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PHYTOCHEMICAL INVESTIGATION AND EVALUATION OF ANTIOXIDANT, AND ANTI-INFLAMMATORY ACTIVITY OF AERIAL PARTS OF *Lindernia antipoda*

A.Y. Sahare[1, ✉], B.K. Chavhan[2], P. S. Dhoke[1] and T.R. Agrawal[3]

[1]Department of Pharmaceutical Chemistry, Bajiraoji Karanjekar College of Pharmacy, Sakoli-441802, Maharashtra, India

[2]Department of Pharmaceutical Sciences, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur-440033, Maharashtra, India

[3]Department of Pharmaceutical Chemistry, Chhatrapati Shivaji College of Pharmacy, Deori-441901, Maharashtra, India

✉Corresponding Author: atish.sahare@gmail.com

ABSTRACT

Plant species always remain a backbone for the discovery of active biomolecules required for public health. The existing scrutiny is outlined to dissect the chemical nature and explore the antioxidant and anti-inflammatory strength of methanol extract of aerial parts of *Lindernia antipoda* by *in vitro* methods. The basic phytochemical evaluation of the extract reflected the existence of essential nitrogenous and phenolic bio-compounds. UV-visible and FTIR (Fourier Transform-Infrared Spectroscopy) spectral analysis supported the chemical nature of reflected compounds. The antioxidant capability was noticed through nitric oxide scavenging assay and ABTS [2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] radical cation scavenging assay while anti-inflammatory competence was examined through the stabilization approach of human erythrocytes membrane against heat-induced membrane lysis. The methanol extract showed nitric oxide scavenging and ABTS radical cation scavenging by IC50 values of 398.38 μg/mL and 513.30 μg/mL respectively while anti-inflammatory activity was observed with the IC50 value of 573.01 μg/mL. The outcomes of each activity of methanol extract are presumably linked to the essential bioactive chemical substances in the extract.

Keywords: *Lindernia antipoda*, Phytochemicals, Nitric Oxide, ABTS, Membrane Stabilization.

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INTRODUCTION

Globally observed oxidative stress fuels the genesis of diverse chronic diseases in the world's population. Enormous oxidative stress may bring out the augmentation of inflammatory cytokines and other pro-inflammatory mediators to offer systemic and local inflammation.[1] In the world, a plant-based medicinal system always remains preferential because of its less or no side effects, modest cost, and multisided potential. The different experimental research justifies the therapeutic efficacy of medicinal plants in inflammatory conditions and oxidative stress in correlation with the phytochemical contents.[2] Secondary plant metabolites become a key subject of research in the world of phytopharmaceuticals due to their health benefits.[3] The crucial role of these compounds in inflammatory conditions is evident by their potential to reduce the prostaglandin level by means of inhibition of prostaglandin-endoperoxide synthase and lipoxygenase. The anti-inflammatory activity of the medicinal plant is always supplemented with its antioxidant competence to avert cellular harm through the removal of reactive oxygen and nitrogen species.[4] *Lindernia antipoda* belongs to the family Linderniaceae and is primitive plant species abundantly found in wet grassland but still unnoticed by researchers. Habitually, the plant is practiced as a remedy to treat rheumatism, limb numbness, prostatic hyperplasia, muscle pain, and hematologic disorders.[5] Scientifically the plant was tested for antioxidant activity by the method of DPPH radical quenching assay and reducing power assay.[6] With the consideration of the medicinal importance of *Lindernia antipoda*, the existing scrutiny was rendered to check out the phytochemical nature as well as the antioxidant and anti-inflammatory potentiality of aerial parts of the plant by *in vitro* methods.

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EXPERIMENTAL

Chemicals

ABTS [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)], ascorbic acid, aspirin (Sigma- Aldrich), Potassium persulfate, sodium nitroprusside (Merck), Napthyletylenediamine dihydrochloride (Oxford Laboratories) and remaining chemicals along with solvents (Loba Chemie) were of typical quality category.

Plant Collection and Extraction

Plants of *Lindernia antipoda* were collected from the bank of Shivanibandh lake in the Bhandara district of Maharashtra, India. The taxonomic characterization of the collected species was validated by the Department of Botany, Manoharbhai Patel College, Sakoli, Maharashtra, India. In this study, extraction was done by using the Soxhlet apparatus. The dried powdered material of aerial parts of *Lindernia antipoda* was defatted with petroleum ether for 8 hours, dried, and continued extraction with methanol (99%) in the same extractor for 28 hours. The extract was made solvent-free by the distillation process and concentrated in an electric water bath at a temperature below 60ºC.[7]

Phytochemical Investigation

Different preliminary tests were conducted as per standard procedures in order to identify the presence of different bioactive phytochemicals in the methanol extract of *Lindernia antipoda*.[8,9] The chemical nature of identified compounds get confirmed by spectral analysis of extract by using Shimadzu 1800 UV-visible double beam spectrophotometer and Jasco FT/IR-4000 Type A model.[10]

Antioxidant Activity by Nitric Oxide Free Radical Scavenging Assay

Exactly 2 mL of sodium nitroprusside (10 mM) in phosphate buffer of pH 7.4 was annexed with half mL of dissimilar concentrations of extract (100-500 ug/mL) and left undisturbed at the temperature of 25°C for 3 hours. Later on, 0.5 mL of solution was treated with sulfanilic acid reagent (1 mL) and stood for 5 min. At last, a 1mL quantity of naphthyl-ethylenediamine (0.1% v/v) was appended and left aside for 30 minutes. Ascorbic acid was employed as standard and absorbance were measured at 540 nm followed by calculations of % inhibition.[11]

Antioxidant Activity by ABTS Radical Cation Scavenging Assay

The alike volume of 20mM ABTS [2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] solution and 70mM potassium persulphate were commingled to provoke the formation of ABTS radical cations and incubated for 24 hours in unlighted condition. The 600 μl of test samples (100-500 μg/mL) were merged separately with 450 μl of ABTS working standard and thereafter absorbance was noted at 734 nm within a few minutes. Outcomes were cited against the samples of ascorbic acid.[12]

Anti-inflammatory Activity (Membrane Stabilization) by Heat-Induced Hemolysis

The methanol extract at different concentrations (100-500 ug/mL) was separately mixed with 1mL of freshly prepared human erythrocytes suspension and warmed in a thermostatic bath at 56 ºC for 30 min trailed by centrifugation (Centrifuge Remi-12C) at 2500 rpm. The upper clear layer from individual centrifuge tubes was detached carefully and absorbance was noted at 560 nm. Samples of aspirin were exercised to compare the results.[13]

RESULTS AND DISCUSSION

Phytochemical Investigation

The different qualitative tests employed for the preliminary analysis of phytochemicals in the extract suggested the existence of divergent bio-compounds including essential nitrogenous and phenolic compounds listed in Table-1. The UV-Visible spectral analysis of the extract (Fig.-1) showed the λ max at 266 nm, 306 nm, 361 nm, 474 nm, 498 nm, and 653 nm with the absorbance of 1.542, 0.341, 0.571, 0.288, 0.254, and 0.076 respectively. In accordance with the priorly published research data, the observation of absorption bands from 234 to 676 nm meant the existence of alkaloids and phenolic compounds. The absorption maxima enclosed within the band of 230 to 285 nm and 300 to 350 nm hinted at flavonoids. The occurrence of peaks in the range of 280 to 330 nm is a depiction of the compounds with phenolic nature while a range of 350–500 nm counseled the tannins. The peak at 653 was perceived as chlorophyll due to its occurrence within the limit of 600 to 700 nm. In furtherance to establish the different functional groups,

the FTIR spectrum (Fig.-2) was studied with respect to captured absorption peaks. The peaks at 3439, 2925, and 1725 cm^{-1} marked the existence of the OH functionality, CH_2 moiety, and C=O group respectively. The peak at 1637 cm^{-1} gave the representation of C-N bonding while the peak at 1461 cm-1 was probably due to the existence of N-H bonding. The peak at 1386 cm^{-1} was credited to the OH group of phenolic nature while the peak at 1272 cm-1 was associated with the vibration of the C–O bond of polyols, akin to hydroxy flavonoids. The peak at 1073 cm^{-1} conveyed the C-H relation, which is a highlighting mark for the aromatic ring whilst the peak at 1039 cm^{-1} was conceivably due to the C–O stretching. The peak at 583 cm^{-1} was due to the C-H in-plane deformation vibrations and the peak at 923 cm^{-1} was doubtful, probably related to the presence of polysaccharides.[14-17] Overall UV-visible and FTIR spectral analysis supported the consequences of preliminary testing for the presence of essential chemical substances.

Table-1: Preliminary Phytochemical Screening

Phytochemicals	Test	Result
Carbohydrates	Molisch's Test	+
	Benedict's Test	+
Proteins	Biuret Test	+
	Millon's Test	+
Alkaloids	Mayer's Test	+
	Wagner's Test	+
Cardiac Glycosides	Keller-Killiani Test	-
Phenolic Compounds and Tannins	Ferric Chloride test	+
	Potassium dichromate Test	+
	Lead acetate Test	+
Flavonoids	Shinoda test	+
	Alkaline reagent Test	+
Phytosterols and Steroids	Salkowski's Test	-
	Libermann-Burchard Test	-
Saponins	Foam Test	-

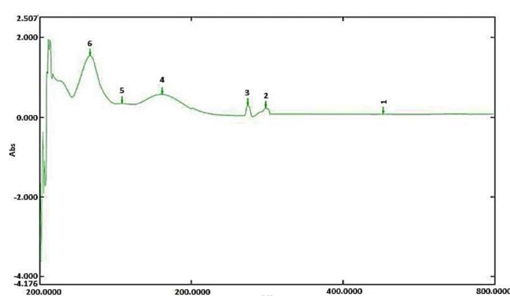

Fig.-1: UV-Visible Spectrum of Methanol Extract of *Lindernia antipoda*

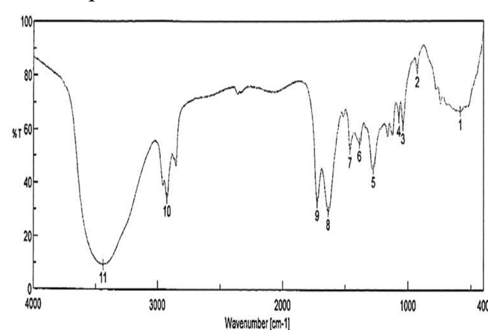

Fig.-2: FTIR Spectrum of Methanol Extract of *Lindernia antipoda*

Antioxidant Activity

The finding of the nitric oxide scavenging assay summarized in Table-2 indicates that methanol extract can prohibit the creation of nitrites in a concentration-dependent aspect and the highest % inhibition by the

extract was observed at 58.81 % with the concentration of 500 µg/mL. A regression graph (Fig.-3) was plotted and IC50 values of the tested extract and ascorbic acid were sequentially computed as 398.38 µg/mL and 136.94 µg/mL. In the body, nitric oxide radicals react with the oxygen to construct nitrite and peroxynitrite anions to induce oxidative damage and lead to a generation of different diseases including the inflammatory condition that can be interrupted with the use of antioxidants.[18] In the ABTS assay, extract, and standard (ascorbic acid) showed the advancement in % inhibition in a dose-dependent manner (Table-2) indicating their potential to scavenge $ABTS^+$ radical cation. The IC50 values determined by plotting the regression graph (Fig.-4) were established as 513.30 and 214.03 µg/mL for extract and standard (ascorbic acid) respectively. In this assay, the discoloration of the $ABTS^+$ is probably linked with the content of essential bioactive chemical substances in the extract. The $ABTS^+$ radical cation scavenging activity is an important mode to study the antioxidant efficacy of the test sample. ABTS on reaction with sodium persulfate assembles a blue-colored radical cation which gets reformed into colorless neutral ions on reaction with an antioxidant sample.[19]

Anti-inflammatory Activity

As the RBC membrane approximates the lysosomal membrane, the anti-inflammatory potential of methanol extract was recognized by the membrane stabilization effect. The tabulated outcomes (Table-2) point out 47.81% as the highest percentage inhibition of hemolysis at 500 µg/mL by the extract whereas that of the standard was 86.34% at 500 µg/mL. The IC50 values calculated from the regression graph (Fig.-5) were obtained as 573.01 µg/mL for the extract and 173.15 µg/mL for the standard. Different scientific data substantiate that lysosomal contents produce tissue damage and cause inflammation. The membrane stability effect of the extract indicates that it can safeguard the RBC and lysosomal membrane during the inflammatory condition. This repercussion is probably due to the coadjuvant interaction of essential phytoconstituents of the methanol extract and membrane components.[20]

Table-2: % inhibition in Antioxidant and Anti-inflammatory Activity by *in vitro* methods

Concentration (µg/ml)	Antioxidant activity by Nitric Oxide Scavenging Assay		Antioxidant activity by ABTS Assay		Anti-inflammatory activity by Heat-Induced Hemolysis	
	% Inhibition (Standard)	% Inhibition (Sample)	% Inhibition (Standard)	% Inhibition (Sample)	% Inhibition (Standard)	% Inhibition (Sample)
100	46.26	24.63	37.54	21.66	42.72	17.23
200	57.42	33.15	50.18	27.15	51.89	22.15
300	65.89	40.96	58.71	35.96	63.65	29.25
400	77.04	49.7	67.78	41.32	76.04	38.7
500	88.86	58.81	79.26	49.48	86.34	44.81

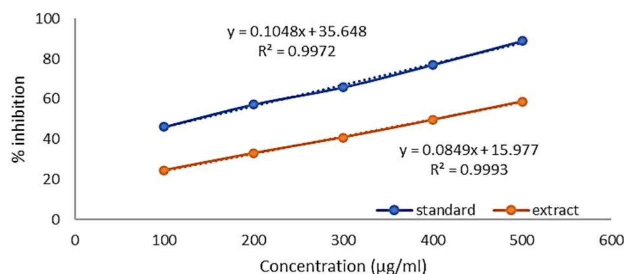

Fig.-3: Calibration curve for Nitric Oxide Free Radical Scavenging by using Standard and Extract

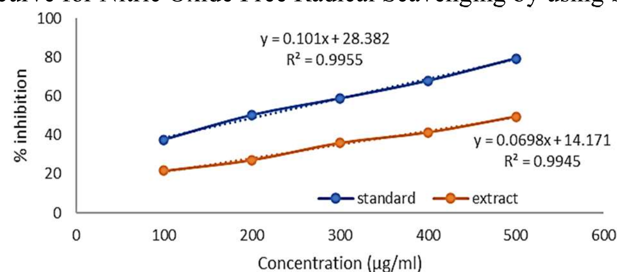

Fig.-4: Calibration curve for ABTS Assay by using Standard and Extract

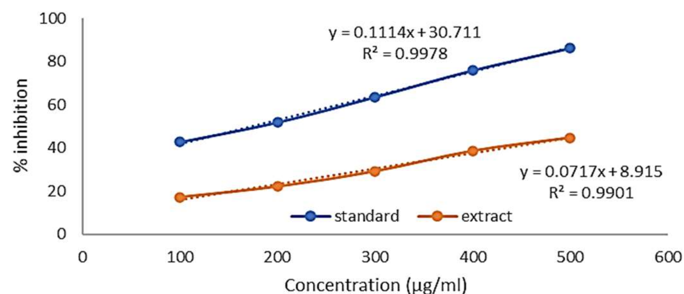

Fig.-5: Calibration curve for Heat-Induced Hemolysis Method by using Standard and Extract

CONCLUSION

The present study indicates the content of essential bioactive chemical substances may be reasonable for the antioxidant and anti-inflammatory potentiality of the tested extract. Furthermore, it is concluded that *Lindernia antipoda* is the potential origin of natural antioxidants, which might be useful to encounter the maturity of different diseases caused by oxidative stress.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

A.Y. Sahare http://orchid.org/0000-0002-4756-1265

B.K. Chavhan http://orchid.org/0000-0001-9287-7106

P.S. Dhoke http://orchid.org/0000-0001-7141-6036

T.R. Agrawal http://orchid.org/0000-0003-1186-9695

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