



Received on 27 July, 2013; received in revised form, 30 August, 2013; accepted, 27 November, 2013; published 01 December, 2013

IN-VITRO EVALUATION OF ANTIOXIDANTS ACTIVITY OF ETHANOLIC LEAVES EXTRACT OF *CELASTRUS PANICULATUS*

Priyanka Sharma*¹ and N.M. Shrivastava²

Department Of Pharmacy, Barkatullah University Bhopal, Madhya Pradesh, India
Gandhi Medical College Bhopal, Madhya Pradesh, India

Keywords:

Reducing power activity, phenolic content, antioxidant activity

Correspondence to Author:

Priyanka Sharma

Department Of Pharmacy,
Barkatullah University Bhopal,
Madhya Pradesh, India

E-mail:

sharma_priyanka30@yahoo.co.in

ABSTRACT: *Celastrus paniculatus* is an important medicinal plant used in India. Traditionally seed oil of this herb is used widely in several medicinal preparations and its bark is used in malaria in folk medicines. It is endangered and vulnerable plant. The investigation used Reducing power assay and Total phenolic content to determine antioxidant potential in the ethanolic extract of the drug. The result showed significant reducing power activity and good phenolic content ability of the sample. The extract of leaves may be considered as good antioxidant herb.

INTRODUCTION: *Celastrus paniculatus* is an unarmed woody climbing shrub commonly known as Malkangni, Kangani, Jyotishmati, Sphuta badhani, Svarnalota, Black-Oil tree, Intellect tree, Climbing –staff plant. It grows throughout India upto a height of almost 1,800-2,000 meters. This deciduous vine can grow to a very large size. It belongs to the class Angiospermae and family Celastraceae. The base stem of this shrub grows up to 10 centimeters in diameter and 6 meters in length.

Being a Rambler by nature, it produces many woody branches that cling to surrounding flora for support. The stem has a rough, pale or reddish brown exfoliating bark covered densely with small elongated white lenticles. The inner bark is light and cork like with yellow sapwood.

The leaves are simple, broad and oval, obvate or elliptic in shape, leathery and smooth, alternately arranged on short petioles with toothed margin¹. The raw drug is collected from the wild since the species is not under cultivation. In Ayurveda, leaves used in menstruation problems whereas seeds are acrid, bitter, hot, appetizer, laxative, emetic, aphrodisiac, powerful brain tonic, cause burning sensation. Oil enriches blood and cures abdominal complain.

According to Unani system of medicine, seeds are bitter, expectorant, brain and liver tonic, cure joint-pains, paralysis and weakness and oil is stomachic, tonic, good for cough and asthma; used in leprosy, cures headache and leucoderma². *C. paniculatus* seeds and oil extracted from them have long been regarded to be highly beneficial and medicinally effective. The seeds yield as much as 52% oil by weight and it is in this oil that numerous alkaloids are found, compounds such as celastrine and paniculatin. The seeds are highly rich in fatty acids. Percentage composition of four lipid fraction of seeds viz. normal triglycerides ester, polar triglycerides, polar non-glyceridic ester and non-

QUICK RESPONSE CODE 	DOI: 10.13040/IJPSR.0975-8232.4(12).4682-84
	Article can be accessed online on: www.ijpsr.com
DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.4(12).4682-84	

polar non-glycosidic ester. Major component acids in these fractions are palmitic, stearic, oleic, linoleic and linolenic. The major molecular species constituting the normal triglycerides are: palmito-oleo-palmitin (6.8%), palmito-oleo-stearin (5.6%), palmitodiolein (14.7%), palmito-oleo-linolein (7.0%), stearo-diolein (6.1%), triolein (8.0%) and dioleo-linolein (7.6%)³⁻⁴.

MATERIAL AND METHODS: The fresh leaves were collected in the month of July-August from dist., Vidisha and authenticated by MPC Park (Vindh herbal) and shade dried and then grind to fine powder.

Preparation of Extract: The coarsely powdered leaves were further macerated for extractive values and then ethanol was chosen for the preparation of extract than extract was prepared in 95% ethanol.

Assessment of Antioxidant Activity:

Reducing power assay: As per the reported method the reducing power of extract was determined. Different concentrations of extract (250–2500 mg/ml) in 1ml of methanol were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferrocyanide (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and FeCl₃ (0.5 ml, 0.1%) and the absorbance was measured at 700 nm and compared with standards. Increased absorbance of the reaction mixture indicated increased reducing power⁵.

Spectrophotometric Quantification of Total Phenolic Content:

Procedure: In the total phenolic amount of extracts was determined with the Folin-Ciocalteu reagent. Gallic acid was used as a standard and the total phenolic were expressed as mg/g gallic acid equivalent (GAE). Concentration of 0.01, 0.02, 0.03, 0.04 and 0.05 mg/ml of gallic acid were prepared in methanol. Concentration of 0.1 and 1mg/ml of plant extract were also prepared in methanol and 0.5ml of each sample were introduced in to test and mixed with 2.5ml of a 10 fold dilute Folin-Ciocalteu reagent and 2ml of 7.5% sodium carbonate.

The tubes were covered and allowed to stand for 30 minutes at room temperature before the absorbance was at read at 760 nm spectrophotometrically. All determination was performed in triplicate. The Folin-Ciocalteu reagent is sensitive to reducing compounds including polyphenols. They produce a blue colour upon reaction. This blue colour was measured spectrophotometrically. Line of regression from Gallic acid was used for estimation of unknown phenol content⁶⁻⁷.

RESULTS:

The Total Phenolics: Phenolic content was determined by statistical method. The phenolic contents in extract was good and expressed as gallic acid equivalents (Fig. 2).

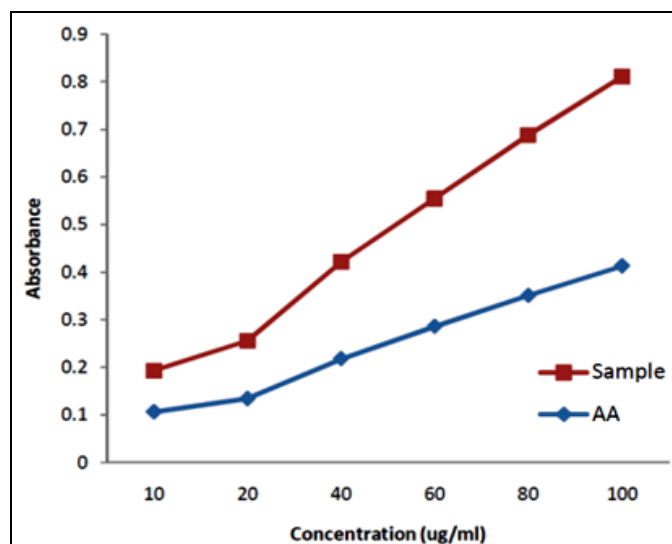
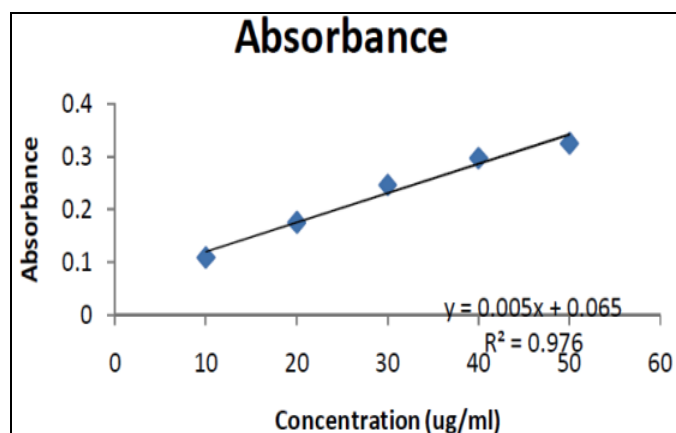
The Reducing Power: The reducing power of each fraction, Fe³⁺ to Fe²⁺ reduction of the extract was investigated as shown in Fig. 1. However *C. paniculatus* showed significant reducing power in the experiments. As absorbance of sample increases with concentration of sample, it indicates reducing power; it may be due to presence of active constituents in the sample.

TABLE 1: REDUCING POWER ASSAY

Sr. No.	Concentration (µg/ml)	Absorbance	
		Ascorbic acid	Sample
1	10	0.106	0.087
2	20	0.134	0.122
3	40	0.218	0.204
4	60	0.286	0.269
5	80	0.351	0.337
6	100	0.413	0.398

TABLE 2: TOTAL PHENOLIC CONTENT

Sr. No.	Absorbance of Extract	Concentration of Extract	Total Phenolic Content mg/g equiv. to Gallic Acid
1.	0.687	1mg/ml	124.4
2.	0.693	1mg/ml	125.6
3.	0.688	1mg/ml	124.6
MEAN±SD			124.8±0.642

**FIGURE 1: REDUCING POWER ASSAY****FIGURE 2: TOTAL PHENOLIC CONTENT**

DISCUSSION: The antioxidant activities of the ethanolic extract of *C. paniculatus* were assayed by using several test systems. Recent investigations have shown differences between the test systems for the determination of antioxidant activity.

How to cite this article:

Sharma P and Shrivastava NM: *In-vitro* evaluation of antioxidants activity of ethanolic leaves extract of *Celastrus paniculatus*. *Int J Pharm Sci Res* 2013; 4(12): 4682-84. doi: 10.13040/IJPSR. 0975-8232.4(12).4682-84

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It is recommended to use at least two methods. In this study, we used several methods. It has Significant reducing power and good phenolic content. It appears that antioxidant activity could be correlated with phenolic content and reducing power.

CONCLUSION: The *in-vitro* antioxidant activity was carried out by reducing power assay and phenolic content. From reducing power assay it shows significant antioxidant effect and good phenolic content. By these results we can suggest that this ethanolic extract of the drug could have some good therapeutic effects.

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