ejpmr, 2015,2(2), 250-254

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 3294-3211

EJPMR

EVALUATION OF FREE RADICAL SCAVENGING ACTIVITY OF SOME MEDICINAL PLANT

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Article Received on 07/12/2014 **Article Revised on 01/01/2015** **Article Accepted on 25/01/2015**

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ABSTRACT

The present study was carried out to evaluate the free radical scavenging activity of various fractions of toluene, ethyl acetate, butan-2-one and n-butyl alcohol of leaves of *Kydia calycina*. DPPH free radical and ABTS free radical scavenging activity were carried out to evaluate the antioxidant potential of the extract. The antioxidant activity of toluene, ethyl acetate, butan-2-one and n-butyl alcoholic extract increased in dose depending manner. In DPPH radical scavenging the IC_{50} value for DPPH scavenging by the toluene, ethyl acetate, butan-2-one and n-butyl alcoholic extracts of *Kydia calycina* were 39.7, 9.4, 45 and 50.2 respectively. The IC_{50} value for ABTS free radical scavenging ability of toluene, ethyl acetate, butan-2-one and n-butyl alcoholic extracts of *Kydia calycina* were found 8.34, 0.96, 10.35 and 7.83 respectively. The obtained results in this study indicate that the ethyl acetate extract can be potential source of natural antioxidant.

KEYWORDS: *Kydia calycina*, antioxidant, DPPH radical, ABTS free radical.

INTRODUCTION

Natural products remain a prolific source for the discovery of new drugs and drugs leads even from Vedic period. Recent data suggests that 80% of drug molecules are natural products or natural compound inspire(Harvey, 2008). Literature from 1981 to 2007 reveals that almost half of the drugs approved since 1994 are based on natural products (Butler, 2008). Indian natural products, particularly those from traditional medicinal plants which are reported in the classic texts like Ayurveda and Charak Samhita, have contributed towards drugs discovery.

Medicinal practitioners have prescribed drugs from herbal origin as a system of medicine in India over centuries. Many of the modern drugs are mainly based on synthetic chemical

compounds have been found to have adverse effects. This has triggered extensive research and development in the field of herbal medicine. In fact, there is a growing demand for herbal medicines in most of the developed and developing countries of the world today. There are several medicinal plants mentioned in Ayurveda, Siddha, Unani, Homeopathy, Naturopathy and Folklore medicine which are used as household remedies. The chemical investigations, biochemical studies and/or pharmacological studies are not fully established for many of the plants. The most of the plants have antioxidant property, so present investigations have been carried out to evaluate the anti-oxidant activity of *Kydia calycina*.

MATERIALS AND METHODS

Plant material

The leaves of *Kydia calycina* were collected in the month of June from thirumal hills, Andhra Pradesh, India. The selected plant was authenticated by Prof. Raju S. Vastavaya, Department of Botany, Kakatiya University, Warangal and voucher specimens were being maintained in the herbarium of University College of Pharmaceutical Sciences, Kakatiya University, Warangal.

Preparation of Extracts

The leaves of *Kydia calycina* (3kg) were made free from the adherent foreign material and air-dried. Then they were coarsely powdered and 2kg of each was macerated with methanol in a round bottom flask for 7 days separately. The content of the flask were stirred intermittently to ensure the efficiency of the extraction. After a week, they were filtered and concentrated under reduced pressure to yield corresponding extracts, and the extracts were kept in a desiccator to remove moisture and stored properly until reuse.

The methanolic extracts of *Kydia calycina* were dispersed in sufficient amount of distilled water separately and fractionated with toluene, ethyl acetate, butan-2-one and n-butyl alcohol in succession. The obtained fractions and the aqueous residues were concentrated under reduced pressure to yield corresponding extracts.

Antioxidant Activity

DPPH free radical scavenging effect (Blois, 1958)

The free radical scavenging activity of MKC fractions were measured by DPPH using the method of Blios[3]**.** Ascorbic acid was used as a reference standard. The methanolic solution of DPPH (0.2mM) was added to different concentrations (100, 200, 400, 600, 800, 1000 µg/ml in methanol) of fractions. After 30 min, absorbance was measured at 517 nm. The degree of discoloration indicates the scavenging potential of the extract. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The IC50 (Inhibitory Concentration) is the concentration of sample required to scavenge 50% of DPPH free radicals.

ABTS free radical scavenging effect (Re *et al.,* 1999)

ABTS was dissolved in water to a 7 mM concentration. ABTS radical cation (ABTS•1) was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12–16 h before use . Because ABTS and potassium persulfate react stoichiometrically at a ratio of 1:0.5, this will result in incomplete oxidation of the ABTS. Oxidation of the ABTS commenced immediately, but the absorbance was not maximal and stable until more than 6 h had elapsed. The radical was stable in this form for more than two days when stored in the dark at room temperature. For the study of antioxidant activity, various concentrations (100, 200, 300, 400, 500µg/ml) of extracts were added to the ABTS•1 solution. Then the mixture was diluted with ethanol to get an absorbance of 0.70 to 0.80 at 734 nm and equilibrated at 30°C. Stock solutions. After addition of 1.0ml of diluted ABTS•1 solution appropriate solvent blanks were run in each assay.

Calculation

$$\text{Percentage O.D.} = \frac{\text{Control O.D.} - \text{Test O.D.}}{\text{Control O.D.}} \times 100$$

RESULTS AND DISCUSSION

Table 1. *In Vitro* DPPH free radical scavenging activity of Selected Fractions MKC.

Compound	IC50 (µg/ml)
STD	4.3
T-MKC	39.7
EA-MKC	9.4
BN-MKC	45.4
BL-MKC	50.2

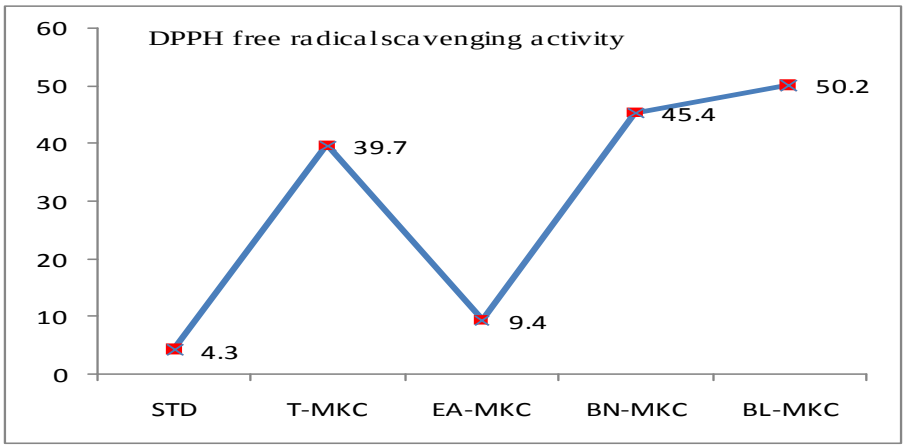

Fig: 1 *In Vitro* DPPH free radical scavenging activity of Selected Fractions MKC

Table 2. *In Vitro* ABTS free radical scavenging activity of Selected Fractions MKC.

Compound	IC50 (µg/ml)
Ascorbic acid	2.42
T-MKC	8.34
EA-MKC	0.96
BN-MKC	10.35
BL-MKC	7.83

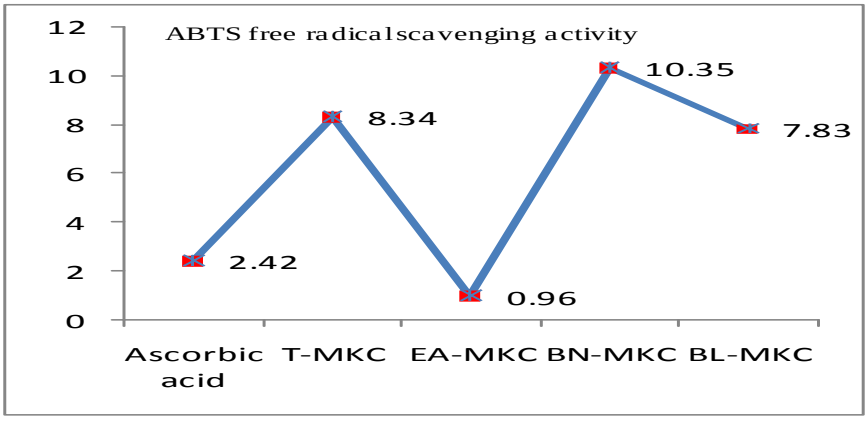

Fig: 2 *In Vitro* ABTS free radical scavenging activity of Selected Fractions MKC

The reduction capability of the DPPH radical induced by antioxidants is determined by the decrease in the absorbance at 517 nm. The decrease in the absorbance of DPPH radical caused by antioxidants, due to the reaction between antioxidant molecules and radical, progresses which results in the scavenging of the radical by hydrogen donation. It is visually noticeable as a change in color from purple to yellow. All the fractions of MKC were evaluated for their free radical activity against DPPH radicals and the results were expressed as IC_{50} values and were shown in Table 1 & Fig 1. This indicated the antioxidant activity of all fractions against DPPH free radical. Among all the fractions showed a maximum

scavenging potential with IC_{50} value of 39.7, 9.4, 45 and 50.2 µg/ml. Among all fractions of MKC, the antioxidant potentials was found as EA-MKC> T-MKC >BN-MKC> BL-MKC.

It is a method for the screening of antioxidant activity. It reported as a decolorization assay applicable to both lipophilic and hydrophilic antioxidants, including flavonoids, hydroxycinnamates, carotenoids, and plasma antioxidants. The pre-formed radical monocation of 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS•1) is generated by oxidation of ABTS with potassium persulfate and is reduced in the presence of such hydrogen-donating antioxidants. The influences of both the concentration of antioxidant and duration of reaction on the inhibition of the radical cation absorption are taken into account when determining the antioxidant activity. All the fractions of MKC were evaluated for their free radical activity against ABTS radicals and the results were expressed as IC_{50} values and were shown in Table 2 & Fig 2. This indicated the antioxidant activity of all fractions against ABTS free radical. Among all the fractions showed a maximum scavenging potential with IC_{50} value of 8.34, 0.96, 10.35 and 7.83µg/ml. The radical scavenging activity of MKC fractions decreased in the following order: EA-MKC> BL-MKC >T-MKC> BN-MKC.

This information encouraged us to evaluate the free radical scavenging effect of MKC fractions by employing widely used methods i.e. DPPH free radical scavenging activity, ABTS assay, radical scavenging activity. MKC fractions showed moderate to strong free radical scavenging activities. These results confirmed that the fractions of the selected plant exhibited a significant antioxidant activity.

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