Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2017, 9(11):25-29
(http://www.derpharmachemica.com/archive.html)

Extraction, Isolation and *In Vitro* Antioxidant Activity of *Butea monosperma* Leaves

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ABSTRACT

Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables. Most of the antioxidant compounds in a typical diet are derived from plant sources and different variety of physical and chemical properties. Butea monosperma is silviculturally important, as it is one of the commonest on the plains of India and capable of thriving where most species will not grow. The leaves of Butea monosperma plant are credited with astringent, tonic, diuretic, and aphrodisiac properties. They are used to cure boils, pimples and tumerous hemorrhoids and are internally given in flatulent colic, worms and piles. The leaves are reported to contain alkaloids, protein, fiber and minerals. In the present study fresh leaves of Butea monosperma plant were isolated for various ingredients i.e., terpenoids and phenolics, fats and waxes, alkaloids, quaternary alkaloids and N-oxides and screened for their antioxidant activity.

Keywords: *Butea monosperma*, Antioxidant activity, DPPH, Alkaloids, Terpenoids

INTRODUCTION

Medicinal plants constitute an effective source of traditional (e.g. Ayurveda, Chinese, Homoeopathy and Unani) and modern medicine. Herbal medicine has been shown to have genuine utility. Recently there has been a shift in universal trend from synthetic to herbal medicine, which we can say 'Return to Nature'. Medicinal plants have been known for 'millennia' and are highly esteemed all over the world as a rich source of therapeutic agents for the prevention of diseases and ailments. Nature has bestowed our country with an enormous wealth of medicinal plants; therefore, India has often been referred to as the medicinal garden of the world. Medicinal plants are of great value in the field of treatment and cure of diseases. Over the years, scientific research has expanded our knowledge of the chemical effects and composition of the active constituents, which determine the medicinal properties of the plants. It has now been universally accepted fact that the plant drugs and remedies are far safer than that of synthetic medicines for curing the complex diseases like cancer and AIDS. Enormous number of alkaloids, glycosides and antibiotics have been isolated, identified and used as curative agents [1-3].

Butea monosperma (Lam.) Taub., also known as flame of the forest is a deciduous tree with a somewhat crooked trunk, up to 15 m in height and 1.6-2.0 m in girth; commonly found throughout India, except in the arid regions. Bark bluish grey or light brown, gum containing; leaves long-petiole, 3-foliate, leaflets coriaceous, obtuse, glabrous above when old, finely silky and conspicuously reticulate veined beneath; flower buds dark brown, flowers bright orange-red, sometimes yellow, in 15 cm long racemes on bare branches; pods pendulous, silky-tomentose, 10-13 cm long, containing one seed at its apex; seeds flat, reniform [4].

Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables. Most of the antioxidant compounds in a typical diet are derived from plant sources and have variety of physical and chemical properties. *B. monosperma* is silviculturally important, as it is one of the commonest on the plains of India and capable of thriving where most species will not grow. The leaves of *B. monosperma* plant are credited with astringent, tonic, diuretic, and aphrodisiac properties. They are used to cure boils, pimples and tumorous hemorrhoids and are internally given in flatulent colic, worms and piles. The leaves are reported to contain alkaloids, protein, fiber and minerals. In the present study fresh leaves of *B. monosperma* plant were isolated for various ingredients i.e., terpenoids and phenolics, fats and waxes, alkaloids, quaternary alkaloids and N-oxides and screened for their antioxidant activity (Chart 1).

MATERIALS AND METHODS

The plant material of *Butea monosperma* were collected seasonally and authenticated by the taxonomists Dr. S.P. Rothe from the Department of Botany, Shri Shivaji College Akola.

Chemicals

Methanol, chloroform, ethyl alcohol and all chemicals used in the study were obtained commercially.

Extraction and isolation

The Leaves of *B. monosperma* plants were shade dried at room temperature and ground in a manual mill to get coarse powder. The coarse powdered material of leaves was treated with methanol and filtered. The residue thus left was again treated with ethyl alcohol and filtered to get fats and waxes. The filtrate was acidified, extracted with chloroform and separated by using separating funnel. The chloroform layer contains terpenoids and phenolics whereas acidic aqueous layer on basic treatment with ammonia followed by chloroform treatment gave alkaloids, whereas methanolic treatment gave quaternary alkaloids and N-oxides. All the isolated constituents were analyzed phytochemically and screened for their antioxidant activity (Figures 1-8) [5].

Study of antioxidant activity by DPPH

The antioxidant activity of the fats and waxes, terpenoids and phenolics, alkaloids, quaternary alkaloids and N-oxides were assessed on the basis 1,1-diphenyl-2-picrylhydrazyl (DPPH). The diluted working solutions of the test extracts were prepared in methanol. 0.004% of DPPH was prepared in methanol and 1 ml of this solution was mixed with 1 ml of sample solution. These solution mixtures were kept in dark for 30 min and optical density was measured at 517 nm using UV Visible spectrophotometer. Methanol (1 ml) with DPPH solution (0.004%, 1 ml) was used as blank. The optical density was recorded and % inhibition was calculated using the formula given below [6].

$$\text{Percentage (\%) inhibition of DPPH (\%AA)} = \frac{A-B}{A} \times 100$$

Where, A=Optical density of the blank and B=Optical density of the sample.

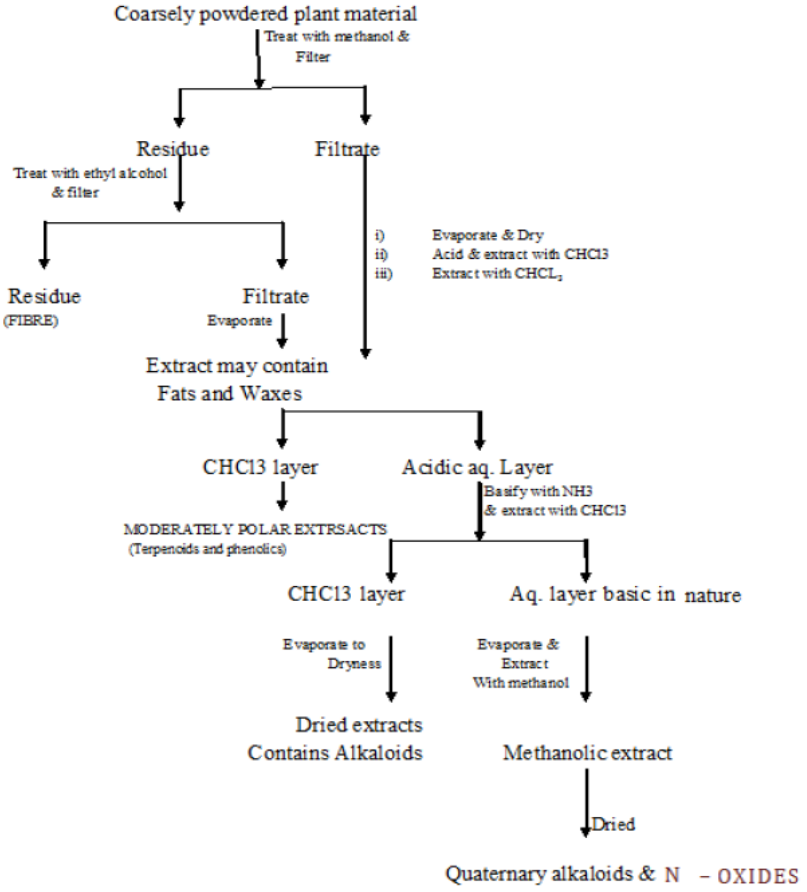

Chart 1: Tree diagram showing extraction and isolation method

RESULTS AND DISCUSSION

The stock solution 3 mg/ml of fats and waxes, terpenoids and phenolics, alkaloids, quaternary alkaloids and N-oxides were prepared. The required dilutions 0.1 mg/ml to 0.9 mg/ml were prepared by appropriate dilutions. The optical density and percent antioxidant activity were calculated and reported in Tables 1-4.

Table 1: Optical density and percent antioxidant activity for fats and waxes O.D. of blank DPPH=0.570

Conc.mg/ml	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
O.D. of sample	0.565	0.559	0.552	0.541	0.540	0.537	0.533	0.530	0.522
% AA	0.877	1.929	3.157	5.087	5.263	5.789	6.491	7.017	8.421

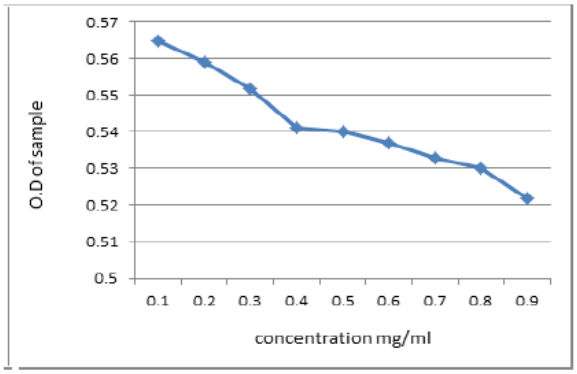

Figure 1: Decrease in O.D of sample with increase in concentration of fats and waxes

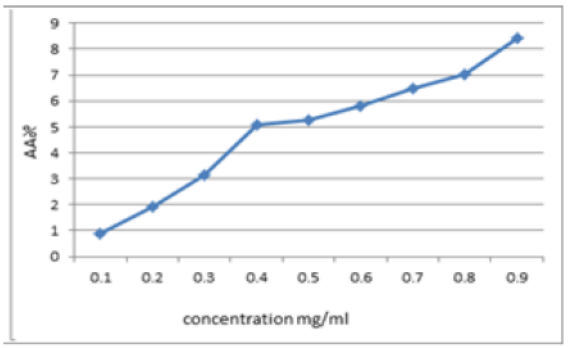

Figure 2: Increase in percent antioxidant activity with increase in concentration of fats and waxes

Table 2: Optical density and percent antioxidant activity for terpenoids and phenolics O.D of blank DPPH=0.570

Conc.mg/ml	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
O.D. of sample	0.498	0.496	0.489	0.484	0.479	0.475	0.470	0.465	0.462
% AA	12.63	12.98	14.21	15.08	15.96	16.66	17.54	18.42	18.94

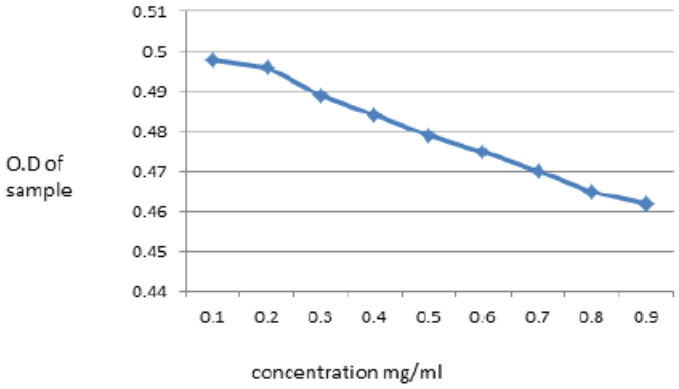

Figure 3: Decrease in O.D of sample with increase in concentration of terpenoids and phenolics

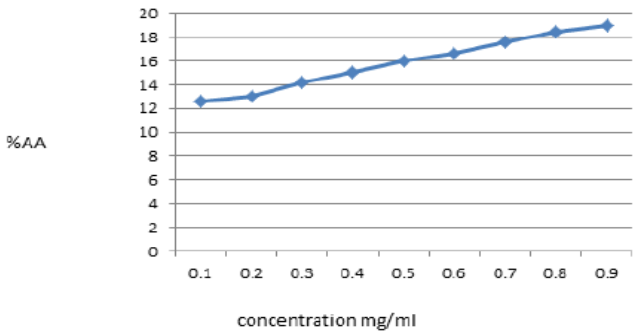

Figure 4: Increase in percent antioxidant activity with increase in concentration of terpenoids and phenolics

Table 3: Optical density and percent antioxidant activity of alkaloids O.D of blank DPPH

Conc.mg/ml	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
O.D. of sample	0.468	0.466	0.459	0.454	0.449	0.445	0.442	0.435	0.432
% AA	17.89	18.24	19.47	20.35	21.22	21.92	22.45	23.68	24.21

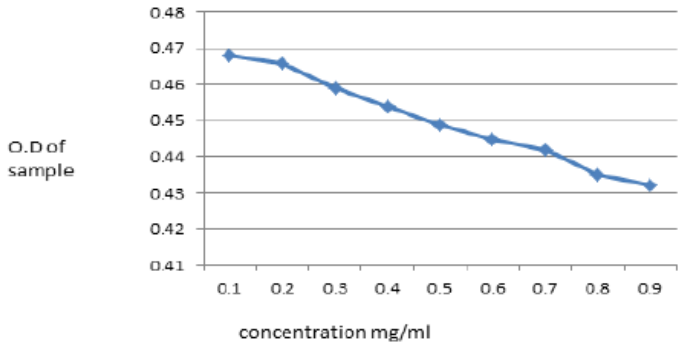

Figure 5: Decrease in O.D of sample with increase in concentration of alkaloids

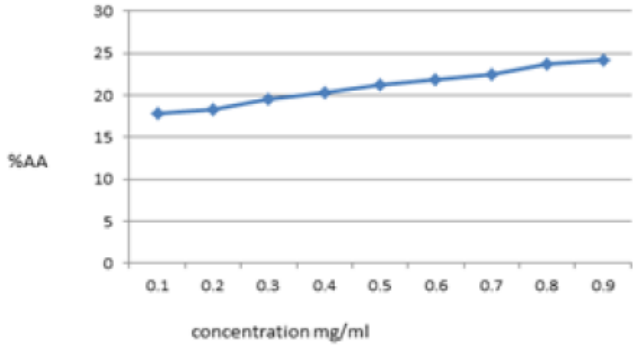

Figure 6: Increase in percent antioxidant activity with increase in concentration of alkaloids

Table 3: Optical density and percent antioxidant activity for methanol extract of quaternary alkaloids and N-oxides O.D.of blank DPPH=1.738

Conc.mg/ml	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
O.D. of sample	0.387	0.381	0.379	0.373	0.366	0.360	0.357	0.349	0.343
% AA	32.10	33.15	33.50	34.56	35.78	36.84	37.36	38.77	39.82

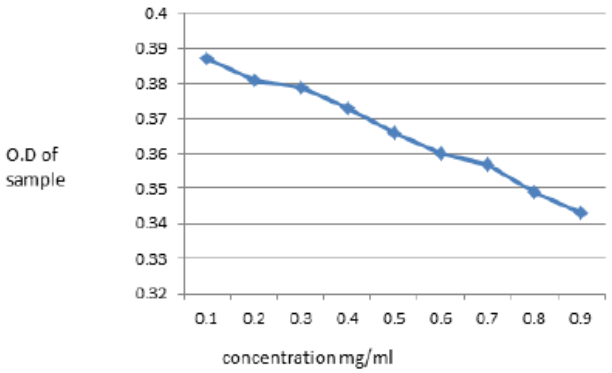

Figure 7: Decrease in O.D of sample with increase in concentration of quaternary Alkaloids and N-Oxides

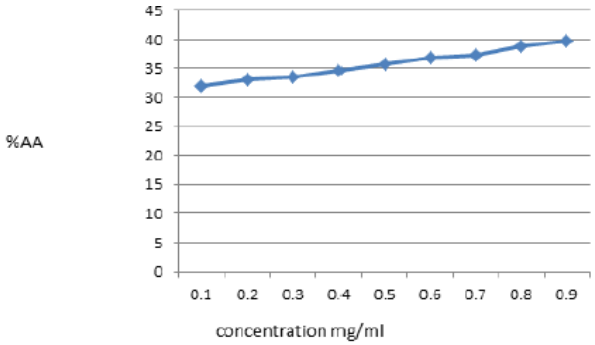

Figure 8: Increase in percent antioxidant activity with increase in concentration of Quaternary alkaloids and N-oxides

CONCLUSION

The results obtained for the antioxidant assay by DPPH performed for various isolated ingredients of *B. monosprma* plants are presented (Tables 1-4). Remarkable decrease in O. D. value of sample for various isolated ingredients were observed from graph, showed good to moderate antioxidant activity [7-9]. The IC_{50} value of the various isolated ingredients such as fats and waxes, terpenoids and phenolics, alkaloids, quaternary alkaloids and N-oxides were calculated from the Figures 2-8.

Table 4: The IC_{50} value of the various isolated ingredients

S. No.	Isolated ingredients	IC_{50}
1	Fats and waxes	0.37 mg/ml
2	Terpenoids and phenolics	0.35 mg/ml
3	Alkaloids	0.41 mg/ml
4	Quaternary alkaloids and N-oxides	0.43 mg/ml

ACKNOWLEDGMENT

The authors are thankful to the Management and Principal of Shankarlal Khandelwal College, Akola for providing necessary facilities. Thanks are also due to Dr. S. P. Rothe, Department of Botany, Shri Shivaji College, Akola.

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