

Antioxidant Activity of Fractions from *Tridax procumbens*

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ABSTRACT

Harmful free radicals are generated in the body during normal metabolism and upon exposure to environmental results such as infectious agents, pollution, UV light and radiation and so on. When harmful free radicals are not neutralized by the body's primary and secondary defense mechanisms, an excess of harmful radicals exists. Clinical studies have also shown that supplemental levels of antioxidant vitamins (Vitamin E, Vitamin C and β -carotene) reduce an individuals risk for certain cancers and cardiovascular disease. *Tridax procumbens* is commonly known as Coat Button or Kansari (Hindi) or Ghamara (in local language) and belongs to family asteraceae. It shows a number of pharmacological activities like hypotensive, insecticidal, leishmanicidal, hair growth promoting, wound healing, anti-inflammatory, hepatoprotective and immunomodulatory, due to the presence of phenolics, tannins, saponins, and glycosides. Hence efforts were taken to evaluate fractions of methanolic extract for antioxidant activity by DPPH method. The Ethyl acetate and n-Butanol fractions have shown significant activity which is comparable to the activity of standard antioxidant Ascorbic acid.

Key words: DPPH Scavenging, *Tridax procumbens*, antioxidant activity

INTRODUCTION

Free radicals are produced in normal and/or pathological cell metabolism. Oxidation is essential to many living organisms for the production of energy to fuel biological processes. However, the uncontrolled production of oxygen derived free radicals is involved in the onset of many diseases such as cancer, rheumatoid arthritis, cirrhosis and arteriosclerosis as well as in degenerative processes associated with aging. Exogenous chemical and endogenous metabolic processes in the human body or in the food system might produce highly reactive free radicals, especially oxygen derived radicals, which are capable of oxidizing biomolecules, resulting in cell death and tissue damage [1]. Awareness of the potential benefits of antioxidant nutrients in health maintenance is growing. Antioxidants are means for the substances or group of the substances that delay or inhibit oxidative damage to a molecule. This defense system is having many modes of

classification such as based on their mechanism of action (chain breaking, preventive) [2-5]. Investigations into the health maintaining properties of plants have resulted in the identification of a wide array of bioactive compounds in plants that include flavonoids, phenolics, limonoids, carotenoids, coumarins, phytosterols, etc. Based on recent research, several compounds from fruits and vegetables were found to possess anticarcinogenic and antioxidant activities [6]. The literature survey reveals that *Tridax procumbens* plant posses good antioxidant activity. [7] Hence efforts were taken to evaluate the fractions obtained from the methanolic extract of the plant for antioxidant activity by DPPH method.

EXPERIMENTAL

Collection and authentication of plant material:

The whole aerial parts of the plant *Tridax procumbens* were collected in the months of August -December from the locality in Maharashtra state, India, and authenticated by the

Dr. A. N. Patil, authority of botany department, Late Karmveer Dr. P.P. Ghogrey Science College, Dhule (M.S.), India and a specimen of the herbarium was stored in the department for future reference.

Herbarium No:- SA-1 - *Tridax procumbens*

Extraction of plant material:

The collected aerial part of the plant was dried under shade and size reduction of plant material was carried out.

Extraction of coarsely powdered plant material i.e. *Tridax procumbens* was carried out with methanol by Soxhletion.

Fractionation of methanolic extract:

The dried methanolic extract was then fractionated by using the solvent according to polarity in ascending order i.e. by using, chloroform, ethyl acetate, n-butanol and remnant was considered as a residual (Water fraction) of methanolic extract. Each fraction was dried in oven at 40^o-50^oC.

Table : * Fractionation of Methanolic Extract

Solvent	Polarity index	Fractionation	% Yield
Chloroform	4.1	Liquid-liquid partitioning	32
Ethyl acetate	4.4	Liquid-liquid partitioning	4
N-butanol	5.3	Liquid-liquid partitioning	18.5
Water fraction	10.2	Remnant	45.5

Evaluation of methanolic extract fractions for antioxidant activity

The free radicals scavenging activity of the *Tridax procumbens* fractions and Ascorbic acid was measured in terms of hydrogen donating or radical-scavenging ability using the stable radical DPPH [8-11]. 0.1 mM solution of DPPH in methanol was prepared and 1.0 ml of this solution was added to 3.0 ml of extract solution in water at different concentrations (10-100 µl/ml). Thirty minutes later, the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The

capability to scavenge the DPPH radical was calculated by using following equation:

$$\text{Scavenging Effect (\%)} = [1 - \text{Abs. of Sample} / \text{Abs. of Control}] \times 100$$

The antioxidant activity of the fractions was expressed as IC₅₀. The IC₅₀ value was defined as the concentration (in µg/ml) of methanolic extract fractions that indicates the formation of DPPH radicals by 50%.

Table 1: Evaluation of methanolic extract fractions from *Tridax procumbens* for antioxidant activity

Fraction	Concentration (mg/ml) and % Inhibition						IC ₅₀ (mg/ml)
	10	20	40	60	80	100	
ASC	28.66± 0.35	47.51± 0.55	64.00± 0.35	77.84± 0.56	89.79± 0.41	96.6± 0.48	21.04
EF	22.60± 0.53	42.01± 0.45	57.22± 0.38	72.18± 0.54	80.88± 0.39	93.12± 0.57	23.81
BF	15.5± 0.42	33.7± 0.34	53.74± 0.39	67.24± 0.49	76.12± 0.41	89.39± 0.48	29.67
AQF	11.2± 0.31	25.7± 0.36	50.10± 0.41	60.12± 0.32	71.11± 0.43	78.60± 0.46	38.91

Legends:ASC – Ascorbic acid,EF – Ethyl acetate fraction,BF – n-Butanol fraction, AQF – Aqueous fraction

Preparation of stock test extract solutions

An accurately weighed quantity of fractions (~100 mg) was dissolved in methanol and volume was made up to 100 ml with methanol (100 µg/ml).

Preparation of working test solutions

The aliquot portion of stock solution of test extract solutions were diluted appropriately with methanol to obtain a concentration range of 10-100 mg /ml.

DISCUSSION AND CONCLUSION:

Many plants exhibit *in vitro* and an *in vivo* antioxidant property owing to their phenolics, proteins, vitamins and pectins contents. In the different literatures, it has been found that the antioxidant activity of plant extracts is responsible for their therapeutic effect against atherosclerosis, CVDs and cancer. Hence, *Tridax procumbens* plant extracts were

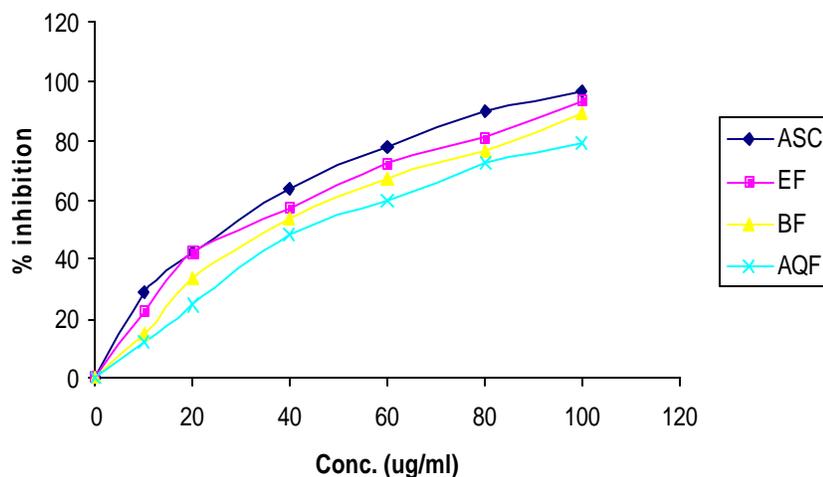


Figure 1: Effect of methanolic extract fractions of *Tridax procumbens* on accumulation of DPPH

evaluated for *in vitro* antioxidant activities. DPPH method provides a good assessment for evaluation of *in vitro* antioxidant activity. It is based on reaction between antioxidant (AH) with nitrogen centered free radical i.e. DPPH (1, 1-diphenyl, 2-picryl hydrazyl). The Ethyl acetate and n-Butanol fractions from methanolic extract have shown significant activity which is comparable to the activity of Ascorbic acid, as shown in Table No. 1 and Fig No.1. Fractionation of the parent extract reduced the complexity of material and provided more accurate idea related to the Phytochemicals, responsible for antioxidant activity of *Tridax procumbens*.

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