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Research article

Evaluation of Anti-Oxidant Activity of *Oorithal Thaamarai Chooranam OTC by* DPPH Assay

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ABSTRACT

The objective of the present study was to evaluate the antioxidant activity of extract of *Hybanthus enneaspermus*. Phytochemical analysis was performed to estimate tannins, flavonoids and alkaloids. Antioxidant activity was per- formed using DPPH . The phytochemical screening revealed that the extract contain terpenes, flavonoids and alkaloids.

Antioxidant activity was performed due to presence of phenolic compounds; flavonoids which may possess significant antioxidant potential. The results suggested that *Hybanthus enneaspermus* has promising antioxidant activity and could serve as potential source of natural antioxidants.

Keywords:

Hybanthus enneaspermus; Antioxidant activity; Flavonoids; DPPH

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INTRODUCTION

Free radicals are chemical species contains unpaired electrons which makes them highly reactive towards biological molecules. Free radicals are produced in the human body due to the various detoxification processes [1]. Ultraviolet light, radiation and metabolic processes can induce the production of free radicals. Free radicals can react with protein, lipid and DNA and may cause tis- sue destruction which leads various diseases. Antioxidants are the compounds which reduce free radical activities and thus used for the treatment of many diseases induced by oxidative destruction of tissue. Antioxidants neutralize or scavenge reactive free radical species by hydrogen donation [2]. Medicinal plants contain phenolic compounds; tannins, flavonoids are known to have antioxidant property [3]. The antioxidant activity of phenolics is principally due to their redox properties, which allow them to act as reducing agents and hydrogen donors. Phenolic compounds play an important in the treatment of cancer, heart diseases and ageing [4].

Hybanthus enneaspermus (Linn) F. Mull is a *violaceae* family plant distributed in the tropical and sub tropical regions, 15-30 cm in height [5], used to treat diarrhea, urinary tract infections and diabetes. Chemically it possess many bioactive components such as phenol, alkaloids and flavanoids [6], due to the presence of phenolics compounds present investigation was carried out which involve phytochemical screening and estimation of antioxidant activity of plant.

MATERIALS AND METHODS

The collected plants were open-air-dried under the shade, pulverized in to a moderately coarse powder and stored for further use.

Preparation of Plant Extract

Powdered plant material was extracted with methanol. After 24 hours the supernatant was collected by filtration and the solvent was evaporated to make the crude extract. The residues obtained were stored in airtight bottles in a refrigerator for further use.

DPPH (2, 2-Diphenyl 1-2 picrylhydrazyl) Assay.

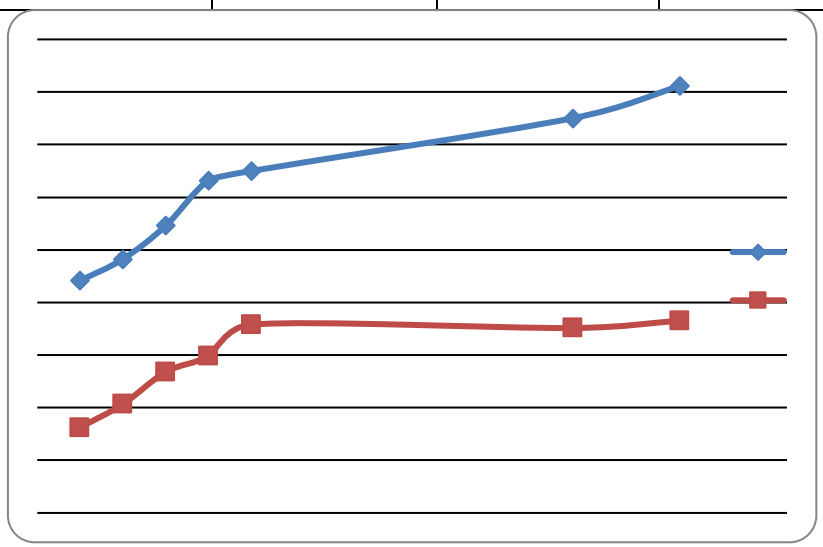
The antioxidant activity of test drug sample *OTC* was determined using the 2,2-diphenyl 1-2 picrylhydrazyl (DPPH) free radical scavenging assay . Sample *OTC* was mixed with 95% methanol to prepare the stock solution in required concentration. From the stock solution the serial dilution the concentration of 10,20,40,60,80,100,250,300 was made respectively. Ascorbic acid were used as standard was prepared in same concentration as that of the sample extract by using methanol as solvent. Final reaction mixture containing 1 ml of 0.3 mM DPPH methanol solution was added to 2.5 ml of sample solution of different concentrations and allowed to react at room temperature. Absorbance in the presence of test sample *OTC* at different concentration of 10,20,40,60,80,100,250,300 was noted after 15 min incubation period at 370C. Absorbance was read out at 517 nm using double-beam U.V Spectrophotometer by using methanol as blank.

% scavenging = [Absorbance of control - Absorbance of test sample/Absorbance of control] X 100.

The effective concentration of test sample *OTC* required to scavenge DPPH radical by 50% (IC50 value) was obtained by linear regression analysis of dose-response curve plotting between %inhibition and concentrations

Results of Analysis of DPPH radical scavenging Assayof *OTC*

S.No	Concentration	Ascorbic acid (Standard)		*OTC*	
		Absorbance	% inhibition	Absorbance	% inhibition
1	20	1.058 ± 0.0017	88.29 %	1.477 ± 0.0018	32.50 %
2	40	0.808 ± 0.0015	96.50%	1.635 ± 0.0018	41.35%
3	60	0.680 ± 0.0020	109.20%	0.907 ± 0.0020	53.62%
4	80	0.468 ± 0.0025	126.40%	0.802 ± 0.0020	59.55%
5	100	0.273 ± 0.0026	130.00%	0.718 ± 0.0027	71.60%
6	250	0.180 ± 0.0028	150.05 %	0.701 ± 0.0029	70.30%
7	300	0.230 ± 0.0031	162.30%	0.650 ± 0.00831	73.10 %
	Ic 50 values		Ic_{50}= 6.1 µg/ml		Ic_{50} = µg/ml



RESULTS

The results of DPPH radical scavenging assay of the sample *OTC* shows that the test drug possesses concentration dependent scavenging activity on DPPH radicals.The value of DPPH free radical scavenging activity of the *OTC* was given in(Table 1 and Figure 1). The extract of *OTC* showed the highest DPPH scavenging activity 73.10 %at conc 300 and the lowest percentage of inhibition(32.50 %) at conc 20 . Ascorbic acid (Standard)showed highest percentage of inhibition(162.30%)at 300 and the lowest percentage of inhibition (%)88.29 at conc 20.

DISCUSSION

Free radicals and other oxidants have gained importance in the field of biology due to their central role in various physiological conditions as well as their implication in a diverse range of diseases. The free radicals, both the reactive oxygen species (ROS) and reactive nitrogen species (RNS), are derived from both endogenous sources (mitochondria, peroxisomes, endoplasmic reticulum, phagocytic cells etc.) and exogenous sources (pollution, alcohol, tobacco smoke, heavy metals, transition metals, industrial solvents, pesticides, certain drugs like halothane, paracetamol, and radiation). Free radicals can adversely affect various important classes of biological molecules such as nucleic acids, lipids, and proteins, thereby altering the normal redox status leading to increased oxidative stress . Large number of medicinal plants has been investigated for their antioxidant properties. Natural antioxidants either in the form of raw extracts or their chemical constituents are very effective to prevent the destructive processes caused by oxidative stress.Substantial evidence has accumulated and indicated key roles for reactive oxygen species (ROS) and other oxidants in causing numerous disorders and diseases. The evidence has brought the attention of scientists to an appreciation of antioxidants for prevention and treatment of diseases, and maintenance of human health . Human body has an inherent antioxidative mechanism and many of the biological functions such as the anti-mutagenic, anti-carcinogenic, and anti-aging responses originate from this property . Antioxidants stabilize or deactivate free radicals, often before they attack targets in biological cells . Recently interest in naturally occurring antioxidants has considerably increased for use in food, cosmetic and pharmaceutical products, because they possess multifacetedness in their multitude and magnitude of activity and provide enormous scope in correcting imbalance. The results of DPPH radical scavenging assay of the sample *OTC* shows that the test drug possesses concentration dependent scavenging activity on DPPH radicals with the highest percentage inhibition of about 73.10 %.

CONCLUSION

Imbalance between the antioxidants and oxidant leads to increased generation of free radicals which in turn causes vigorous damage to macromolecules such as nucleic acids, proteins and lipids. This leads to tissue damage in various disease conditions such as diabetes mellitus, neurodegenerative diseases, cancer, cardiovascular diseases, cataracts, rheumatoid arthritis, asthma etc. and thus severely hastening the disease progression. From the result obtained from the present investigation it was concluded that the formulation *OTC* possess significant antioxidant property and may act therapeutically in treating several oxidative stress related disorder's. Further present investigation had generated an evidence based data with respect to purity, standards and antioxidant potential of the formulation *OTC.*

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