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IN-VITRO ANTIOXIDANT ACTIVITIES OF AERIAL PARTS EXTRACTS OF *CAESALPINIA MIMOSOIDES LAMK*

V. V. Lakshmi[*1]

[1*]Department of Pharmaceutical Chemistry, University College of Pharmacy, Cheruvandoor campus, Kottayam, Kerala - 686 631, India.

ABSTRACT

In vitro antioxidant activities of different extract (pet. ether, chloroform, ethylacetate, alcohol residue) of aerial parts of *Caesalpinia mimosoides Lamk* was investigated. The free radical scavenging activity to evaluate by Iron chelating method, Nitric oxide method. In iron chelating assay the IC_{50} value of the standard ascorbic acid was found to be 49.70µg/ml and it was comparable with the IC_{50} value of EAE (IC_{50} 58.32µg/ml). The nitric oxide scavenging ability of ethyl acetate extract IC_{50} value was found to be 61.77µg/ml which is comparable with the ascorbic acid (32.37µg/ml). Ethyl acetate extract exhibited good antioxidant activity when compared to other extracts. The results of the study showed that aerial parts of *caesalpina mimosoides Lamk.* Have strong radical scavenging and reducing capacities.

KEYWORDS

Caesalpinia mimosoides, Anti-oxidant, Iron chelating assay and Nitric oxide method.

Author for Correspondence:

Lakshmi V V,
Department of Pharmaceutical Chemistry,
University College of Pharmacy,
Cheruvandoor, Kottayam, Kerala, India.

Email: lakshmivrv.m@gmail.com

INTRODUCTION

Free radicals which are atomic or molecular chemical species with unpaired electrons are highly unstable and can react with other molecules by giving out or accepting single electron. Oxidation processes are one of the most important routes for producing free radicals in food, drugs and even living systems. These unstable molecules are capable of causing cellular damage, which leads to cell death and tissue injury. Free radicals are linked with the majority of human diseases like ageing, atherosclerosis, cancer, diabetes, liver Cirrhosis, cardiovascular disorders etc.

Anitoxidants have the ability to protect the body from oxidative damage by scavenging the free radicals and inhibiting peroxidation and other radical mediated processes. Traditionally the plants are used as medicine due to their antioxidant activity and play important role to alleviate many diseases.

Caesalpinia mimosoides Lam is a member of Leguminosae family. A prickly climbing shrub, with branches bearing small prickles is mainly distributed in the south of China and grows in countries like India, Myanmar, as well as in northern and north-eastern parts of Thailand. Young sprouts and leaves are edible and sour and are traditionally used as a carminative and a remedy for dizziness. The methanolic extract of *C. mimosoides* shoot tips was reported to exhibit antioxidant activity. Moreover, the aqueous and the ethanol extracts contained gallic acid, the antioxidative compound. This plant showed moderate antioxidant activity, and high tannin and total phenolics contents, which led us to examine it further for other biological activity. The present study was designed to investigate the anti-oxidant activity of aerial part extract of *Caesalpinia mimosoides* by *in vitro* model.

MATERIAL AND METHODS[6-8]

Plant Materials

Aerial parts of *Caesalpinia mimosoides*, was collected from Kottayam district, Kerala during the month of March 2013 and was identified and authenticated by the botanist, Mr. Rogimon P. Thomas, Department of Botany, C.M.S. College, Kottayam, Kerala. A voucher specimen (No. 265) was preserved at C.M.S. College, Kottayam.

Preparation of Extract

Shade dried and powdered aerial parts (100g) of *Caesalpinia mimosoides* was soaked in rectified spirit in a round bottom flask. After soaking it for one day, it was refluxed with ethanol 95% (2Litre) for 3 hours and the clear solution was decanted off. The extraction was repeated thrice. The combined extract was concentrated to a semisolid consistency. Thus total ethanolic extract was obtained. The fractionation of the ethanolic extract was carried out using solvents in the increasing order of polarity i.e. petroleum ether (PEE), chloroform (CHE) and ethyl acetate (EAE). Each fraction was concentrated, weighed and stored for further studies.

Evaluation of Antioxidant activity by *in vitro* Techniques

Iron Chelating Assay[8]

In this method 2ml of various concentrations of extract and ascorbic acid solutions were incubated with 1ml *o*-phenanthroline solution (0.05%) and 2ml 2µM ferric chloride solution (3.24mg in 100 ml distilled water) at ambient temperature for 10 minutes. After incubation, the absorbance of solutions was measured at 510 nm. The blank used here was a mixture of methanol and distilled water. A control was also prepared omitting the sample. The experiments were performed in triplicate. The results were tabulated[8].

Calculation of IC_{50} (50 % inhibitory concentration)

$$\%\text{scavenging} = \frac{(\text{Absorbance of test-Absorbance of control}) \times 100}{\text{Absorbance of test}}$$

Nitric Oxide Scavenging Assay[7]

In this assay 0.5 ml Sodium nitroprusside (10mM/L) in phosphate buffered saline pH 7.4, was mixed with different concentration of the samples (20, 40, 60, 80, 100 and 120 mcg/ml from a stock concentration of 100mg/ml methanol) and incubated at 25°C for 3 hr. A control without the test compound, but an equivalent amount of ethanol was taken. After 3 hrs, 1.5 ml of Griess reagent (1% sulphanilamide, 2% phosphoric acid and 0.1% N-1-naphthyl ethylene diamine dihydrochloride) was added and incubated for 30 minutes for colour development. Absorbance of the chromophore formed during diazotization of the nitrate with sulphanilamide and subsequent coupling with N-1naphathyl ethylene diamine dihydrochloride was measured at 546 nm and the percentage scavenging activity was measured with reference to the standard. The experiments were performed in triplicate. The results were tabulated.

$$\%\text{ scavenging} = \frac{(\text{Absorbance of control}-\text{Absorbance of test} \times 100}{\text{Absorbance of control}}$$

RESULTS AND DISCUSSION

Iron Chelating Assay

In the present antioxidant activity studies using Iron chelating assay, antioxidative ability of *C.mimosoides* was estimated by assessing its iron chelating capacity. The presence of *C.mimosoides* extract in the reaction

mixture interferes the reaction by chelating the Fe^{2+} ion. In this assay the IC_{50} value of the standard ascorbic acid was found to be 49.70µg/ml and it was comparable with the IC_{50} value of EAE (IC_{50} 58.32µg/ml). The IC_{50} values of various extracts of *C.mimosoides* and standard ascorbic acid are given in Table No.1 and graphically represented (Figure No.1).

Nitric Oxide Scavenging Assay

Nitric oxide (NO) is known to be a ubiquitous free-radical moiety, which is distributed in tissues or organ systems and is supposed to have a vital role in neuromodulation or as a neurotransmitter in the CNS.

In addition to reactive oxygen species, nitric oxide is also implicated in inflammation, cancer and other pathological conditions. Oxygen reacts with the excess of nitric oxide to generate nitrite and peroxy nitrite anions which act as free radicals. Anti-oxidants compete for oxygen and reduce the production of nitric oxide. The results of Nitric oxide scavenging activity represents that stable NO radical was effectively scavenged by the various extracts. The nitric oxide scavenging ability of ethyl acetate extract IC_{50} value was found to be 61.77µg/ml which is comparable with the ascorbic acid (32.37µg/ml). The result is given Table No.2 and graphically represented (Figure No.2).

Table No.1: IC_{50} values using iron chelating assay

S.No	Sample	IC_{50}Value (µg/ml)
1	Standard (Ascorbic acid)	49.70
2	PEE	93.17
3	CHE	87.41
4	EAE	58.32
5	ALE	69.43

Table No.2: IC_{50} values using nitric oxide scavenging assay

S.No	Sample	IC_{50} Value (µg/ml)
1	Standard (Ascorbic acid)	32.37
2	PEE	101.66
3	CHE	86.01
4	EAE	61.77
5	ALE	77.62

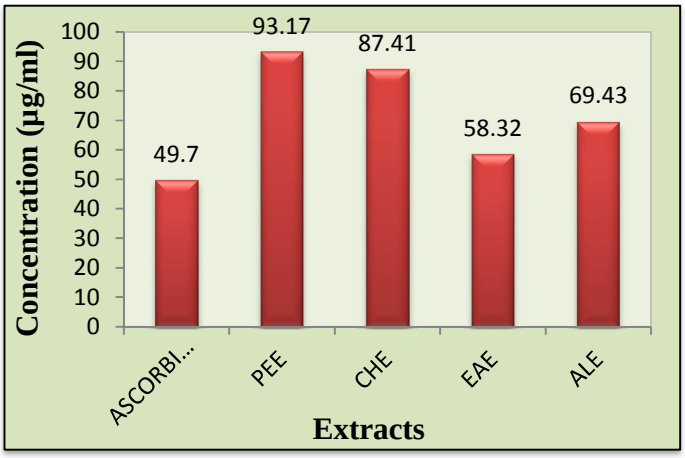

Figure No.1: Comparison of IC50 values of Standard ascorbic acid Vs Extracts using Iron chelating assay

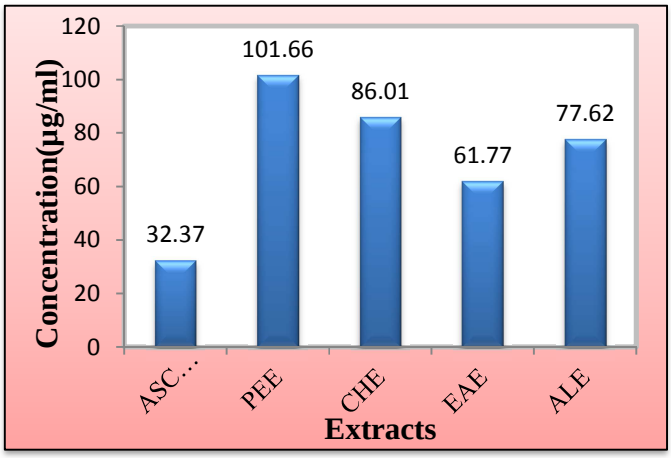

Figure No.2: Comparison of IC50 values of Standard ascorbic acid Vs Extracts using nitric oxide scavenging assay

CONCLUSION

The results of the above investigation indicated that the ethyl acetate extract of aerial parts of *Caesalpina mimosoides* showed strong antioxidant activity. However, Phytochemical screening of plant extract showed presence of Tannins, Phenolic compound, and Flavonoids, carbohydrates. So it can be concluded that these components might be involved in the antioxidant activity of *caesalpina mimosoides.*

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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