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In-vitro antioxidant activity of the ethyl acetate extract of gum guggul (*Commiphora mukul*)

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ABSTRACT

Free radicals are implicated for more than 80 diseases including Diabetes mellitus, arthritis, cancer, ageing etc. In treatment of theses diseases, antioxidant therapy has gained an utmost importance. Current research is now directed towards finding naturally occurring antioxidant of plant origin. In Indian system of medicine *Commiphora mukul* is an important medicinal plant its resinous part has been used in various ailments and as health tonic. To understand the mechanisms of pharmacological actions, the in vitro antioxidant activity of ethyl acetate extract of *Commiphora mukul* was investigated for reducing power activity and total antioxidant activity. The ethyl acetate extract *C. mukul* exhibited good reducing power activity at higher concentration, which was measured at 700 nm. Gallic acid was used as standard antioxidant. Total antioxidant activity was determined by thiocyanate method, which was measured at 500 nm. α-tocopherol was used as standard, the standard, showed 76.38 % inhibition at 500 µg/ml concentration while *C.mukul* extract showed 51.16 % inhibition at the same concentration. In these testing, a significant correlation existed between concentrations of the extract and percentage inhibition of free radicals and reducing power. The antioxidant property may be related to the antioxidant vitamins, phenolic acids and micronutrients present in the extract. These results clearly indicate that *Commiphora mukul* is effective against free radical mediated diseases

Keywords: *Commiphora mukul* , reducing power activity, thiocyanate method

INTRODUCTION

Antioxidants are radical scavengers, which protect the human body against free radicals that may cause pathological conditions such as ischemia, anaemia, asthma, arthritis, inflammation, neuro-degenertion, Parkinson's diseases, mongolism, ageing process and perhaps dementias (Polterat, 1997). Flavonoids and flavones are widely distributed secondary metabolites with antioxidant and antiradical properties (Nakayoma and Yamada, 1995). Plants are potent biochemical factories and have been components of phytomedicine since times immemorial; man is able to obtain from them a wondrous assortment of industrial chemicals. The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. The medicinal actions of plants are unique to particular plant species or groups are consistent with this concept as the combination of secondary products in a particular plant is taxonomically distinct. Antioxidant-based dugs/formulations for the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease, and cancer have appeared during the last 3 decades (Devasagayam *et al* 2004*)*. This has attracted a great deal of research interest in natural antioxidants. The majority of the active antioxidant compounds are flavonoids, isoflavones, flavones, anthocyanins, coumarins, lignans, catechins, and isocatechins. In addition to the above compounds found in natural foods, vitamins C and E, beta-carotene, and tocopherol are known to possess antioxidant potential (Prior, 2003). With this background and abundant source of unique active components harbored in plants, the present study was taken up on *Commiphora mukul belongs* to the family burseraceae. In Indian ethno medicine, this plant is locally known as guggulu, and whole

plant have long been used as Ayurvedic remedy for lowering the blood cholesterol.

MATERIALS AND METHODS

Plant Material

Resins of *C. mukul* were collected from local area of Jhansi and authenticated by the head of the Botany department, B.U. Jhansi. A voucher specimen has been preserved at Institute of Pharmacy, Bundelkhand University Jhansi.

Preparation of Extract

100 gm air-dried resins were extracted with 500 ml of ethyl acetate for 5 days .The extract was filtered with Whatman no.2 filter paper. The filtrate was collected and solvent was evaporated under reduced pressure using vacuum evaporator. The extract was subjected to qualitative chemical tests for steroids, terpenoids, flavonoids, alkaloid, etc. (Evans, 1997; Harborne, 1998).

Antioxidant activity

Reducing power assay:

The reducing power of *C. mukul* extract was determined according to the method of Oyaizu (1986). 1.0 ml of plant extract solution (final concentration 50-500 µg/ml) was mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml potassium ferricyanide $[K_3Fe(CN_6)]$ (10g/l), then mixture was incubated at 50 degree C for 20 minutes. Two and one-half, 2.5 ml of trichloro acetic acid (100g/l) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. Finally, 2.5 ml of the supernatant solution was mixed with 2.5 ml of distilled water and 0.5 ml $FeCl_3$ (1g/l) and absorbance measured at 700 nm in UV-Visible Spectrophotometer. As a standard, gallic acid was used (final concentration 10 mg/ml). Increased absorbance of the reaction mixture indicates stronger reducing power.

Total antioxidant activity:

The antioxidant activity of *C.mukul* was determined according to the thiocyanate method (Mitsuda *et al.*, 1996). About 10 mg of extract was dissolved in 10 ml distilled water. Various concentrations (50, 100, 250 and 500 µg/ml) of extract were added to linoleic acid emulsion (2.5 ml, 0.04 M, pH 7.0) and phosphate buffer (2 ml, 0.04 M, pH 7.0). The linoleic acid emulsion was prepared by mixing 0.2804 g of linoleic acid, 0.2804 g of Tween 20 as emulsifier and 50 ml phosphate buffer and then the mixture was homogenized. The final volume was adjusted to 5ml with potassium phosphate buffer (0.04 M, pH 7.0). Further on the mixed samples were incubated at 37^0 C in a glass flask for 60 hours to accelerate the oxidation process. Each 12 hours, 1 ml of the incubated sample was removed and 0.1 ml of $FeCl_2$ (0.02 M) and 0.1 ml of ammonium thiocyanate (30%) were added. The amount of peroxide was determined by measuring the absorbance at 500 nm. Alpha tocopherol was used as the reference compound. to eliminate the solvent effect, the control sample, which contains the same amount of solvent added into the linoleic acid emulsion in the test sample and reference compound was used. All the data are expressed as mean of triplicate determinations. The percentage of inhibition of lipid peroxide generation was measured by comparing the absorbance values of control and those of test samples.

$$\% \text{ Inhibition} = \frac{(\text{Control Absorbance} - \text{Test Absorbance})}{\text{Control Absorbance}} \times 100$$

RESULTS AND DISCUSSION

Recently much attention has been focused on reactive oxygen species and free radicals, which play an important role in the genesis of various diseases such as inflammation, cataract, liver cirrhosis and ischemia/reperfusion injury (Halliwell, 1994). Herbal drugs containing radical scavengers are gaining importance in the prevention and treatment of such diseases. Phenolic compounds and flavonoids are the major constituents in most plants reported to possess antioxidant and free radical scavenging activity. Preliminary phytochemical screening of ethyl acetate extract gave positive tests for flavonoids and steroids. The reducing power of ethyl acetate extract of *Commiphora mukul* along with that of gallic acid at concentrations between 50-500 µg/ml. The reducing power of

the plant extract was determined by the method of Oyaizu (1986). High absorbance indicates high reducing power. The reducing power of the plant extract of *C. mukul* was increased as the amount of extract increases [Table-1]. However, this reducing power is lower than that of gallic acid which was used as standard. Therefore, the absorbance of gallic acid in a sample was (100 µg/ml) 1.25 while at the 500 µg/ml ethyl acetate extract concentration it was 1.05. The most commonly used method for determining antioxidant activity is to measure the inhibitory degree of auto-oxidation of linoleic acid. The different concentration of extract 50, 100, 250 and 500 µg/ml showed antioxidant activities in a dose dependent manner and had 31.99, 38.01, 45.46 and 51.16 % inhibition respectively on lipid peroxidation of linoleic acid system. At the same time α-tocopherol at the concentration 500 µg/ml showed 76.38 % inhibition [Table-2]. Against the backdrop of many known medicinal properties of this plant, results from the present work suggest that relatively low values of antioxidant and reducing power may not imply a low medicinal value. Emerging trends in antioxidant research point to the fact that low levels of phenolics (and other phytochemicals) and low value of antioxidant indices in plants do not translate to poor medicinal properties. The present investigation indicates that though *C.mukul* has been described as plant of low economic values, this is not worthless. This study suggested that the *Commiphora mukul* plant extract possess antioxidant activity, which might be helpful in preventing or slowing the progress of various oxidative stress- related diseases. Further investigation on the isolation and identification of antioxidant component(s) in the plant may lead to chemical entities with potential for clinical use.

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Table 1. Reducing Power Assay

Concentration µg / ml	**Absorbance (700 nm)**	
	C. mukul	**Gallic acid**
50	0.470 ± 0.002	0.988 ± 0.001
100	0.674 ± 0.003	1.251 ± 0.003
200	0.793 ± 0.001	1.743 ± 0.003
300	0.916 ± 0.003	1.833 ± 0.001
400	0.979 ± 0.002	1.856 ± 0.002
500	1.050 ± 0.003	1.905 ± 0.004

All values are means ± SD (n = 3)

Table 2. Total Antioxidant activity

Concentration µg / ml	**% Inhibition (500 nm)**	
	C. mukul	**α-TPh**
50	31.99 ± 0.08	60.20 ± 0.09
100	38.02 ± 0.08	65.54 ± 0.16
250	45.46 ± 0.27	68.58 ± 0.11
500	51.16 ± 0.26	76.38 ± 0.09

All values are means ± SD (n = 3)