ORIGINAL ARTICLE

Phytochemical investigation, *in vitro* antioxidant, and *in vivo* antidepressant activity of ethanolic leaf extract *Antigonon leptopus*

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Abstract

Aim: The present investigation deals with the preliminary phytochemical screening, *in vitro* antioxidant, and *in vivo* antidepressant activity of ethanolic leaf extract of *Antigonon leptopus*. **Materials and Methods:** *In vitro* antioxidant activity was evaluated using the parameters such as free radical scavenging using phosphomolybdenum antioxidant assay, nitric oxide scavenging activity, and hydrogen peroxide scavenging. Ascorbic acid (AA) with the same concentration was used as an standard antioxidant. Extract was investigated further for its antidepressant activity using the forced swim test, tail suspension test, and locomotor activity using digital photoactometer. AA, fluoxetine (10 mg/kg), and imipramine (4 mg/kg, p.o) were used as reference drugs for comparison in the antioxidant and antidepressant experiments, respectively. **Results and Discussions:** Preliminary phytochemical screening of the extracts revealed the presence of alkaloids, carbohydrates, glycosides, phenolic compounds, tannins, saponins, and flavonoids. The presence of these bioactive constituents is associated with the antioxidant and antidepressant activity of the plant. The IC_{50} values of nitric oxide and hydrogen peroxide radicals were found to be 216 and 20.8 ug/ml for extract and 24.54 and 33.1 ug/ml for AA. It has been observed from our study that the extract showed significant ($P < 0.01$) reduction in immobility in tail suspension and forced swim model of depression comparable to imipramine. In locomotor activity testing, it showed psychostimulant effect comparable to that of standard fluoxetine. **Conclusion:** The results indicated that dose-dependent *in vitro* antioxidant activity against phosphomolybdenum antioxidant assay, nitric oxide scavenging activity, and hydrogen peroxide scavenging by ethanolic leaf extract of *A. leptopus* comparable with that of standard AA. The antioxidant and antidepressant effect of *A. leptopus* seems to be mediated due to the presence of various phytochemical constituents.

Key words: *Antigonon leptopus*, antioxidant, ascorbic acid antidepressant, forced swim test, free radicals, phytochemical screening, scavengers, tail suspension test

INTRODUCTION

During the past decade, considerable attention has been focused on the involvement of reactive oxygen species in various diseases. Generation of free radicals causes cumulative damage of DNA, proteins, and lipids led to oxidative stress. This oxidative stress has been suggested to be the cause of aging and various human diseases such as cancer, hepatic disorders, and diabetes. DNA damage mediated by free radicals may result in mutation or chromosomal aberrations leading to carcinogenesis.[1] The use of medicinal herb in the treatment and prevention of diseases is attracting attention by scientists worldwide.[7-8] Active oxygen species and free radicals play an important role in the initiation and evolution of numerous diseases. The use of compounds with antioxidant activity is expected to be useful for the treatment of these diseases. Therefore, there has been a growing interest in finding novel antioxidants to meet the requirements of pharmaceutical industries.[2] Scanty work has reported an antimicrobial activity of whole plant. *Antigonon leptopus* has been used for the treatment of the ulcers, depression, inflammation cancer,

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lithiasis, hepatotoxicity, and diabetes.[3-6] Thus, the study is aimed to demonstrate and determine the antioxidant and antidepressant effects of *A. leptopus* leaves on various free radical scavengers.

According to the World Health Organization report, mood disorders are the second leading cause worldwide of disability-adjusted life years and the leading cause of years lived with disability in all ages. Each drug used to treat this disorder has a success rate of about 60%. In addition, most therapies require several weeks of treatment before improvement of signs and symptoms is observed and there are numerous side effects caused by antidepressants.[9-11] Thus, the high prevalence of depression and the fact that a significant proportion of individuals do not respond well to any currently marketed antidepressants or treatments support the need for new therapeutics to treat depression. Numerous antidepressant compounds are now available, presumably acting through different mechanisms including serotonergic, noradrenergic, and/or dopaminergic systems.[3]. Medical plant therapies may be effective alternatives in the treatment of depression and has progressed significantly in the past decade.[4]

Therefore, the present work aimed to evaluate the antioxidant and antidepressant-like effect of the ethanolic leaf extract of *A. leptopus*.

MATERIALS AND METHODS

Collection of Plant Material

Collection of *A. leptopus* leaves was done from Peddapuram area of East Godavari district of Andhra Pradesh. The plant authentication was done by Dr. T. Raghuram Taxonomist, Maharani College, Peddapuram.

Extract Preparation

A. leptopus leaves were shade dried at room temperature for 4–5 days. The dried leaves were then powdered in a mixture and weighed. 100 g powder was taken from the obtained fine powder, and it is macerated in 200 ml of ethanol for 3 days. The hot percolation process is carried out for about 3 h. later on the filtration was done and distillation is performed to get concentrated product.

Phytochemical Analysis [Table 1]

The evaluation of powdered leaf was done for qualitative determination of major phytoconstituents, i.e., alkaloids, carbohydrates, glycosides, phenolic compounds, saponins, steroids, tannins, and flavonoids.[12]

Antidepressant Activity Using TST, FST, and Spontaneous Locomotor Activity [Tables 4 and 5].

Chemicals and Instruments

1. Drugs used: Acacia (1%), ascorbic acid (AA), ethanol (90%), fluoxetine, imipramine, and normal saline.
2. Instruments used: Digital photoactometer, pH meter, and ultraviolet spectrophotometer.

Experimental Animals

Either sex of albino rats (75–100 g) was used in the entire study. The animals were maintained under normal laboratory conditions such as humidity, temperature, and light. Standard laboratory diet and water were maintained for the animals.

In vitro Antioxidant Activity

Phosphomolybdenum antioxidant assay

The extracts of *A. leptopus* was evaluated by the phosphomolybdenum method for antioxidant activity according to the procedure.[13] The assay is based on the reduction of Mo (VI)–Mo (V) by the extract and subsequent formation of a green phosphate/Mo (V) complex at acid pH. Extracts of 0.3 ml (0.05, 0.1, 0.3, and 0.5 mg/ml) were combined with 3 ml of the mixture (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes containing the reaction solution were incubated at 95°C for 90 min. The absorbance of the solution was measured at 695 nm against blank using spectrophotometer.

Nitric oxide generation and assay of nitric oxide scavenging

Nitric oxide was generated from sodium nitroprusside and measured by the Greiss reaction as described previously. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions that can be estimated by the use of Greiss reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide.[14] Sodium nitroprusside (5 mM) in phosphate-buffered saline was mixed with different concentrations of the extracts (0.05, 0.1, 0.3, and 0.5 mg/ml) dissolved in the suitable solvent systems and incubated at 25°C for 150 min.

The samples were added with Greiss reagent (1% sulfanilamide, 2% H3PO4, and 0.1% naphthyl ethylenediamine dihydrochloride). The chromophore absorbance synthesized during the nitrite diazotization with sulfanilamide and subsequent reaction with naphthyl ethylenediamine was read at 546 nm, and the absorbance of standard solutions of AA was carried out in similar. All the tests were performed in triplicate. As a reference compound, AA was used. The percentage decrease in absorbance was calculated.

Nitric oxide scavenged (%)= $[(A_o - A_1)/ A_o] \times 100$

Where A_o is the absorbance of the control reaction (containing all reagents except the sample extract), and A_1 is the absorbance of the sample extract. AA was used as positive controls.

Scavenging of hydrogen peroxide

Hydrogen peroxide to scavenge the ability of the extracts was determined.[15] Hydrogen peroxide (40 mM) solution was prepared in phosphate buffer (pH 7.4). The concentration of hydrogen peroxide was determined by absorption at 230 nm using a spectrophotometer. Extracts (0.05, 0.1, 0.3, and 0.5 mg/ml) in distilled water were added to a hydrogen peroxide solution (0.6 ml and 40 mM). The absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The hydrogen peroxide scavenging percentage by the extracts and standard compounds was calculated as follows:

$$\% \text{ Scavenged } [H_2O_2] = [(A_o - A_1)/ A_o] \times 100$$

Where A_o is the absorbance of the control reaction (containing all reagents except the sample extract), and A_1 is the absorbance of the sample extract. AA was used as positive controls.

Antidepressant Activity

Experimental design for antidepressant activity

The rats were divided into five groups ($n = 3$). Drugs/vehicle was administered to the animals 60 min before the study.

Group I: Negative control, administer saline 2 ml/kg orally.

Group II: Receive ethanolic leaf extract *A. leptopus* 150 mg/kg orally.

Group III: Receive ethanolic leaf extract *A. leptopus* 300 mg/kg orally.

Group IV: Receive standard drug Imipramine (4 mg/kg orally).

Forced swim test (FST)

Rats of either sex were forced to swim in an container (diameter 10 cm and height 25 cm) containing 19 cm of water at 25 ± 1°C. 60 min before the study, all animals were forced to swim for 6 min and the immobility duration was observed and measured during the final 4 min interval of the test. Immobile means when the animal ceased struggling and remained in the water floating motionless, making only those movements to keep its head above water. A decrease in the duration of immobility is indicative of an antidepressant-like effect.[16]

Tail suspension test (TST)

The tail suspension method used in this study was similar to those described by Steru *et al*.[17] Treatment was given 60 min before the study as described by the study design. Mice were suspended on the edge of the table, 50 cm above the floor, with the help of adhesive tape placed approximately 1 cm from the tip of the tail. The total duration of immobility induced by tail suspension was recorded during a 6 min of the 10 min period. Animal was considered to be immobile when it did not show any movement of the body, hanged passively, and completely motionless.

Statistical Analysis

A statistical analysis was performed using one-way analysis of variance followed by Dunnett's multiple tests. Results are expressed as mean ± standard deviation for six rats in each group. Differences among groups were considered statistically significant at $P < 0.001$ level.

RESULTS

Phytochemical Analysis [Table 1]

Table 1: Results of phytochemical analysis of ethanolic leaf extract of *A. leptopus*

Compounds	Tests	Results
		Ethanolic Extract
Alkaloids	Dragendorff's	+ve
	Mayer's test	+ve
	Hager's test	+ve
Glycosides	General test	+ve
	Legal's test	+ve
	Modified Borntrager's test	+ve
Flavonoids	Lead acetate test	+ve
	Zinc hydrochloride test	+ve
	NaOH test	+ve
Saponins	Froth formation test	+ve
Triterpenoids	Salkowski test	+ve
	Liebermann–Burchard test	+ve
Tannins	Ferric chloride test	+ve
Carbohydrates	Molisch's test	+ve
	Benedict's test	+ve
	Fehling's test	+ve
Proteins	Xanthoproteic test	+ve
	Millon's test	+ve
	Biuret test	+ve

+ve: Indicates Presence. *A. leptopus: Antigonon leptopus*

In vitro Antioxidant Activity [Table 2 and 3]

Antidepressant Activity Using Tst, Fst, and Spontaneous Locomotor Activity

The antidepressant effect of *A. leptopus* (150 and 300 mg/kg) and imipramine was studied by observing the changes in the duration of immobility in the three models: FST, TST, and locomotory activity by digital photoactometer. In TST, *A. leptopus* 150 and 300 mg/kg p.o produced a significant reduction in immobility period 102 ± 2.3** and 115 ± 0.34**, respectively, when compared with that of control group animals receiving only vehicle of immobile period 148.5 ± 2.2. The standard drug imipramine exhibits immobility period 91 ± 2.4*. The results are statistically significant when compared with control with $P < 0.001$. In FST, *A. leptopus* 150 and 300 mg/kg p.o produced a significant reduction in immobility period of 45 ± 1.35* and 58 ± 2.37**, respectively, when compared with that of control group animals receiving only vehicle of immobile period 140.33 ± 1.2. The results are statistically significant when compared with control with P<0.001. In spontaneous locomotor activity, *A. leptopus* 150 and 300 mg/kg, p.o, produced significant reduction in immobility period 793 ± 3.2 and 890 ± 2.56, respectively, when compared with that of control group animals receiving only vehicle of immobile period 77.83 ± 2.53. The results are statistically significant when compared with control with $P < 0.001$. The extract (300 mg/kg) was found to be effective and exhibits the activity similar to that of conventional drug imipramine, and the results are tabulated in Table 4.

DISCUSSION

H_2O_2 is highly important because of its ability to penetrate biological membranes. H_2O_2 itself is not very reactive, but it can sometimes be toxic to cell because it may give rise to hydroxyl radical in the cells.[18] Table 2 indicates that ethanolic leaf extract of *A. leptopus* had strong hydrogen peroxide radical scavenging activity similar to that of AA. Nitric oxide radical generated from sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide.[19,20] Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide. Ethanolic leaf extract of *A. leptopus* had comparably more nitric oxide radical scavenging activity than AA as presented in Table 2. The total antioxidant capacity of ethanolic leaf extract of *A. leptopus* was determined by phosphomolybdenum assay, and the highest absorbance was recorded at 0.5 mg/ml as shown in Table 3. The antioxidant capacity of the ethanolic extract was measured by phosphomolybdenum method, which is based on the molybdenum reduction by the sample analyte and the subsequent formation of green phosphate/Mo (V) compounds with a maximum absorption at 695 nm. The antioxidant capacity of extracts was found to increase with increase in concentration.

Depression is an important psychiatric disorder that affects individuals' quality of life and social relations directly. Depression is characterized by emotional symptoms such as hopelessness, apathy, loss of self-confidence, sense of guilt, indecisiveness, and a motivation, as well as biological symptoms such as psychomotor retardation, loss of libido, sleep disturbances, and loss of appetite. When the symptoms are very severe, major depression is considered.

Medications such as tricyclic antidepressants, selective serotonin reuptake inhibitors (SSRIs), selective reversible inhibitors of monoamine oxidase A, and specific serotonin–noradrenaline reuptake inhibitors (SNRIs) are clinically employed for drug therapy.[21] However, these drugs can impose a variety of side-effects including cardiac toxicity, hypopiesia, sexual dysfunction, body weight gain, and sleep disorder.[22-25] There will be the prevention of reuptake of noradrenaline and serotonin resulting in their increased availability in the synapse and therefore an increase in adrenergic and serotonergic neurotransmission by imipramine.[26]

Table 2: *In vitro* antioxidant potential of ethanolic leaf extract of *A. leptopus* and AA against nitric oxide and hydrogen peroxide

Sample	Concentration (µg/Ml)	Nitric oxide		Hydrogen peroxide	
		(%) Inhibition	IC_{50}	(%) Inhibition	IC_{50}
Test sample	50	32.75±0.2	216 **	74.70±0.3	20.8*
	100	43.40±0.05		85.48±0.1	
	300	55.72±0.03		88.81±0.2	
	500	58.055±0.1		93.20±0.04	
AA	50	69.47±0.05	24.54	55.36±0.2	33.1
	100	82.58±0.2		60.32±0.03	
	300	83.46±0.01		70.85±0.1	
	500	87.65±0.04		80.32±0.04	

IC_{50} Values *P<0.01 when compared with standard values; **P<0.001 when compared with standard values. *A. leptopus: Antigonon leptopus*, AA: Ascorbic acid

Table 3: *In vitro* antioxidant potential of ethanolic leaf extract of *A. leptopus* and AA by phosphomolybdate

Sample	Concentration (µg/Ml)	Absorbance
Test sample	50	0.117±0.02*
	100	0.168±0.03*
	300	0.212±0.05**
	500	0.236±0.06**
AA	50	0.181±0.02
	100	0.381±0.01
	300	0.621±0.04
	500	0.973±0.05

*P<0.01 when compared with standard values; **P<0.001 when compared with standard values. *A. leptopus: Antigonon leptopus*, AA: Ascorbic acid

Table 4: Effect of ethanolic leaf extract of *A. leptopus* on duration of immobility time in the TST and FST

	102±2.3**	45±1.35*
A. leptopus (300 mg/kg, p.o)	115±0.34**	58±2.37**

Test solutions were administered orally 60 min before the test. Values represented mean±S.E.M. (*n*=3), **P<0.01, ***P<0.001 versus control. SEM: Standard error of the mean, *A. leptopus: Antigonon leptopus,* TST: Tail suspension test, FST: Forced swim test

Table 5: Effect of ethanolic leaf extract of *A. leptopus* on locomotor activity in photoactometer

Treatment	Photoactometer score in 10 min
control	77.83±2.53
Fluoxetine (10 mg/kg) IP	540.23±2.26 ***
Ethanolic extract of *A. leptopus* (150 mg/kg)	793±3.2***
Ethanolic extract of *A. leptopus* (300 mg/kg)	890±2.56***

Test solutions were administered orally 60 min before the test. Values represented mean±standard error of the mean. (*n*=3), **P<0.01, ***P<0.001 versus control. *A. leptopus: Antigonon leptopus*

In this study, we used two animal models, FST and TST. Both the paradigms are widely accepted behavioral models for assessing pharmacological antidepressant activity. Characteristic behavior scored in these tests is termed immobility, reflecting behavioral despair as seen in human depression.[17] In addition, it is well known that many antidepressant drugs are able to reduce the immobility time in rodents.[16] Ethanolic leaf extract of *A. leptopus* produced a marked reduction in immobility time at doses of 150 and 300 mg/kg in the rat FST and TST, with a profile comparable to that observed for the classical antidepressant drug ESC and imipramine. FST has not traditionally been viewed as a consistently sensitive model for detecting SSRI activity, whereas these antidepressants are generally reported as active in the TST.[27]

CONCLUSION

Ethanolic leaf extract of *A. leptopus* shows dose-dependent antioxidant activity. This study showed that ethanolic leaf extract of *A. leptopus* possesses antidepressant effects. As the effect of extract was similar to that of imipramine, it may be concluded that this effect might be related to inhibition of norepinephrine uptake which eventually leads to increased availability of norepinephrine in synapses. Further research is underway.

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