



Free radical scavenging activity, antioxidative ingredients, and possible anti-inflammatory abilities of selected traditional medicinal plants from ayurveda

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Abstract

The ethanol extract (50%, v/v) of *Smilax china* Linn. (Smilacaceae), *Barleria prionitis* Linn. (Acanthaceae), *Calotropis gigantea* Linn. (Asclepiadaceae), *Trigonella foenum-graecum* Linn. (Papilionaceae), *Ricinus communis* Linn. (Euphorbiaceae), *Curcuma amada* Linn. Roxb. (Zingiberaceae), and *Piper nigrum* Linn. (Piperaceae) were evaluated for their free radical scavenging activity, antioxidative ingredients, and possible anti-inflammatory activity. All the plant extracts under study were found to be effective scavengers of DPPH radicals along with considerable reducing ability. Except for *Ricinus communis* and *Piper nigrum*, all other samples were active towards scavenging of OH radicals. Considerable amounts antioxidative substances such as Vit. C and phenolics were estimated from all the plant extracts under study. All the tested plant extracts showed considerable PPO inhibitory activity. Anti-inflammatory related studies such as inhibition of dien conjugates, trypsin and β -glucuronidase were carried out. Significant inhibition of dien conjugate hydroperoxides was observed with all the plants under study. The plants under study were found to have poor to moderate anti-proteolytic activity towards trypsin induced hydrolysis of bovine serum albumin (BSA). With few exceptions all plants showed moderate inhibition of β -glucuronidase. In a critical situation wherein the standardization of herbal formulations has remained a key issue to be addressed to the consumers, the results of the present study may find useful for strengthening the process of standardization of Ayurvedic preparations involving the usage of medicinal plants considered in present studies.

Key words: Ayurveda, medicinal plants, antioxidants, anti-inflammatory and enzyme inhibition.

1. Introduction

Biological oxidation reactions and production of free radicals are an integral part of life and the body's metabolism to the extent where they may be deliberately produced to serve an important

biological function [1]. Free radicals are electrically charged molecules that have an unpaired electron. This electron imbalance renders them highly reactive and capable of

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widespread oxidation of lipids, proteins, DNA, and carbohydrates. These radicals have an inherent ability to cause random, irreversible change in biological system that can have very adverse serious physiological effects [2]. An extreme adverse manifestation of free radicals may cause disruption of cell membranes, leading to release of cell contents and death [3]. To combat with these renegade radicals, cells are also equipped with an impressive repertoire of endogenous as well as exogenous (mostly derived from fruits and vegetables) antioxidant molecules [4]. The imbalance between protective antioxidants (enzymatic and non enzymatic) and damaging free radicals results in the generation of oxidative stress: a cause for various ailments in humans [5].

It is worth stating that as vitamins go, antioxidants have ignited the research interest of academic researchers and medical experts in recent years. Clinical and epidemiological evidence supports the 'antioxidant therapy' as an attractive, alternative and safe new pharmacological approach for the amelioration of diverse ailments of humans such as cancer [6], cardiovascular diseases [7], inflammation, shock ischemia injury [8], diabetes, cataract [9,10], Alzheimer's disease [11] in the treatment of acute central nervous system injury [12]. It is interesting to note that the several reputed nonsteroidal anti-inflammatory drugs (NSAIDs) and phenolic compounds with anti-inflammatory activity are reported to have free radical scavenging activity [13].

Medicinal plant is an important element of indigenous medical systems in India as well as abroad. Ayurveda: a holistic approach for management of health and diseases is one of the great traditional complementary and alternative medical systems. Ayurveda contributes Rs. 3500 crores (US \$813 million) annually to the internal market [14]. Ayurveda

has also mentioned the Sanskrit word Vata-Vridhhi (oxidative stress) as the cause of majority of human ailments if corrective measures to ameliorate the disorders in imbalanced physiological state are not taken up in time [15]. The realization of oxidative stress as the cause of several diseases in modern medical system has, therefore started various global programmes to harness and harvest natural antioxidant rich resources [16-19]. In recent years, the use of traditional medicine information on plant research has attracted both academic as well as industrial researchers.

The plants selected in the present study have been described in Ayurvedic medicines and traditionally used for management of diverse ailments of humans. The tubers of *Smilax china* Linn. (Smilacaceae), rich in tannins, steroidal saponins, and other compounds, are used in venereal diseases, rheumatoid disorders, and chronic skin infections [20]. *Barleria prionitis* Linn. (Acanthaceae), possess antiseptic properties. The dried bark of this plant is given in whooping cough. The leaves are given as antipyretic, and in urinary/stomach disorders. The fresh leaves are also use for rheumatic pain and itch [21]. Flowers of *Calotropis gigantean* Linn. (Asclepiadaceae), are given as digestive, stomachic and tonic, also useful in anorexia, asthma, cold and cough. Leaves are applied to painful joints or swelling, skin eruptions, toothache and wounds. Latex is effective in abortion, when a stick smeared with latex is applied locally to induce uterine contractions [22]. *Trigonella foenum-graecum* Linn. (Papilionaceae), is one of the major vegetables used all over India. Seeds are useful as diuretic, carminative, emenagogue, aphrodisiac and as a tonic. It is also a useful remedy for diarrhoea, dropsy, anorexia, rheumatism, anaemia, and diabetes [23]. *Ricinus communis* Linn. (Euphorbiaceae), is a famous plant for well-known purgative 'Castor oil', which is derived

from the seeds. Leaves are anodyne and galactagogue, externally applied to boils and sores in the form of poultice [24]. The rhizomes of *Curcuma amada* Linn. Roxb. (Zingiberaceae), are useful as carminative and stomachic also used on contusions and sprains. The fruits of *Piper nigrum* Linn. (Piperaceae) has been given as a stimulant following the weakness due to fever. Externally valued as a rubefacient and as a local application for relieving sore throat, piles, and cutaneous troubles. Also recommended as a remedy for prevention of periodic fever in malaria [20].

In recent years the mainstream in pharmaceutical research is deviating from single molecule or single target approach to combinations and multiple target approaches [25]. Moreover the standardization of herbal formulations has remained a key issue to be addressed to the consumers in the growing herbal market. The present study is an attempt to provide supplementary scientific information on selected traditional Ayurvedic botanicals, which may strengthen the process of standardization and scientific validation of herbal formulations containing the plants under study. Moreover it may also provide insights for future study and development of Ayurvedic preparations in modern scientific perspective.

2. Materials and Methods

The leaves of *Barleria prionitis*, *Calotropis gigantea*, and *Ricinus communis* were collected in the month of July 2006 from the nearby field of Nanded city (MS), the collected plants were identified and authenticated by RNG, Department of Botany, School of Life Sciences and the voucher specimens were deposited in the herbarium of the School of Life Sciences of the host institute. The collected samples were dried at room temperature and were made into fine powder for extraction process. The powder form of seeds of *Trigonella foenum-graecum*,

fruits of *Piper nigrum* and rhizomes of *Curcuma amada* were purchased from Yogesh Pharmacy Ltd., A-6/6 M. I. D. C. Nanded (MS), India. DPPH and β -glucuronidase (EC 3.2.1.31, 25,000 units, source: E. Coli), were obtained from Sigma-Aldrich Co. (St. Louis MO, USA), p-nitrophenyl- β -D-glucopyranosiduric acid was purchased from CALBIOCHEM (EMD Biosciences Inc. La Jolla CA), trypsin was obtained from SISCO Research Lab. Ltd. Mumbai. L-DOPA (3,4 dihydroxy phenyl L-alanine) and glutathione (reduced form) were purchased from s. d. Fine Chemicals Ltd. Mumbai. Blood sample (containing 2 mg/ml EDTA) was collected from the local slaughterhouse at Nanded City (MS) for the preparation of RBC membrane solution. Apples (for extraction of PPO) were purchased from the local market at Nanded city. All other reagents and solvents used were obtained from commercial sources and were of analytical grade.

2.1 Preparation of 50% ethanol extract of selected medicinal plants

The 50% ethanol extract of selected plants was prepared by extracting approximately 10 g of powdered samples in 50% (v/v) ethanol using Soxhlets extraction apparatus. The extracts were air dried and maintained at 4°C for further studies.

2.2 Determination of DPPH radical scavenging activity

The DPPH radical scavenging assay was carried out according to a method described elsewhere [26]. The reaction mixture contained different concentrations of individual plant extract (in absolute ethanol) and DPPH radical (1 mM in absolute ethanol) solution. The contents of the reaction mixture were observed spectrophotometrically at 517 nm after 20 min. The O. D. of the control was considered as a 100 % unreduced DPPH and IC_{50} values were

Table 1. Effect of ethanol extract of selected medicinal plants on scavenging of DPPH and OH radicals, reducing ability (RA) and inhibition of dien conjugates (OOH).

Sr. No.	Name of the plant	DPPH	RA	OH	OOH
		IC ₅₀ (mg/ml)			
1	<i>Smilax china</i>	0.85*	2.2	0.87*	0.654
2.	<i>Barleria prionitis</i>	1.1	2.2	0.91*	0.089*
3.	<i>Calotropis gigantea</i>	1.7	1.9	1.1	0.530
4.	<i>Trigonella foenum-graecum</i>	0.98*	1.7*	0.79*	0.378*
5.	<i>Ricinus communis</i>	0.95*	1.6*	NR	0.734
6.	<i>Curcuma amada</i>	1.025	1.7*	0.88*	0.279*
7.	<i>Piper nigrum</i>	0.978*	1.8	NR	0.756
8.	Glutathion	0.48*	ND	ND	ND
9.	Ascorbic acid	ND	0.051*	ND	ND
10.	Coumarin	ND	ND	0.98*	0.035

The results presented are the mean values of duplicate measurements. * Indicates significant difference when the value is compared to the control ($P < 0.05$). NR- no reaction, ND-not determined.

determined as the concentration of the individual plant extract required to achieve 50% reduction of the DPPH radicals. Glutathione (1 mM, IC₅₀ = 0.48 mg/ml) was used as a standard compound.

2.3 Determination of reducing ability

The principle of the method used for the determination of reducing ability is based on the ability of antioxidants to reduce Fe³⁺ of K₃Fe(CN)₆ to Fe²⁺, the reducing ability of the plant samples was determined by the decrease in absorption of K₃Fe(CN)₆ at 420 nm [27]. The reaction mixture contained 500 µl solution of individual plant extract in 3 ml, 1 mM potassium ferricyanide solution and the absorbance was recorded at 420 nm after 10 min. The O. D. of control sample (without plant extract) was considered as 100% potassium ferricyanide, the IC₅₀ values were calculated as the amount of plant samples required to reduce 50% potassium ferricyanide. Ascorbic acid (1 mM, IC₅₀ = 0.051 mg/ml) was used as a reference compound.

2.4 OH radical scavenging activity

As per the method described by Christos & Dimitra [28], the Fe³⁺/ascorbic acid system was utilized for generation of hydroxyl radicals. The formaldehyde produced from the oxidation of dimethyl sulfoxide (DMSO) was measured for the detection of OH radicals generated in the Fe³⁺/ascorbic acid system. The reaction mixture contained 0.1 mM EDTA, 167 µM Fe³⁺, 33 mM DMSO in phosphate buffer of 50 mM pH 7.4. 0.1 ml different concentrations of individual plant extract and 150 µl of ascorbic acid (10 mM in phosphate buffer) were added finally to start the reaction. Trichloroacetic acid (17%, w/v) was used to terminate the reaction after 30 min. The formaldehyde produced was detected spectrophotometrically at 412 nm. The IC₅₀ values were determined as the concentration of the individual plant sample required to achieve 50% of formaldehyde production as compared to control (without sample). Coumarin (1 mM, IC₅₀ = 0.98 mg/ml) was used as a reference drug for comparative study.

Table 2. Profile of Vit. C and total phenolic contents of ethanol extract of selected plants.

Sr. No.	Name of the plant	Vit. C (mg/100 g)	Phenolics (mg/g)
1	<i>Smilax china</i>	17.37	180.0
2.	<i>Barleria prionitis</i>	30.40	48.35
3.	<i>Calotropis gigantea</i>	26.06	122.5
4.	<i>Trigonella foenum-graecum</i>	18.47	31.8
5.	<i>Ricinus communis</i>	35.16	34.87
6.	<i>Curcuma amada</i>	21.72	17.4
7.	<i>Piper nigrum</i>	13.03	18.0

The results summarized are the mean values of n=2.

Table 3. Inhibitory effect of ethanol extract of selected plants on the activity of PPO, β -glucuronidase and trypsin induced hydrolysis of BSA.

Sr. No.	Name of the plant	b-glucuronidase	Trypsin	PPO
		IC ₅₀ (mg/ml)		
1.	<i>Smilax china</i>	0.084*	0.275*	1.6
2.	<i>Barleria prionitis</i>	0.038*	0.650	1.8
3.	<i>Calotropis gigantea</i>	NR	0.600	1.4*
4.	<i>Trigonella foenum-graecum</i>	0.051*	0.310	1.38*
5.	<i>Ricinus communis</i>	0.043*	0.150	1.2*
6.	<i>Curcuma amada</i>	0.049*	0.058*	1.58
7.	<i>Piper nigrum</i>	NR	0.090*	1.5
8.	Salicylic acid	0.058*	0.056*	ND
9.	L-cysteine	ND	ND	0.95*

The results summarized are the mean values of duplicate measurements. * Indicates significant difference when the value is compared to the control ($P < 0.05$). NR- no reaction, ND-not determined.

2.5 Conjugated dien hydroperoxides assay

The blood sample was collected with addition of EDTA (2 mg/ml) as an anticoagulant. RBC Membrane solution was prepared as per the method described elsewhere [29] with slight modification in buffer [30]. Membrane solution (1.0 ml) was mixed with 5 ml of chloroform: methanol (2:1) followed by centrifugation at 1000 g for 15 min for separation of the two phases. The chloroform layer was taken in a test tube and dried at 45°C in water bath. The

left over lipid residue was dissolved in 1.5 ml of cyclohexane and the hydroperoxides generated were detected at 233 nm against a cyclohexane blank. Coumarin (1 mM, IC₅₀ = 0.035 mg/ml) was used as a standard drug.

2.6 Estimation of Vit. C content

Oxalic acid (4%, w/v) was used for extracting the plant samples. The method described by Sadasivam & Manickam [31], was followed for estimation of Vit. C (L-ascorbic acid) content in the selected plant samples. In this method L-

ascorbic acid reduces the 2,6-dichlorophenol indophenol (a blue colored dye) to a colorless leuco-base. The ascorbic acid gets oxidized to dehydroascorbic acid. The dye is pink colored in acid medium. Oxalic acid was used as the titrating medium. The Vit.C content of selected plant extracts was expressed as mg/100 g of individual plant sample.

2.7 Estimation of total polyphenols

A method described by Bray & Thorpe [32], was used for estimation of total polyphenols from the ethanol extracts of the selected medicinal plants. The method is based on the principle that, phenols react with an oxidizing agent phosphomolybdate an ingredient of Folin-Ciocalteu reagent, resulting in the formation of a blue colored complex having λ_{\max} at 660 nm. The standard curve was constructed using serial dilutions of catechol (500 $\mu\text{g/ml}$). The total amount of polyphenol was expressed as mg/g of samples.

2.8 Polyphenol oxidase (PPO) inhibition assay

A crude preparation of PPO from apple was used to understand the effect of individual plant extract on PPO activity. An experimental protocol described by Gacche *et al.* [33] was used for the extraction and preparation of semipure PPO solution. The reaction mixture contained L-DOPA (1 ml, 2 mM), 0.5 ml enzyme, 1 ml individual concentration of plant extract and citrate buffer (0.5 ml, pH 4.8, 0.1 M). After 5 min the contents of the reaction mixture were observed spectrophotometrically at 470 nm [34]. The O. D. of the control was considered as 100% activity of PPO and the concentration of plant samples required to achieve 50% PPO activity were calculated as IC_{50} values. L-cysteine (1 mM, $\text{IC}_{50} = 0.95 \text{ mg/ml}$) was used as a standard PPO inhibitor.

2.9 Studies on inhibition of β -glucuronidase

A method described by Demetrios, *et al.* [35], was carried out to study the effect of individual

plant extract on activity of β -glucuronidase. Different concentrations of plant extract (0.1 ml) in 0.1 M acetate buffer pH 7.4 for 5 min at 37°C were preincubated with 0.8 ml of 2.5 mM p-nitrophenyl- β -D-glucopyranosiduronic acid. After this 0.1 ml of β -glucuronidase was added. The mixture was incubated for 30 min and later the reaction was terminated by addition of 2 ml of 0.5 N NaOH. The contents were observed spectrophotometrically at 410 nm. The reaction rate of β -glucuronidase in the absence of inhibitor was considered as 100% activity and the amount of plant samples required to show 50% activity was then calculated as IC_{50} values. For comparative studies salicylic acid (1 mM, $\text{IC}_{50} = 0.058 \text{ mg/ml}$) was used as a standard compound.

2.10 Inhibition of trypsin induced hydrolysis of bovine serum albumin

A method described by Tandon *et al.* [36] was utilized to understand the anti-proteolytic activity of ethanol extract of selected medicinal plants. The method is based on the measurement of inhibition of trypsin induced hydrolysis of BSA. Trypsin (0.075 mg/ml) was initially incubated with individual concentrations of plant extract of 0.1 ml for 20 min. The substrate, BSA (6 g/100 ml, in 0.1 M phosphate buffer, pH 7.6) was added after completion of 20 min. The reaction mixture was incubated for 25 min at 37°C. The reaction was terminated by the addition 3 ml of CCl_3COOH (5 %, w/v). The acid soluble fractions were obtained by centrifuging the contents at 5000 RPM for 15 min. The obtained samples were treated so as to observe them spectrophotometrically at 660 nm [37]. The trypsinization activity in the absence of inhibitor was considered as 100%, and accordingly the concentration of each test sample giving 50% inhibition (IC_{50}) was then calculated. Salicylic acid ($\text{IC}_{50} = 0.056 \text{ mg/ml}$) was used as a reference drug.

3. Results and discussions

Results summarized in Table 1 indicate that the extract of the plant samples under study were reactive towards the scavenging of DPPH radicals. Amongst the tested plants the extract of *Smilax china* ($IC_{50} = 0.85$ mg/ml) was found to be the most effective DPPH reducing agent. Other samples showed the activity in an IC_{50} range of 0.95-1.7 mg/ml. Considerable reduction of Fe^{3+} of $K_3Fe(CN)_6$ by selected plant samples was observed, the extract of *Ricinus communis* ($IC_{50} = 1.6$ mg/ml) can be graded as most potential reducing agent as compared to all other samples. The DPPH and Fe^{3+} of $K_3Fe(CN)_6$ reduction assays were performed to understand the reducing ability of the test plant samples. There exist a positive correlation between the reducing ability and antioxidant/anti-inflammatory activity, moreover in general, highly reducing compounds can be considered for designing possible inhibitors of cyclooxygenase: a major enzyme implicated in recruiting the process of inflammation [35].

The results summarized in Table 1 indicate that, except the extract of *Ricinus communis* and *Piper nigrum*, all other plants showed significant OH radical scavenging activity. *Trigonella foenum-graecum* ($IC_{50} = 0.79$ mg/ml) followed by *Smilax china* ($IC_{50} = 0.87$ mg/ml) and *Curcuma amada* ($IC_{50} = 0.88$ mg/ml) were found to be effective towards interaction with OH radicals. Hydroxyl radicals have been reported to interact with various biomolecules found in the living cells and may alter the normal physiological functions of the cells [38]. Moreover the OH radicals are also implicated in the membrane damage of the cells in the inflamed region [39]. The DPPH, OH radical scavenging activity and the reducing ability can be attributed with the presence of redox active substances such as ascorbic acid, glutathione, flavonoids, tocopherols, carotenoids, hydroxycinnamic

acids, coumarins etc. reported to occur in plant kingdom [40].

The results of the inhibition of dien conjugates (hydroperoxides) summarized in Table 1, shows that all the plant samples under study were found to be active towards inhibition of formation of hydroperoxides. The extract of *Barleria prionitis* ($IC_{50} = 0.089$ mg/ml) was found to be the most effective candidate for inhibition of hydroperoxides formation. All other plants showed the inhibitory potential in an IC_{50} range of 0.279-0.756 mg/ml. Generation of hydroperoxides or conjugated diens is an intermediate step in the process of lipid peroxidation. The process of lipid peroxidation is thought to play a central role in many inflammatory disorders. This process is proved to be initiated by reactive oxygen species which ultimately results in the loss of membrane integrity and consequently damages the cell [41]. The inhibition of hydroperoxide formation may be due to the presence of a wide range of antioxidant phytochemicals such as flavones, coumarins, chalcones, cinnamic acids, etc. which may exert their effect by reducing hydroperoxides [17].

The results of the total phenolic and ascorbic acid (Vit. C) contents in the selected plants are shown in Table 2. The extract of *Smilax china* (180 mg/100 g) followed by *Calotropis gigantean* (122.5 mg/100 g) were found to contain highest concentration of total polyphenol, whereas the extract of *Ricinus communis* (35.16 mg/g) was found to contain highest concentration of ascorbic acid. All other plants under study were found to possess significant amount of total polyphenols and ascorbic acid.

Plethora of clinical and epidemiological studies supports the therapeutic and antioxidant reputation of Vit. C. Apart from its major physiological role, it acts as the first line of

defense during oxidative stress. It is also important for maintaining the levels of antioxidant Vit. E by reducing the Vit E radical (the oxidized form of Vit. E). Thus apart from radical scavenging activity of Vit. C, it also acts synergistically [42]. Moreover Vit. C has been reported to scavenge different types of free radicals such as reactive oxygen species like superoxide anion, singlet oxygen, H_2O_2 and acts as a chain-breaking antioxidant [43]. Vast body of literature has been focused in favor of plant polyphenols describing the presence of antioxidant and free radical scavenging activity which arise due to their suitable structural peculiarities, high reactivity as hydrogen donors and metal chelation abilities [44-46].

Polyphenol oxidase (EC 1.14.18.1), is a copper containing metalloenzyme ubiquitously present in plant kingdom. In present studies PPO has been studied as a representative of metal containing model-oxidizing enzyme. The activity profile of PPO in the presence of different plant samples has been summarized in Table 3. The results obtained shows that all the test samples under study were found to inhibit the activity of PPO. The overall IC_{50} range of PPO inhibition was in between 1.2-1.8 mg/ml. whereas the extract of *Ricinus communis* (IC_{50} = 1.2 mg/ml) showed maximum PPO inhibitory activity. PPO catalyses the oxidation of variety of phenolic compounds to corresponding quinones which are highly reactive [47]. It has been reported that transition metal mediated generation of free radicals are implicated in the variety of human diseases such as rheumatoid arthritis, cancer, iron overload etc. [38]. In general physiological notion, a system involving transition metal ions and molecular oxygen may become sensitive for generation of free radicals and address deviations in normal physiological functions. Several plant polyphenolic compounds have been found to act as metal chelators [48], chelation of copper

(present in the active center of PPO) by specific polyphenols may be the probable cause for inactivation of PPO activity.

The results of the β -glucuronidase inhibition assay are summarized in Table 3. The extract of *Calotropis gigantea* and *Piper nigrum* showed no activity against β -glucuronidase. Amongst the tested plants the extract of *Barleria prionitis* (IC_{50} = 0.038 mg/ml) was observed to be the most effective agent for inhibition of β -glucuronidase. All other plant samples showed inhibitory activity in an IC_{50} range of 0.043-0.084 mg/ml. The enzyme β -glucuronidase is present in the lysosomes of the polymorphonuclear neutrophils and has been described as one of the contributory factor for inducing the process of inflammation [49,50].

Trypsinization of bovine serum albumin was studied as a model proteolytic system for investigating anti-proteolytic activities of the plants under study. The results tabulated in Table 3, shows that the extract of *Curcuma amada* (IC_{50} = 0.058 mg/ml) was graded as the most effective anti-proteolytic sample as compared to all other samples which showed activity in an IC_{50} range of 0.09-0.65 mg/ml. Proteolytic enzymes especially serine proteases (SP) have been described as mediators of inflammation, moreover serine protease inhibition (trypsin is a member of SP) has been considered as one of the target for designing anti-inflammatory agents [51]. Plant polyphenols especially flavonoids have been reported to inhibit several enzymes mediating inflammation. A progressive inactivation of proteolytic enzymes by flavonoids has been investigated in neutrophils [52]. The inhibition of β -glucuronidase and trypsin might be due to interaction of these enzymes with specific flavonoids, as this group of phytochemicals is recognized as anti-inflammatory in action [53].

4. Conclusion

In recent years there is renewed interest in the medicinal plant research all over the world. Plant based drugs contribute major share and forms the basis of complementary alternative and traditional medicine system. Moreover natural product drug discovery remains a significant hope in the mainstream of pharmaceutical research. The results of the present study may add to the process of

standardization of plant drugs, which happens to be a key issue to be addressed in the currently growing herbal market.

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