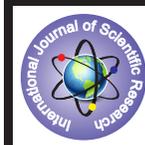


## Evaluation of antioxidant activity of *Artocarpus hirsutus* methanolic fruit extract: An *in vitro* study



### Chemistry

**KEYWORDS :** Artocarpus hirsutus, Antioxidants, DPPH, Reducing power, Methanolic fruit extract.

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### ABSTRACT

*Antioxidants are substances that can scavenge free radicals, thereby protecting the body against their potentially damaging effects. Research suggests that there is increased oxidative stress in combination with reduced antioxidant status; this results in greater vulnerability to the damaging effects of free radicals. High levels of reactive oxygen species (ROS) can initiate impaired functioning and destruction of neurons and other bodily cells. Many plants and herbs have potent antioxidant activity, Artocarpus hirsutus show potency of antioxidant activity, but the effects of these have not been studied previously. The methanolic fruit extract of Artocarpus hirsutus was found to be effective in DPPH radical scavenging activity. The DPPH radical scavenging activity of the extract was increased with the increased concentration of crude plant methanolic fruit extract. The reducing power of the extract was carried out with ascorbic acid as a standard reducing agent. The methanolic fruit extract was potentially exhibited concentration dependent DPPH radical scavenging and reducing power is an increased quest to obtain natural antioxidants with broad-spectrum actions. The majority of the rich diversity of Indian medicinal plants is yet to be scientifically evaluated for such properties. In present work an attempt has been carried out to evaluate antioxidants of Artocarpus hirsutus widely found in Western Ghats regions.*

### Introduction

*Artocarpus hirsutus* (Family: Moraceae) is found throughout tropical and subtropical region of India. It is widely found in Western Ghats areas of Chikmagalur. The deciduous trees are used in folk medicines and also it is reported to have antipyretic, antibacterial, antiviral, antifungal, antiarthritic activity. There is need to develop alternative antibiotic drugs from plants one approach is to screen local medicinal plants, which represent rich source of novel antiagents. A better understanding of medicinal value of plants now has been growing interest, regarding thousands of bioactive compounds that has been produced by plants are referred to as phytochemicals. They include Antioxidants, Steroids, Tannins, Terpenoids, Flavanoids, Anthocyanins, Phlobatannins, Saponins etc., These phytochemicals are found to provide protection against premature ageing, cardiovascular diseases, several common cancers and some chronic diseases.

Major disease preventative mechanisms of plants which have been identified in human dietary studies due to the action of Phytochemicals include; Antioxidant Activity, Detoxification of Carcinogens and Harmful Chemicals, Stimulation of the Immune System, Altered Hormone Metabolism, Blood Pressure Reduction, Antibacterial and Antiviral Properties, Maintain Normal DNA Repair, Inhibit Tumor Growth, Decreases Processes which Promote Cardiovascular Disease. Agents inhibit or neutralize potentially harmful compounds known as free radicals. Free radicals are produced during metabolic activity. Flavonoids and flavones are widely distributed secondary metabolites with antioxidant and antiradical properties. (Nakayama and Yamada, 1995). If cellular constituents do not effectively scavenge free radicals, they lead to disease conditions as described above. Antioxidant-based drugs/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. Sub-

sequently, a worldwide trend towards the use of natural phytochemicals present in berry crops, tea, herbs, oilseeds, beans, fruits, and vegetables has increased (Wang and Jiao 2000). Several herbs and spices have been reported to exhibit antioxidant activity, including rosemary, sage, thyme, nutmeg, turmeric, white pepper, chili pepper, ginger, and several Chinese medicinal plants extracts (Lee et al., 2003). 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). Free radicals, also known as reactive oxygen species, play a role in the etiology of several major diseases, including cancer, atherosclerosis, and diabetes. Based on available literature in phytochemistry we have taken up this research to explore natural potency of plants in antioxidant research.

### Materials and methods

#### Plant material

The selected species viz. *Artocarpus hirsutus* was collected from Western Ghats of Chikmagalur District Karnataka. Further plants were identified by Prof. Krishna V, Head and Chairman, Department of Biotechnology, Kuvempu University and voucher specimens were deposited at the same Department of Kuvempu University, Karnataka, India. The fruits were separated from the plant dried under shade for eight days.

#### Preparation of fruit extract

At first the fruit of the plant separated and shade dried. It was made into fine powder using blender. Powder was filtered through 30 mesh Filtered powder was taken in a blotting paper bag the apparatus was washed with methanol to remove water. Then the paper bag containing filter powder is placed inside the apparatus. The apparatus consists of a bulb, an extractor and the condenser. The ¾ of the bulb is filled with methanol. The ex-

tractor in the middle contains blotting paper bag and was filled with methanol [only half portion]. The condenser is meant for condensing the vapors. The temperature of the apparatus is adjusted to the boiling point of the solvent used. The temperature is set to 60°C. When methanol begins to boil the vapor moves through extractor and condensed in the condenser. The liquid falls on the blotting paper bag and extract from the material. The methanol level increases in the extractor and also simultaneously in the side tube. When the methanol level rises to maximum the entire solution falls down to the bulb. This constitutes one cycle. This is repeated until fruit contents are extracted or as per requirement of the experiment.

**Antioxidant assay**

The antioxidant activity of methanolic extract of *Artocarpus hirsutus* was determined by different in-vitro methods such as, DPPH free radical scavenging assay methods and the reducing power. The methanolic extract was dissolved in methanol at the concentration of 1mg/mL, all the assays were carried out in triplicate and average value was considered to calculate antioxidant activity.

**DPPH radical scavenging activity:**

DPPH scavenging activity of methanolic fruit extract of *Artocarpus hirsutus* was conducted according to the standard protocol of (H Makari et al., 2007) 0.2 mL of methanolic fruit extract at different concentration (20- 100µg/ mL) was mixed with 0.8 mL of Tris HCl buffer (100Mm, pH 7.4). One mL DPPH (500 M in methanol) solution was added to above mixture. The mixture was shaken vigorously and incubated for 30min in room temperature. Absorbance of the resulting solution was measured at 517nm UV-Visible Spectrophotometer (Systronics UV-Visible Spectrophotometer 117, INDIA). All the assays were carried out in triplicates with BHA (Butylated Hydroxy Anisole) as a positive control. Blank was prepared without the addition of DPPH and for control 0.2 mL of methanol (without fruit extract) was added. Percentage of DPPH radical- scavenging activity determined as follows.

$$\% \text{ DPPH radical-scavenging} = \frac{[(\text{Absorbance of control} - \text{Absorbance of test}) / (\text{Absorbance of control})] \times 100}{}$$

Sample) / (Absorbance of control)] X 100

Control was the DPPH solution without fruit extract.

Purified fruit sample 2mg/mL in Methanol of *Artocarpus hirsutus* was taken for antioxidant activity with a standard BHA (Butylated Hydroxy Anisole) antioxidant. Decreased absorbance of the reaction mixture stronger DPPH radical-scavenging activity.

**Reducing Power**

This was carried out as described previously (H Makari et al., 2007, Yildirim et al., 2001; Lu Y and Foo, Y 2000). 2 mg/1 mL of fruit extract solution (final concentration 100-500 mg/L) was mixed with 2.5 mL phosphate buffer (0.2 M, pH 6.6) and 2.5 mL potassium ferricyanide 10g/L, and then mixture was incubated at 50°C for 20 minutes. 2.5 mL of trichloroacetic 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<sub>3</sub> (1g/L) and absorbance measured at 700nm in UV-Visible Spectropho-

tometer (Systronics UV-Visible Spectrophotometer 117, INDIA). Ascorbic acid was used (final concentration 10 mg/mL). Increased absorbance of the reaction mixture indicates stronger reducing power.

**Results and Discussions**

**Antioxidant activity**

**DPPH radical scavenging activity**

The Percentage of DPPH radical scavenging activity of methanolic fruit extract of *Artocarpus hirsutus* presented in Table. 1. DPPH radical scavenging activity of the extract increases with increased concentration, 27.6% DPPH radical scavenging was present for 250µL. Although this fruit extract shows lower scavenging activity in comparison to BHA. Fruit extract exhibited antioxidative potential and increased concentration of fruit extract has shown increased antioxidative potential.

Concentration	OD 517nm		%of activity	
	Test	Standard	Test	Standard
50µl	1.060	2µl 0.873	4.5	21.35
100µl	1.040	4µl 0.679	6.3	38.83
150µl	0.903	6µl 0.482	18.6	56.58
200µl	0.890	8µl 0.359	19.8	67.66
250µl	0.803	10µl 0.274	27.6	75.32

**Table 1: Control OD AT 517nm - 1.110 (OD of METHANOL)**

**Reducing power**

Methanolic fruit extract of *Artocarpus hirsutus* exhibited good reducing power. The reducing power of ascorbic acid at concentrations between 100-500 mg/mL was determined. The reducing power of the fruit extract was determined by the method of (Ozaizu 1986, Makari H K et al., 2007). High absorbance of *Artocarpus hirsutus* indicates high reducing power. The reducing power of the methanolic fruit extract of *Artocarpus hirsutus* increases with the amount of extract increased. However, this reducing power is lower than that of ascorbic acid which was used as control. Therefore, the absorbance of ascorbic acid in a test was (10 mg/L) 1.10 while at the 500mg/L methanolic fruit extract concentration it was 0.46.

Test	Concentration (mg/l)	Absorbance (700 nm)
*Control	0	0.07 ± 0.06
Artocarpus hirsutus	100	0.10 ± 0.013
	200	0.27 ± 0.047
	500	0.46 ± 0.07
Ascorbic acid	5	0.39 ± 0.006
	10	0.76 ± 0.006
	15	1.10 ± 0.006

**Table 2: The control was test without plant extract. High absorbance of Artocarpus hirsutus indicated high reducing power.**

**Conclusion**

In the present investigation, the methanolic fruit extract of *Artocarpus hirsutus* showed potent antioxidant activity compared to reference standard BHA and ascorbic acid. Further study need to be upgraded in the direction of presence of secondary metabolites harbored in this plant. However, *Artocarpus hirsutus* can be used as a potent antioxidative agent and plants can be explored extensively for the future research.

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