Journal of Current Pharma Research
(An Official Publication of Human Journals)
An International Peer Reviewed Journal For Pharmacy, Medical & Biological Science
DOI: 10.25166 CODEN: JCPRD6 NLM ID: 101744065
ISSN: Print: 2230-7834 Online: 2230-7842
SJIF Impact Factor: 6.913

Human Journals
Research Article
September 2021 Vol.:12, Issue:4

Evaluation of *In-Vitro* Antioxidant Activity of *Leea macrophylla* Standardized Extracts

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Submitted: 20 August 2021
Accepted: 25 August 2021
Published: 30 September 2021

Keywords: *Leea macrophylla*; Antioxidant

ABSTRACT

The present study was designed to investigate the antioxidant properties of standardized aqueous and methanolic extract of *Leea macrophylla* using *in-vitro* models. The results indicate antioxidant potentials of the extracts, hence plant *L. macrophylla* could serve as an effective free radical inhibitor or scavenger which may be a good candidate for pharmaceutical plant-based products. However, further exploration is necessary for effective use in both modern and traditional systems of medicines.

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INTRODUCTION:

Leea macrophylla (Roxb.), belonging to the Leeaceae family, is a herb or herbaceous shrub with a very big size leaf like an elephant-ear. Ethnobotanical survey of this plant shows some important therapeutic uses in cancer, dysentery, body-ache, and sexual disability [1]. It has some other traditional uses for tonsillitis, tetanus, nephrolithiasis, rheumatism, arthritis, snake bites, soreness, pain, and blood effusion [2,3]. Recently the plant has been studied for its seed, leaf, and roots. These are extensively used by ayurvedic physicians in the preparation of seasonal tonic modaka preparation [4]. Leaf juice is recognized as a local anti-inflammatory agent and used in boils, arthritis, gout, and rheumatism [4,5].

Therefore, in this study, we evaluated the comparative antioxidant activity of *L. macrophylla* extracts from different solvents and made a probable correlation between phenolic contents and antioxidant activity.

MATERIALS AND METHODS:

Preparation of *Leea macrophylla* Extracts: The plant species *L. macrophylla* was collected from Kalgaon village Taluka. Patan, District- Satara and authenticated at Botanical department of Yashwantrao Chavan college of science, Karad. The plant was dried under sunlight and fine powder of the plant was prepared by using a hand grinder.

Preparation of Aqueous Extract [LMAE]: powder was mixed with 30ml distilled water boiled for 30 minutes in a round bottom flask attach with a reflux condenser. The material was filtered with Whatman filter paper no 40 and filtrate was collected.

Preparation of Alcoholic Extract [LMEE]: powder was mixed with 30 ml alcohol and 10 ml distilled water boiled for 30 minutes in a round bottom flask attach with a reflux condenser. The material was filtered Whatman filter paper no 40 and filtrate was collected. The filtrate was collected in a porcelain dish. Alcohol was evaporated and then 4ml distilled water was added.

Chemicals

All the chemicals used in the study were of extra pure analytical grade.

Evaluation of the antidiabetic activity of *L. macrophylla* extracts using various in vitro methods

1 Quantitative DPPH radical-scavenging assay

Scavenging activity on DPPH free radicals by the extract was assessed according to the method reported with slight modifications Awah et al [6]. Briefly, a 2.0 ml solution of the extract at different concentrations diluted two-fold in methanol was mixed with 1.0 ml of 0.3 mM DPPH. in methanol. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 25 min. Blank solutions were prepared with each test sample solution (2.0 ml) and 1.0 ml of methanol while the negative control was 1.0 ml of 0.3 mM DPPH solution plus 2.0 ml of methanol. L-ascorbic acid was used as the positive control.

Thereafter, the absorbance of the assay mixture was measured at 518 nm against each blank with a UV-visible spectrophotometer.

2. *In vitro* nitric oxide radical (NO.) scavenging assay [7]

Nitric oxide generated from sodium nitroprusside (SNP) was measured according to the method briefly, the reaction mixture (5.0 ml) containing SNP (5 mM) in phosphate-buffered saline (pH 7.3), with or without the plant extract at different concentrations, was incubated at 25˚C for 180 min in front of a visible polychromatic light source (25W tungsten lamp). The NO radical thus generated interacted with oxygen to produce the nitrite ion (NO.) which was assayed at 30 min intervals by mixing 1.0 ml of incubation mixture with an equal amount of Griess reagent (1% sulfanilamide in 5% phosphoric acid and 0.1% naphthyl ethylene diamine dihydrochloride). The absorbance of the chromophore (purple azo dye) formed during the diazotization of nitrite ions with sulphanilamide and subsequent coupling with naphthyl ethylenediamine dihydrochloride was measured at 546 nm. The nitrite generated in the presence or absence of the plant extract was estimated using a standard curve based on sodium nitrite solutions of known concentrations. Each experiment was carried out at least three times and the data was presented as an average of three independent determinations.

Statistical analysis- All the determinations were carried out in triplicates and data were analyzed by ANOVA followed by student's T-test. Values were considered at $P<0.05$.

RESULTS

Effect of *L. macrophylla* extracts on *in vitro* DPPH assay

Scavenging the stable DPPH radical model is a widely used method to effectively evaluate antioxidant activities. The DPPH test measures the hydrogen atom or electron-donating capacity of the *L. macrophylla* extract to the stable radical DPPH formed in the solution. The ability of the *L. macrophylla* extracts to scavenge DPPH radicals was determined quantitatively. The effect of the *L. macrophylla* plant extracts on the DPPH assay is summarized in Table 1. The addition of the extract to the DPPH solution caused a rapid decrease in the optical density at 517 nm indicating the good scavenging capacity of the extract. The extract showed substantial antioxidant activity in a dose-dependent manner similar to that of ascorbic acid which was used as a control standard antioxidant. Almost complete inhibition of the DPPH radical activity was observed when 1000 µg/ml of the extract was used.

The antioxidant compounds in the *L. macrophylla* extract and the standard neutralized the free radical character of DPPH by transferring either electrons or hydrogen atoms to DPPH, thereby changing the colour from purple to the yellow colored stable diamagnetic molecule diphenyl picryl hydrazine. The degree of discoloration indicated the scavenging potential of the extracts in terms of hydrogen donating ability.

Effect of *L. macrophylla* extracts on *in vitro* nitric oxide radical scavenging assay

Nitric oxide (NO) released from SNP has a strong NO + character which can alter the structure and function of many cellular components. The *L. macrophylla* extracts exhibited good NO scavenging activity leading to the reduction of the nitrite concentration in the assay medium. The detailed effects of *L. macrophylla* are summarized in table 2. The NO scavenging capacity was concentration-dependent with 1000 µg/ml scavenging most efficiently. The *L. macrophylla* in SNP solution significantly inhibited ($p < 0.05$) the accumulation of nitrite, a stable oxidation product of NO liberated from SNP in the reaction medium with time compared to the standard. The toxicity of NO increases when it reacts with superoxide to form the peroxynitrite anion, which is a potential strong oxidant that can decompose to produce OH and NO2. The present study shows that an *L. macrophylla* has a potent nitric oxide scavenging activity.

DISCUSSION

Antioxidants are tremendously important substances that possess the ability to protect the body from damage caused by free radical-induced oxidative stress. The antioxidant potential of *L. macrophylla* extracts was investigated in the search for new bioactive compounds from natural resources. It became clear that *L. macrophylla* extracts present the highest antioxidant activity compared with reference antioxidant Vitamin C for DPPH scavenging activity. Polyphenols were found in both extracts. The obtained results for DPPH are in agreement with the phenol contents determined for each sample as indicated in table 3. Plant polyphenols act as reducing agents and antioxidants by the hydrogen-donating property of their hydroxyl groups [8]. Hence, we could conclude that these polyphenols are responsible for the observed antioxidant activity in this study.

Nitric oxide reacts with free radicals, thereby producing the highly damaging peroxynitrite. Nitric oxide injury takes place for the most part through the peroxynitrite route because peroxynitrite can directly oxidize LDLs, resulting in irreversible damage to the cell membrane [9–10]. Hence, *L. macrophylla* extract could be used to overcome various health problems caused by nitric oxide injury. Concerning the nitric oxide scavenging profile of *L. macrophylla* it showed a suitable potential for the nitric oxide scavenging effect assay.

CONCLUSION

These findings show that the *L. macrophylla* extracts possess antioxidant activity. The assay revealed that leaf extract had the highest antioxidant activity comparable with Vitamin C. The *L. macrophylla* extracts are a promising candidate for use as a natural product-based antioxidant for the health of the human being.

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Table 1: Antioxidant activity of ethanol and aqueous extract of leaves, stem and root of *Barleria gibsoni* by DPPH method

Plant extracts and standard	Concentrations (µg/ml)	% Scavenging (Mean ± SEM) of triplicates
Ethanolic [LMEE]	200	42.89 ± 0.13
	400	56.66 ± 0.41
	600	64.76 ± 1.03
	800	71.28 ± 1.10
	1000	75.79 ± 1.10
Aqueous [LMAE]	200	31.65 ± 0.91
	400	42.34± 0.95
	600	43.25 ± 0.94
	800	51.15 ± 0.96
	1000	56.34 ± 0.98
Ascorbic acid	200	38.03 ± 0.99
	400	51.28 ± 0.91
	600	53.24 ± 0.99
	800	58.32 ± 1.02
	1000	61.33 ± 0.98

Table 2: Antioxidant activity of ethanol and aqueous extract of leaves, stem and root of *Barleria gibsoni* by Nitric oxide scavenging method

Plant extracts and standard	Concentrations (µg/ml)	% Scavenging (Mean ± SEM) of triplicates
Ethanolic [LMEE]	200	38.92 ± 0.91
	400	46.23 ± 0.92
	600	52.76 ± 0.94
	800	56.19 ± 0.94
	1000	60.19 ± 0.91
Aqueous [LMAE]	200	36.25 ± 0.89
	400	49.28 ± 0.94
	600	56.25 ± 0.91
	800	61.29 ± 0.93
	1000	65.29 ± 0.91
Ascorbic acid	200	32.06 ± 0.99
	400	49.24 ± 0.91
	600	58.26 ± 0.99
	800	64.36 ± 1.02
	1000	65.35 ± 0.98

Table 3: Preliminary phytochemical screening of extracts

Test	Extracts	
	Aqueous [LMAE]	Ethanolic [LMEE]
Flavonoids	++	++
Steroids	++	++
Terpenoids	++	++
Alkaloids	--	--
Tannins	--	--
Glycosides	-	-
Saponins	+	--

Interpretation of results: (−) absent; (+) low; (++) good