found throughout the plant kingdom (18). They contain a range of compound that include structures such as simple aromatic phenols, hydroxy and substituted benzoic acids and aldehydes, hydroxy and substituted cinnamic acids, coumarins, tannins, and perhaps a few of the flavonoids (19). Flavonoids are present in most plant tissues and often in vacuoles (20). Flavonoids are the most common and widely distributed group of plant phenolic compounds and are usually very effective antioxidants (21).

Table 6. HPTLC alkaloid profile of *L. uniflora*

Rf	Height	Area	Assigned substance
0.40	299.6	9517.2	Colchicine standard
0.04	11.4	72.4	Unknown
0.09	11.4	112.2	Unknown
0.14	77.7	1986.9	Alkaloid 1
0.16	51.0	496.7	Alkaloid 2
0.25	105.6	5158.0	Alkaloid 3
0.32	174.0	7326.5	Alkaloid 4
0.35	115.5	1496.9	Unknown
0.38	119.6	2732.5	Alkaloid 5
0.46	156.1	8198.9	Alkaloid 6
0.56	221.0	12141.3	Alkaloid 7
0.61	164.8	3538.3	Unknown
0.65	208.2	5283.8	Unknown
0.68	236.5	5019.4	Unknown
0.82	740.1	106185.0	Unknown

Table 7. HPTLC phenolic profile of *L. uniflora*

Rf	Height	Area	Assigned substance
0.61	738.5	22805.1	Quercetin standard
0.12	12.4	192.1	Unknown
0.27	12.1	231.7	Unknown
0.63	427.1	24316.5	Phenolic 1
0.70	392.4	20834.1	Phenolic 2
0.77	283.3	13509.7	Phenolic 3
0.84	250.9	10838.1	Phenolic 4
0.92	423.8	24796.2	Unknown

Hydrogen/electron transfer from antioxidants to DPPH radical and Mo (VI) complex occur in the DPPH radical and phosphomolybdenum assays, respectively (22). This reduction ability was relatively shown by the active extracts of *L. uniflora*. The results of H_2O_2 scavenging activity showed that extracts of *L. uniflora* seem to be an effective scavenger of H_2O_2. H_2O_2 can be converted to more reactive species (e.g. the hydroxyl radical. Hence, the results indicated that *L. uniflora* can act as a good scavenger against such harmful radicals.

The results of the phytochemical analysis of petroleum ether, chloroform, acetone, methanolic and aqueous extracts of *L. uniflora* revealed the presence of various primary and secondary metabolites with varied degrees. Alkaloids rank among the most efficient and therapeutically significant plant substances. They comprise the largest single class of secondary plant substances containing 5,500 alkaloids (23). Tannins are one of

the major phytochemicals found in many higher plants. Tannins have a characteristic strange smell and astringent taste and could bind to proteins through the effective formation of strong complexes with proteins and other macromolecules (24). In the present study, phytochemical analysis was conducted on different extracts of *L. uniflora* and provided useful information which may help in authenticating the plant along with nature of phytoconstituents present in it.

UV-Vis and FTIR spectrum is a most credible method to validate and identify the mix-substance systems such as traditional medicine and herbal medicine (25). The results of the present FTIR spectrum study also revealed the presence of functional constituents in the crude powder and methanolic extracts of *L. uniflora* which supplemented the previous observations and provided the similarity in functional groups. These results on UV-Vis and FTIR spectroscopic profile will act as pharmacognostical tools to determine the authenticity of the plant.

Chromatographic fingerprint is a rational option to meet the need for more effective and powerful quality assessment of traditional system of medicine throughout the world (26). The optimized chromatographic fingerprint is not only an alternative analytical tool for authentication, but also an approach to express the various patterns of chemical ingredients distributed in the herbal drugs and to preserve such "database" for further multifaceal sustainable studies (27, 28). Of which, HPTLC method can be used for phytochemical profiling of plants and quantification of compounds present in plants, with increasing demand for herbal products as medicines. The results of the present study provide some information about HPTLC profile of alkaloids, tannins and phenolic compounds which provide distinct bands with varied R_f values. Thus, it will help in identification, standardization and quality control of *L. uniflora* in the pharmaceutical industry. It is hoped that the present work revealed the bio-potentials of *L. uniflora* plant extracts for field applications. Further investigations are needed to elucidate the active ingredients of the extract responsible for various pharmacological properties.

Table 8. HPTLC tannin profile of *L. uniflora*

Rf	Height	Area	Assigned substance
0.41	386.8	14437.9	Gallic acid standard
0.37	13.7	282.2	Unknown
0.67	426.6	22183.7	Tannin 1
0.75	175.5	8429.8	Tannin 2
0.82	95.8	3201.7	Tannin 3
0.93	324.9	17389.8	Tannin 4

Conflict of interests

The authors declared no conflict of interests.

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