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PHYTOCHEMICAL CONSTITUENTS AND RADICAL SCAVENGING ACTIVITIES OF STEM BARK EXTRACTS OF *DREGEA VOLUBILIS* (LINN. F.) BENTH EX. HOOK.F.

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ABSTRACT: Over time, ethnomedicinal plants have been praised hugely for the presence of diverse pharmacologically active phytochemicals and have been used traditionally in primary medication to cure various human diseases or disorders. In the present study, different phytochemical constituents were analysed and free radical scavenging activities of *Dregea volubilis* stem bark (DvSB) extracts were assayed. Different *in vitro* scavenging tests such as DPPH[•] scavenging assay, O₂^{•-} scavenging assay, OH[•] scavenging assay and H₂O₂ scavenging assay were employed to estimate the free radical scavenging potentiality. Results revealed that methanolic extract had superior scavenging activity over the hot water extract. Qualitative analysis of phytochemicals showed the presence of reducing sugars, phenolic compounds, flavonoids, resins, glycosides, cardiac glycosides and steroids; and quantitative phytochemical analysis for polyphenols, flavonoids, flavonols, proanthocyanidins and alkaloids were made by standard procedures. Hence the study reveals that DvSB has significant scavenging properties, thus validating the ethnic usage for treating stress-related complications and restoration of good health.

INTRODUCTION: Medicinal plants and plant-derived medicable agents have long been attracted worldwide attention because of their multifarious activity and superior safety regards. The usage of plants in primary medication is practiced traditionally and universally. Over time, people from different societal clusters have relied on plant-based remedies for both acute and chronic health problems¹. Investigation in recent times is continuing to establish that ethnomedicinal plant-based therapeutics and their chemical constituents may serve as complementary and alternative medicine (CAM) as these may act in our body system like the action of modern drugs with no or less side effects².

The ethnomedicinal plant *Dregea volubilis* (Linn. f.) Benth ex. Hook.f. [Syn: *Wattakaka volubilis* (Linn. f.) Stapf; *Marsedenia volubilis* (Linn. f.) Cooke] is commonly known as “Tita kunga” or “Jukti” in Bengal and is used by different tribal communities of North Bengal. The plant is being used very specifically in indigenous systems of medicine such as Ayurveda, Siddha and Unani and unorganized system like folk-medicine. Different parts of this asclepidacean woody vine are traditionally used to cure various diseases such as diabetes mellitus, boils, abscesses, inflammations, eye ailments, tracheitis, stomach ache and general debility^{3,4}.

The tribal people mainly from the Oraon community of North Bengal use the stem bark as food additive in preparing a traditional fermented beverage namely *Jhara* or *Harhia* and in controlling the complications of diabetes. Many pieces of work have been done to explore the biological activities of leaf, flower, fruit and root extracts of *Dregea volubilis*^{5,6,7,8,9}, till now there

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is no scientific report on phytochemical investigations and radical scavenging activities of *DvSB* extracts, hence the present study has been attempted.

MATERIALS AND METHODS:

Plant sample and its collection: Fresh stems of *Dregea volubilis* along with some flowering twigs and branches were collected from a tribal village located in Dooars region of North Bengal, West Bengal, India. The specimen was identified and authenticated taxonomically at North Bengal University Herbarium. During collection, the plant materials were kept in zip lock plastic bags and brought to the laboratory, washed initially with tap water to remove any debris and finally washed with double distilled water. The cleaned stems were then blotted off with tissue paper and the bark was peeled from them. Bark sample was dried in hot air oven for 48 h at 45 °C and used to obtain fine powder for extraction.

Preparation of lyophilized extract: For methanolic extract (MEE), under normal room temperature, about 10 g of fine powder of stem-bark of *Dregea volubilis* was extracted thrice with degassed methanol (1:10, w/v) by stirring at 150 r min⁻¹ for 3 x 24 h intermittently with fresh solvent each time and filtered through Whatman No. 1 filter paper, following a modified method of Cheesbrough¹⁰. For hot water extract (HWE), method of Aliakbarlu & Tajik¹¹ was followed with some modifications. Ten g of powdered sample was extracted for 30 min under darkness by refluxing with HPLC grade deoxygenated water (1:10, w/v) at 100 °C in a temperature controlled water bath shaker with gentle agitation. After cooling, each sample was filtered through Whatman No. 1 filter paper and the solid residues obtained were further treated with same procedure twice. In each extraction process the filtrate fractions were pooled and concentrated *in vacuo* at 40 °C, followed by lyophilisation to obtain the lyophilized crude extracts. The extracts were dissolved in the respective solvent and preserved at -20°C for future analysis.

Determination of solvent extractive value: The solvent extractive (methanol soluble extractive, MSE and water soluble extractive, WSE) value was expressed in percentage (%) and was determined using the formula:

$$\% \text{ MSE or } \% \text{ WSE} = \left(\frac{\text{Weight}_{\text{lyophilised crude extract}}}{\text{Weight}_{\text{initial plant material}}} \right) \times 100.$$

Determination of total moisture content: Moisture content in bark sample was determined according to the method of AOAC¹², using the following formula:

$$\text{Total moisture content (\%)} = \left[\frac{(\text{Weight}_{\text{initial}} - \text{Weight}_{\text{final}})}{\text{Weight}_{\text{initial}}} \right] \times 100.$$

Analysis of phytochemical constituents:

Qualitative analysis: Phytochemical analysis of the crude plant extracts for identifying different active chemical constituents was done according to the methods as described by Dev Chaudhuri *et al.*¹³.

Quantitative analysis:

Total polyphenol content: The total polyphenol content was assayed with Folin-Ciocalteu reagent (FCR) using gallic acid as the standard as followed by Taga *et al.*¹⁴. An aliquot of 0.1 mL of extract was added to 2.0 mL of 2% aqueous solution of sodium carbonate. After 2 min, 0.1 mL of 50% FCR was added and the mixture was shaken thoroughly and left to stand for 30 min in the dark at RT. Absorbance was read spectrophotometrically at 750 nm. TPC was calculated as gallic acid equivalents (GAE) from a calibration curve of gallic acid standard solutions and expressed as mg of GAE g⁻¹ of lyophilized extract. The experiment was performed in triplicate (*n* = 3).

Total flavonoid content: Total flavonoids were estimated using method described by Ordon-ez *et al.*¹⁵. To 0.5 mL of extract, 1.5 mL of methanol, 0.1 mL of 10% aluminium trichloride hexahydrate, 0.1 mL of 1.0 M potassium acetate solution and 2.8 mL of ddH₂O water was added. After one and half hour of incubation at RT, the absorbance was measured spectrophotometrically at 420 nm against a blank. TFC was calculated as catechin equivalents (CAE) from a calibration curve of (+)-catechin standard solutions and expressed mg of CAE g⁻¹ of lyophilized extract. The experiment was performed in triplicate (*n* = 3).

Total flavonol content: Total flavonols in the extract were estimated using the method of Kumaran & Karunakaran¹⁶. To 2.0 mL of extract or standard, 2.0 mL of 2% aluminum trichloride (in methanol) solution and 3.0 mL of aqueous solution

of sodium acetate (50 g L^{-1}) were added. The absorption at 440 nm was read spectrophotometrically after 2.5 h at 20°C . Total flavonols (TFO) content was calculated as quercetin equivalents (QRE) and expressed as mg of QRE g^{-1} of lyophilized extract. The experiment was performed in triplicate ($n = 3$).

Total alkaloid content: Determination of total alkaloid content was made following the methodology of Harborne¹⁷. In a 250 mL beaker, 100 mL of 10% acetic acid in ethanol was taken and to it 5 g of powdered sample was added. The beaker was covered and allowed to stand for 4 h. This was filtered using Whatman filter paper and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue was the alkaloid which was dried in hot air oven at 40°C and weighed after complete dryness. The experiment was performed in triplicate ($n = 3$).

Total proanthocyanidin content: Proanthocyanidins reactive to vanillin were analyzed by the vanillin method as described by Broadhurst & Jones¹⁸. 1.0 mL of extract solution was placed in a test tube together with 2.0 mL of vanillin (1% in 7 M H_2SO_4) in an ice bath and then incubated at 25°C . After 15 min the absorbance of the solution was read at 500 nm. Total proanthocyanidin (TPA) content was calculated as catechin equivalents (CAE) from a calibration curve of (+)-catechin standard solutions and expressed mg of CAE g^{-1} of lyophilized extract. The experiment was performed in triplicate ($n = 3$).

Determination of *in vitro* radical scavenging activities:

Free radical scavenging activity using DPPH: The 2, 2 - Diphenyl-1-picrylhydrazyl (DPPH) scavenging activity was monitored after 30 min at 517 nm using the method of Yen & Duh¹⁹, with minor modifications. Briefly, different aliquots (0.1 mL) of extract were added to 2.9 mL of freshly prepared solution of DPPH• ($6 \times 10^{-5} \text{ M}$ in methanol). The mixtures were vortexed thoroughly for 15 s, and left in the dark at RT for 30 min. After

incubation, the decrease in absorbance was recorded spectrophotometrically at 517 nm for all samples. Methanol was used to zero the spectrophotometer. Absorbance of the radical without antioxidant (control) was measured daily. L-ascorbic acid (AsA) was used as reference. Free radical scavenging activity ($\text{FRSA}_{\text{DPPH}\cdot}$) expressed as percentage inhibition (% I) of the DPPH• radical was calculated according to the formula: $\text{FRSA} (\% \text{ I}) = [(A_c - A_s) / A_c] \times 100$, where A_c refers to the absorbance (λ_{517}) of control ($t = 0 \text{ min}$) and A_s is the absorbance (λ_{517}) of sample plus DPPH• ($t = 30 \text{ min}$). The experiment was performed in triplicates ($n = 3$).

Superoxide anion scavenging activity: The superoxide anion radical ($\text{O}_2\cdot^-$) scavenging measured according to the method described by Nishikimi *et al.*²⁰. Reaction mixture contained 1.0 mL of sample extract, 1.0 mL of NBT solution ($312 \mu\text{M}$ prepared in phosphate buffer, pH 7.4) and 1.0 mL of NADH solution ($936 \mu\text{M}$ prepared in phosphate buffer, pH 7.4). Finally, the reaction was accelerated by adding 0.1 mL PMS solution ($120 \mu\text{M}$ prepared in phosphate buffer, pH 7.4) to the mixture. The reaction mixture was incubated at 25°C for 5 min and absorbance at 560 nm was measured against a methanolic blank. L-ascorbic acid (AsA) was used as a positive control. The decrease of absorbance at 560 nm with antioxidants indicates the consumption of superoxide anions in the reaction mixture indicating the increased scavenging activity. Percentage of superoxide anion radicals scavenged was measured using the formula: Superoxide anion scavenging (SAS) activity (% I) = $[(A_c - A_s) / A_c] \times 100$, where A_c was the absorbance (λ_{560}) of the control, and A_s was the absorbance (λ_{560}) of the extract or L-ascorbic acid. The experiment was performed in triplicate ($n = 3$).

Hydroxyl radical scavenging activity: The hydroxyl radical ($\text{OH}\cdot$) scavenging activity was measured according to the sodium salicylate method by Smirnoff & Cumbes²¹ and Manda *et al.*²² using sodium salicylate, a procedure based on the reaction between sodium salicylate and residual $\text{OH}\cdot$. Briefly, the reaction mixture (2.1 mL) contained 1.0 mL of FeSO_4 (1.5 mM), 0.7 mL of H_2O_2 (6 mM), 0.3 mL of sodium salicylate (20 mM) solutions and the extract to be tested. The

reaction mixture was incubated for 1 h at 37 °C, after which the absorbance of the hydroxylated salicylate complex was measured at 562 nm with methanol as the blank. L-ascorbic acid (AsA) was used as the positive control. HRS activity was calculated in terms of percent scavenging effect by using the formula: OH[•] scavenging (HRS) activity (%) = $[1 - (A_1 - A_2) / A_0] \times 100$, where A_0 was the absorbance (λ_{562}) of the control (without extract or standard), A_1 was the absorbance (λ_{562}) in the presence of the extract or standard and A_2 was the absorbance (λ_{562}) without sodium salicylate. The experiment was performed in triplicate ($n = 3$).

Hydrogen peroxide scavenging activity: The hydrogen peroxide (H₂O₂) scavenging assay was carried out following the procedure of Ruch *et al.*²³. The principle of this method is that there is a decrease in absorbance upon oxidation of H₂O₂. A solution of 43.0 mM H₂O₂ was prepared in 0.1M phosphate buffer (pH 7.4). 1.0 mL extract in 3.4 mL phosphate buffer (0.1 M, pH 7.4) was added to 0.6 mL of 43.0 mM H₂O₂ solution and the absorbance of the reaction mixture was recorded at 230 nm after 10 min incubation at room temperature. A blank solution contained the sodium phosphate buffer (0.1 M, pH 7.4) without H₂O₂. The percentage of H₂O₂ scavenging by the extracts and standard solutions of L-ascorbic acid (AsA) were calculated using the following equation: H₂O₂ scavenging (HPS) activity (%) = $[(A_c - A_s) / A_c] \times 100$, where A_c was the absorbance (λ_{230}) of the control (without test sample) and A_s was the absorbance (λ_{230}) in presence of test sample. The experiment was performed in triplicate ($n = 3$).

Statistical analysis: Experimental analyses were carried out in three different observations ($n=3$) and results were expressed as mean value \pm standard deviation (SD). Statistical analysis was carried out by SPSS statistic software version 21 (IBM SPSS, USA).

RESULTS AND DISCUSSION: The present investigation was set to evaluate the phytochemical screening and radical scavenging activities of stem bark extracts of *Dregea volubilis*. The solvent soluble extractive value plays an important role in evaluation of crude plant extracts as different solvents has different level of polarity and thus

capacity of phytochemical extraction. The study revealed that the methanol soluble extractive (MSE) value (12.93 ± 0.21 %) was quite higher than that of water soluble extractive (WSE) value (8.61 ± 0.15 %). The total moisture content of *DvSB* was analysed and it was observed the total moisture content (TMC) was 5.71 ± 0.20 % (**Fig. 1**).

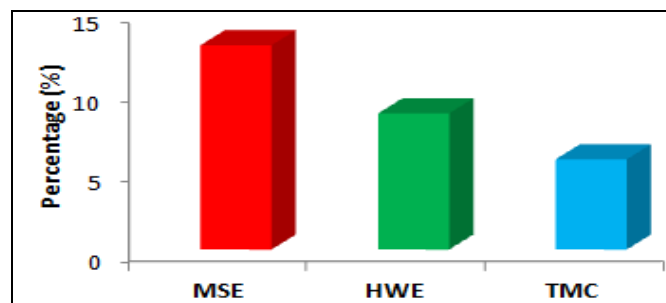


FIG. 1: ANALYSIS OF SOLVENT EXTRACTIVE VALUES AND TOTAL MOISTURE CONTENT. MSE: methanol soluble extractive, WSE: water soluble extractive and TMC: Total moisture content. Results are expressed in percentage

Preliminary phytochemical screening is helpful in finding out chemical constituents present in the plant material that leads to their quantitative determination²⁴. Screening of phytochemical constituents showed the presence of major plant secondary metabolites *viz.* reducing sugars, phenolic compounds, flavonoids, resins, glycosides, cardiac glycosides and steroids in both methanolic and hot water extracts (**Table 1**).

TABLE 1: QUALITATIVE DETECTION OF PHYTOCHEMICALS DVSB EXTRACTS

Phytochemicals	MEE	HWE
Reducing sugars	+	+
Polyphenols	++	+
Flavonoids	+	+
Resins	++	+
Free amino acids	-	+
Anthraquinones	+	-
Tannins	+	-
Triterpenoids	+	-
Alkaloids	++	-
Glycosides	++	+
Steroids	+	+
Saponins	++	-
Cardiac glycosides	+	+
Essential oils	-	-
Phlobatannins	+	-
Cardenolides	+	-

Occurrence of phytochemicals depicted as ++: moderate amount, +: trace amount, -: absent. MEE: Methanolic extract and HWE: Hot water extract

The quantitative estimation of different secondary metabolites presented in **Table 2**. Plant secondary metabolites have significant contribution in pharmacological activities of regarding antioxidant, antimicrobial, hypoglycemic, antidiabetic, anti-inflammatory activities^{25, 26, 27} etc.

TABLE 2: QUANTITATIVE ESTIMATION OF PHYTOCHEMICALS PRESENT IN *D. VOLUBILIS* STEM-BARK

TPC ^a	TFC ^b	TFLC ^c	TALC ^d	TPAC ^e
2.54 ± 0.03	1.58 ± 0.13	0.64 ± 0.07	2.1 ± 0.03	1.67 ± 0.05

a. Total polyphenol content in mg GAE g⁻¹ of MEE, b. Total flavonoid content in mg CAE g⁻¹ of MEE, c. Total flavonol content in mg QRE g⁻¹ of MEE, d. Total alkaloid content in mg per 100 g of dry weight, e. Total proanthocyanidin content in mg CAE per 100 g of MEE. Here, MEE: methanolic extract. Values are presented as mean ± SD unit (n = 3)

The contents of total polyphenols, total flavonoids and total flavonols were more or less within the range of values reported earlier⁷. It was found that one milligram of methanolic fruit extracts of *D. volubilis* contained 95.03 µg of pyrocatechol equivalents of phenols. Generally, same species of medicinal plants but with different habitat and growth conditions shows difference in their composition of phytochemicals and contents²⁸. Furthermore, the extraction process is another critical factor to determine the various phytochemical contents in the resulting crude extract.

Of late, much attention has been paid to explore the role of natural antioxidants mainly phenolic compounds for their health benefits and therapeutic attributes. Medicinal plants are a rich source of different phytochemicals, including polyphenols and flavonoids which have been reported as scavenger of free radicals due to their redox properties and chemical structures. Flavonoids, including flavonols and proanthocyanidins, on the other hand, a diverse group of plant secondary metabolites, exhibit high antioxidant activity owing to the presence of free hydroxyl (OH) groups, especially 3-OH²⁹.

The antioxidant activity in terms of free radical scavenging potentiality was evaluated by different *in vitro* test models such as DPPH· scavenging activity, H₂O₂ scavenging activity, O₂^{·-} scavenging activity and OH· scavenging activity. The results

showed that the scavenging activity of methanolic extract showed significantly higher than the hot water extract. It may be of the reason that methanol have higher polarity than water that enable it to extract greater amount of phytochemicals. However, the radical scavenging potentiality for both the extracts at different concentrations was quite low as compared to standard reference, L-ascorbic acid (AsA). The scavenging activities of extracts were ranging from 53.50% to 70.70% at 1 mg mL⁻¹ concentration. The results were presented in **Fig. 2** and **3**.

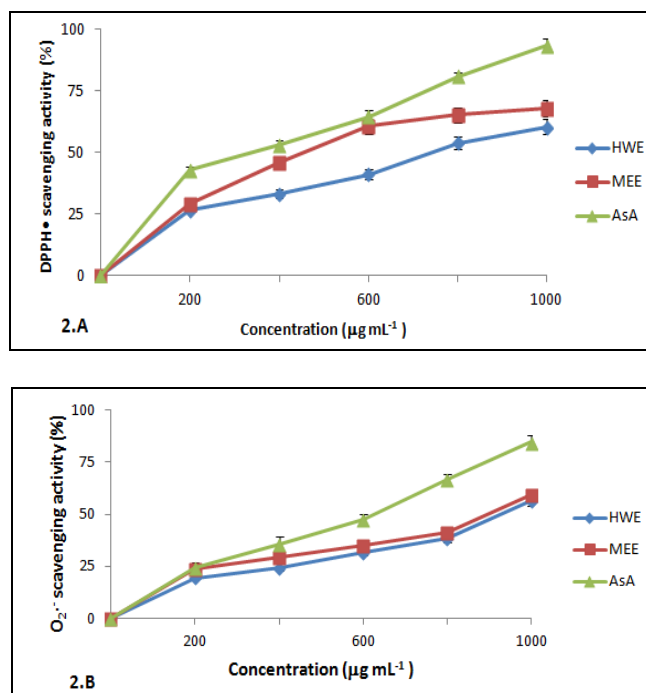


FIG. 2: COMPARATIVE PRESENTATION OF DPPH·-FREE RADICAL SCAVENGING ACTIVITY (A) AND SUPEROXIDE ANION RADICAL (O₂^{·-}) SCAVENGING ACTIVITY (B). Results are expressed as percentage of inhibition (%) ± SD. MEE: methanolic extract, HWE: hot water extract, and AsA: L-ascorbic acid.

The DPPH· activity estimated as 70.70% and 61.35% for methanolic extract and hot water extract respectively (**Fig. 2.A**). Superoxide anion radical scavenging activity of methanolic extract was observed 59.63% and that of hot water extract was 56.62% (**Fig.2.B**). Hydroxyl radical scavenging potentiality was measured as 63.41% for methanolic extract and 53.50% for hot water extract (**Fig. 3.A**), whereas hydrogen peroxide scavenging efficiency was found to 61.48% for methanolic extract and 56.84% for hot water extract (**Fig.3.B**).

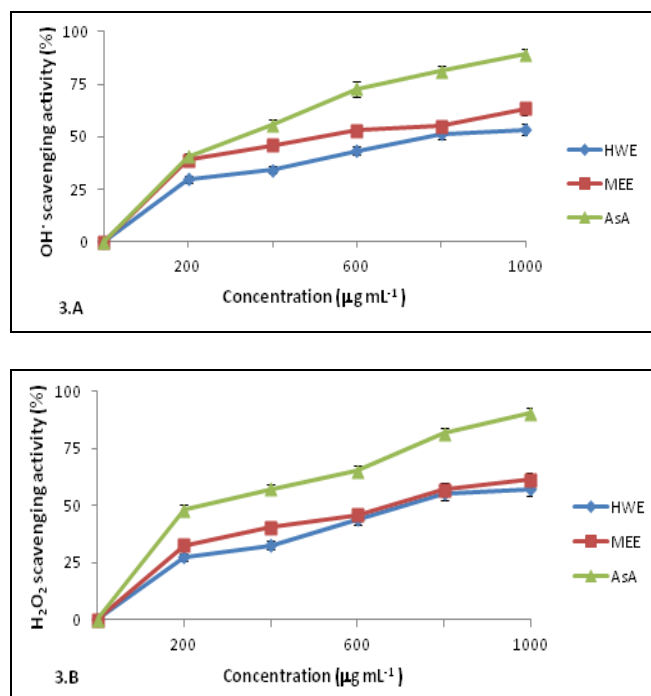


FIG. 3: COMPARATIVE ANALYSIS OF HYDROXYL RADICAL (OH[·]) SCAVENGING ACTIVITY (A) AND HYDROGEN PEROXIDE (H₂O₂) SCAVENGING ACTIVITY (B). Results are expressed as percentage of inhibition (%) ± SD. MEE: methanolic extract, HWE: hot water extract, and AsA: L-ascorbic acid.

Previous study on methanolic fruit extract of *D. volubilis* showed scavenging efficiency of 84.81% and 89.92% in DPPH[·] and superoxide anion scavenging assays⁸. Results revealed that stem showed comparatively less efficiency than fruits of the plant due to accumulation of lesser amount of secondary metabolites in that part of plants.

The ethnomedicinal plant *D. volubilis* is an excellent source of plant secondary metabolites. A plethora of phytoconstituents like steroids, cardiac glycosides, reducing sugars, triterpenoids, flavonoids, phenolic compounds and some alkaloids are found to be present in the plant. Stem and leaves of the plant contain taraxerol, a pentacyclic triterpenoid; Kaempferol, and saponins. From the stems, leaves and bark drevogenin A, drevogenin P, D-cymarose and L-oleandrose have been isolated⁵. Many reports have been available that the antioxidant properties of plants are mainly due to the presence of high content of secondary metabolites, phenolic compounds and flavonoids^{30, 31, 32}. Extract of *D. volubilis* that contained considerable amount of polyphenolic compounds and flavonoids, exhibited significant scavenging activity which in turn may protect from

deteriorative changes in the biological system usually caused by free radicals.

CONCLUSION: Results obtained in the study indicated that both the methanolic extract and hot water extract of stem bark of *D. volubilis* contain polyphenols and flavonoid compounds which exhibit radical scavenging activities. The methanolic extract showed significantly higher activity than the water extract. Hence, in conclusion, the scavenging activities of the plant may contribute to its pharmacological potentiality and demonstrates the scientific proof to the traditional claims of therapeutic values. Finally further studies are recommended for isolation and characterisation of active principles present in plant for better understanding of biological activities.

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CONFLICT OF INTEREST: The authors declare no conflict of interest.

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