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IN VITRO ANTIOXIDANT ACTIVITY OF ULTRA-SONIC BATH ASSISTED ETHANOL EXTRACT OF *ABUTILON INDICUM L.* LEAF

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ABSTRACT

The present study was undertaken to evaluate the invitro antioxidant activities of ultra-sonic bath sonicator assisted extract of ethanol extract of *Abutilon indicum* leaf was tested for Total phenolic content and DPPH (2,2-diphenyl, 2-picryl hydrazyl) radical scavenging assay. The result shows that extract of *Abutilon indicum L*. leaf possesses antioxidant activity when compared with standard shows the same.

Keywords: *Abutilon indicum L.*, Total phenolic content, DPPH radical scavenging assay.

INTRODUCTION

Antioxidants are a type of complex compounds found in our diet that act as a protective shield for our body against certain disastrous enemies (diseases) such as arterial and cardiac diseases, arthritis, cataracts and also premature ageing along with several chronic diseases. Antioxidants interact with and stabilize free radicals and may prevent some of the damage-free radicals might otherwise cause. Antioxidants come in a variety of forms include Vitamin E, Vitamin C, Carotenoids [1,2].

Abutilon indicum L. Sweet is an erect woody undershrub. Leaves ovate, cordate irregularly toothed, covered on both surfaces with white down. Flowers solitary, axillary; calyx 5-lobed, tubular below, lobes ovate-acute; corolla yellow, petals 5, connate below and adnate to the tube of the stamens. Ripe carpels 15-20, longer than the calyx, truncate or shortly awned. Throughout the Philippines in thickets and waste places in and about towns at low and medium altitudes. A common garden plant. Certainly introduced. Now pan tropic. *Abutilons* are popular garden plants in subtropical areas. The hardiest species, *A. vitifolium* from Chile, is hardy in warm temperate areas with moderate frost down to about −10 °C (14 °F). *Abutilon hybridum* is a popular group of hybrids that are semi-tropical, frost-tender shrubs typically growing 2–3 m tall. The lantern-like buds open to solitary, pendulous, bell- to cup-shaped flowers to 8 cm diameter with five overlapping petals and significant staminal columns typical of the mallow family. Flowers come in red, pink, yellow, white and pastel shades. Lobed, maple-like, light green leaves are often variegated with white and yellow [3].

MATERIALS AND METHODS

Plant material

The plant material was collected from Boduppal, Hyderabad district, and Andhra Pradesh, India. It was identified by Prof. B. Badharaih Department of Botany, Osmania University, and Hyderabad. Voucher no. 0097 of the plant was deposited in the Department of Botany, Osmania University, and Hyderabad. Air-dried under the shade at room temperature. Dried plant material was pulverized and the powder kept in polyethylene bags.

Preparation of plant extracts

Accurately weighed plant material was soaked in the conical flask by using ethanol solvent. Extraction was done by using ultra-sonic bath sonicator. Solvent recovery done by using simple distillation method. Extract was collected and stored in refrigerator. The percentage yield of prepared extract was around 10.5% w/w.

ANTIOXIDANT ACTIVITY

DPPH method

This assay is based on the measurement of the scavenging ability of antioxidant test substances towards the stable radical. The free radical scavenging activity [4] of the extract *Abutilon indicum L.* was examined in vitro using DPPH radical. The radical scavenging activities of the plant extract against DPPH radical (Sigma Aldrich) was

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determined by LABINDIA UV 3000$^+$ UV/VIS Spectrophotometer at 517 nm. Hydrogen atom or electron-donating ability of the leaf extract was measured from the bleaching of the purple-colored methanol solution of DPPH. This spectrophotometric assay uses stable DPPH radical as a reagent [5]. One ml of various concentrations of the extract was added to 3ml of methanol followed by 0.5 ml of1mM methanolic solution of DPPH. After incubation period at room temperature, the absorbance was read against a blank (A blank solution was prepared containing the same amount of methanol and DPPH except the test compound). Ascorbic acid (Vitamin-C) was used as the antioxidant standard at concentrations of 0.5 µg/ml-16 µg/ml. The radical scavenging activity (Inhibition of DPPH free radical in percent) was calculated using the following formula:

% DPPH radical scavenging = [(Absorbance$_{control}$ – Absorbance$_{sample}$) ÷ Absorbance$_{control}$] × 100

Determination of total phenolic content [6]

The content of total phenolic in leaf of *Abutilon indicum L.* extract was determined spectrometrically using Folin-Ciocalteu reagent by the method of MacDonald et.al. with modifications. Calibration curve was prepared by mixing ethanolic solution of Gallic acid (1ml; 0.010-0.400mg/ml) with 5ml Folin-Ciocalteu reagent (diluted 10 fold) and sodium carbonate solution in distill water(4ml,0.7M). The absorption was measured at 765nm using a LABINDIA UV 3000$^+$ UV/VIS Spectrophotometer. 1ml of plant extract (10gm/l=10mg/ml) was mixed instead of 1ml Gallic acid with the same reagent as described above in 3 different test tubes and after 1 hour the absorption was measured to determine the total phenolic contents. The absorbance was measured against a reagent blank, which was composed of the same reagents except test extract. The Gallic acid standard calibration curve was established by plotting concentration (mg/ml) vs. absorbance (nm)(y=0.05X-0.101,R^2 =0.986),where y is absorbance and X is concentration. Total content of phenolic in the leaf extract was expressed as Gallic acid equivalents (mg of GAE/g sample) and was calculated by the formula:

T= (C x V)/M

Where, T= total phenolic contents of the compounds, mg /gm. leaf extract, in GAE; C = The concentration of Gallic acid established from the calibration curve, mg/ml; V= The volume of extract, ml; M= The weight of ethanolic leaf extract, gm.

RESULTS AND DISCUSSIONS

DPPH method

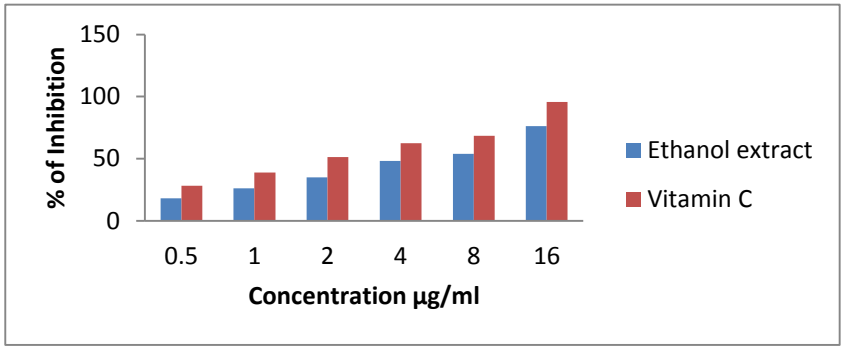
Radical Scavenging Increased with Antioxidant. The results of scavenging effect of tested plant extract on DPPH radical are given in Table 1. These results indicated that the extract of *Abutilon Indicum L.* leaf have notably reduced the stable free radical of DPPH Graph 1.to the yellow-colored Diphenyl picryl hydrazyl with an IC_{50} value (the concentration that Inhibits 50% of the DPPH radical)

5.4 µg/ml. showed in (Table 1) comparison with Ascorbic acid(Vitamin-C) (IC_{50} value of 1.9 µg/mL).

Table 1. Scavenging effect of ethanol extract of *Abutilon indicum L.* leaf and standard vitamin C on 1, 1'-Diphenyl-2-picryl hydrazyl (DPPH) radical. Results are mean ± S.D of five parallel measurements

Concentration	Ethanol extract	Vitamin-C
0.5 µg/ml	18.03	28.13
1.0 µg/ml	26.25	38.79
2.0 µg/ml	35.07	51.33
4.0 µg/ml	48.12	62.36
8.0 µg/ml	53.78	68.40
16.0 µg/ml	76.33	95.72

Graph 1. Scavenging effect of ethanol extract of *Abutilon indicum L.* leaf and standard vitamin C on 1, 1'-Diphenyl-2-picryl hydrazyl (DPPH) radical.Results are mean ± S.D of five parallel measurements



Determination of total phenolic content

The total phenolic content of the *Abutilon indicum L.* leaf extract measured by Folin-Cicalteu reagent in terms of Gallic acid equivalent (GAE) was 22.34 mg/gm. The phenolic compounds may contribute directly to antioxidative action. It is suggested that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans, when up to 1.0 g daily ingested from a diet rich in fruits and vegetables [7]. From the results suggested that phenolic compounds appear to be responsible for antioxidant activity of ethanol extract of *Abutilon indicum* leaf.

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