Academic Sciences International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 6, Issue 3, 2014

Research Article

EVALUATION OF ANTIOXIDANT ACTIVITY OF LEAF EXTRACTS OF *HOLOPTELEA INTEGRIFOLIA* (ROXB) PLANCH

RAVINDRA C. SUTAR[1*], V. K. KALAICHELVAN[2]

[1]Department of Pharmacology, Sanjivani College of Pharmaceutical Education and Research, Kopargaon. At- Sahajanandnagar, Post-Shinganapur 423603,Tal- Kopargaon, Dist-Ahmednagar, Maharashtra, India, [2]Department of Pharmacy, Annamalai University, Annamalai Nagar, Chidambaram 608002, Tamil Nadu, India. Email: ravi_sutar1980@yahoo.com

Received: 19 Feb 2014, Revised and Accepted: 29 April 2014

ABSTRACT

Holoptelea integrifolia (Roxb.) Planch has been used from the long time in traditional medicine. The main objective of the work was to evaluate the antioxidant activity of leaves of Holoptelea integrifolia (Roxb.) Planch. The antioxidant activity of petroleum ether and methanolic extract of Holoptelea integrifolia (HI) leaves was evaluated using Hydroxyl Radical Scavenging Activity and Total Reduction Capability models. Preliminary Phytochemical investigation of the petroleum ether extract (PEHI) of Holoptelea integrifolia leaves reveals the presence of steroids, terpenoids, alkaloids, glycosides, flavonoids, proteins, tannins, and carbohydrate while methanolic extract (MHI) of Holoptelea integrifolia showed the presence of steroids, alkaloids, flavonoids, proteins and carbohydrates. In case of Hydroxyl Radical Scavenging Activity both the HI extracts showed dose dependant increase in percent inhibition i. e. Hydroxyl radical scavenging activity and thereby showed antioxidant activity however PEHI is more potent than MHI in this regard, while in case of Total Reduction Capability both the HI extracts have shown dose dependent increase in absorbance thereby dose dependant total reduction capacity indicating antioxidant activity. Further trend suggests that PEHI has more potent antioxidant potential than that of MHI. The results indicate that petroleum ether and methanol extracts contained such phytochemial compounds which are active in case of antioxidant activity using Hydroxyl Radical Scavenging Activity and Total Reduction Capability. Which support the ethnomedicinal application of the plant as an antioxidant agent.

Keywords: *Holoptelea integrifoila* leaves, Antioxidant, Hydroxyl Radical Scavenging Activity, Total Reductioncapabilityy.

INTRODUCTION

Oxygen is essential for the survival of all on this earth. During the process of oxygen utilization in normal physiological and metabolic processes, approximately 5% of oxygen gets univalently reduced to oxygen derived free radicals[1,2] like superoxide, hydrogen peroxide, hydroxyl and nitric oxide radicals. All these radicals known as reactive oxygen species (ROS) exert oxidative stress towards the cells of human body rendering t each cell to face about 10000 oxidative hits per second.[3] When generation of ROS overtakes the antioxidant defense of the cells, the free radicals start attacking the cell proteins, lipids and carbohydrates [4-6] and this leads to a number of physiological disorders. Free radicals are involved in the development of degerative diseases.[6] They have also been implicated in the pathogenesis of diabetes, liver damage, nephrotoxicity, inflammation, cancer, cardiovascular disorders, neurological disordes and in the process of ageing.[7] Many plants often contain substantial amounts of antioxidants including vitamin C and E, carotenoids, flavonoids and tannins etc. and thus can be utilized to scavenge the excess free radicals from the body.[8]

In traditional system of medicine, bark and leaves of *Holoptelea integrifoila* (HI)used as bitter, astringent, acrid, thermogenic, antiinflammatory, digestive, carminative, laxative, anthelmintic, depurative, repulsive, urinary astringent and in rheumatism.[9,10] The plant Holoptelea integrifolia is used traditionally for the treatment of inflammation, gastritis, dyspepsia, colic, intestinal worms, vomiting, wound healing, leprosy, diabetes, hemorrhoids, dysmenorrhoea and rheumatism.[11] But, the antioxidant activity of *Holoptelea integrifoila* leaves is not yet validated scientifically as on date. Hence in the current dissertation the antioxidant activity of petroleum ether and methanol extract of leaf of Holoptelea integrifolia leaves is evaluated.

MATERIALS AND METHODS

Plant introduction

Holoptelea integrifoila belongs to the family ulmaceae commonly called as Indian Elm and frequently used in India by the tribal people for its medicinal properties. The mucilaginous bark is boiled and the juice squeezed out and applied to rheumatic swellings.[12] leaves of *Holoptelea integrifoila* were collected in the month of August from the agricultural fields of Tirunelveli district, TamilNadu, India. The plant was identified and leaves of Holoptelea integrifolia were authenticated and confirmed from Dr. V. Chelladurai, Research Officer, Botany, C. C. R. A. S. (Retired), Govt. of India by comparing morphological features (leaf and stem arrangement, flower /inflorescence arrangement,fruit and seed morphology etc.). The collected plant material was shade dried to retain its vital phytoconstituents and then subjected to size reduction for further extraction process.

Fig. 1: Habitat of *Holoptelea integrifolia* (Roxb.) Planch.

Preparation of petroleum ether and methanol extract

Successive extraction of leaves of *Holoptelea integrifolia* was prepared on the basis of solvent polarity. For extraction the powder of *Holoptelea integrifoila* leaves was charged in to the thimble of a Soxhlet apparatus and extracted using petroleum ether. Appearance of colourless solvent in the siphon tube was the indication of exhaustive extraction and based on that the further extraction was terminated. The extract was then transferred into the previously weighed empty beaker and evaporated to a thick paste on the water bath, maintained at 50^0 C to get petroleum ether extract. The extract was finally air dried thoroughly to remove all traces of the solvent and the percentage yield was calculated. Perfectly dried extract was then stored in an air tight container in a refrigerator below 10 ^{0}C. After obtaining petroleum ether extract the marc was pressed and it is air dried and again it was extracted using methanol. Appearance of colourless solvent in the siphon tube was the

indication of exhaustive extraction and based on that the further extraction was terminated. The extract was then transferred into the previously weighed empty beaker and evaporated to a thick paste on the water bath, maintained at 50^0 C to get semi solid mass of methanol extracts. The extract was stored in an airtight container in a refrigerator below 10^0C.

The Petroleum ether and Methanol extracts of *Holoptelea integrifolia* leaves were subjected to the following investigations,

1. Preliminary photochemical screening.

2. Antioxidant activity

Drugs

Phosphate buffer, hydrogen peroxide and vitamine- C, all the chemicals used in the study were of analytical grade and procured from Merck India Pvt. Ltd.

Preliminary phytochemical screening of extracts

The extracts were subjected to follow chemical tests to detect the phytochemical constituents present in them. 0.5 gm of extract was dissolved in 5 ml of distilled water and filtered. The filtrate was used to determine the presence of various phytoconstituents.[13]

Assessment of antioxidant activity

Hydroxyl radical scavenging activity of HI extracts

Stock solutions of EDTA (1 mM), FeCl3 (10 mM), ascorbic acid (1 mM), H_2O_2 (10 mM) and deoxyribose (10 mM) were prepared in distilled de ionized water. The assay was performed by adding 0.1 ml EDTA, 0.01 ml of $FeCl_3$, 0.1 ml of H_2O_2, 0.36 ml of deoxyribose, 1.0 ml of the extract (10 – 100 µg/ml) dissolved in distilled water, 0.33 ml of phosphate buffer (50 mM, pH 7.4) and 0.1 ml of ascorbic acid in sequence. The mixture was then incubated at 37 °C for 1 h. A 1.0 ml portion of the incubated mixture was mixed with 1.0 ml of 10% TCA and 1.0 ml of 0.5% TBA (in 0.025M NaOH containing 0.025% BHA) to develop the pink chromogen measured at 532 nm. The hydroxyl radical scavenging activity of both the extracts is reported as % inhibition of deoxyribose degradation and is calculated as

$$\% \text{ Inhibition} = [(A_0\text{-}A_1) / A_0 \times 100]$$

Where, A_0 was the absorbance of the control (blank) and A_1 was the absorbance in the presence of different extracts. [14,15]

Total reduction capability

Total reduction capability of both the HI extracts (20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml and 100µg/ml) and Vit C (20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml and 100µg/ml) were estimated by using the method of Oyaizu. Different concentrations of HI extracts and Vit C were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide (1%, 2.5 ml). The mixture was incubated at 50º C for 20 min. A portion of (2.5 ml) trichloroacetic acid (10 %) was added to the mixture. Then it was centrifuged for 10 min at 1000 g. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and ferric chloride (0.5 ml, 0.1%) and the absorbance was measured at 700 nm by using spectrophotometer. Higher absorbance of the reaction mixture indicated greater reducing power.[16, 17]

RESULTS

Phytochemical examination of extracts.

Preliminary phytochemical analysis of petroleum ether extract of *Holoptelea integrifolia* showed the presence of steroids, terpenoids, alkaloids, glycosides, flavonoids, proteins, tannins and carbohydrates while methanolic extract of *Holoptelea integrifolia* showed the presence of steroids, alkaloids, flavonoids, proteins and carbohydrates.

Hydroxyl radical scavenging activity of HI extracts

Both the HI extracts showed dose dependant increase in percent inhibition i.e. hydroxyl radical scavenging activity and thereby showed antioxidant activity however PEHI is more potent than MHI in this regard. The observations are given in table no.1

Table 1: Hydroxyl radical scavenging activity of HI extracts at different concentrations

Concentration	Control	PEHI (% inhibition)	MHI (% inhibition)	Vitamin- C (% inhibition)
20 µg/ml	0.1011	0.0911 (09.89)	0.0925 (08.41)	0.0905 (10.39)
40 µg/ml	0.1029	0.0920 (10.59)	0.0944 (08.26)	0.0862 (21.16)
60 µg/ml	0.1021	0.0875 (14.29)	0.909 (10.96)	0.0771 (30.45)
80 µg/ml	0.1030	0.0865 (16.01)	0.0914 (11.26)	0.0755 (28.80)
100 µg/ml	0.1050	0.0835 (20.47)	0.0902 (14.09)	0.0770 (49.67)

Where PEHI: Petroleum ether extract of *Holoptelea integrifolia* leaves,

MHI: Methanol extract of *Holoptelea integrifolia* leaves. Vitamin- C was used as the positive control

Table 2: Reducing power assay of hi extracts at different concentrations

Concentration	PEHI	MHI	Vitamin-C
20 µg/ml	0.5317	0.5112	0.7143
40 µg/ml	0.6009	0.5850	0.7710
60 µg/ml	0.7132	0.6476	0.8440
80 µg/ml	0.8816	0.7015	0.9122
100 µg/ml	1.0124	0.9018	1.1023

Where PEHI: Petroleum ether extract of *Holoptelea integrifolia* leaves,

MHI: Methanol extract of *Holoptelea integrifolia* leaves. Vitamin- C was used as the positive control

Total reduction capability

Both HI extracts have shown dose dependent increase in absorbance thereby dose dependant total reduction capacity indicating antioxidant activity. Further trend suggests that PEHI has more potent antioxidant potential than that of MHI. The observations are given in table no.2

DISCUSSION

Free radicals are chemical entities that can exist separately with one or more unpaired electrons. The propagation of free radicals can bring about thousands of reactions and thus may cause extensive tissue damage. Lipids, proteins, and DNA are all susceptible to attack by free radicals.[4,5] Antioxidants may offer resistance against oxidative stress by scavenging the free radicals, inhibiting lipid peroxidation etc.

In the present investigation, preliminary phytochemical analysis and the earlier scientific studies have shown that petroleum ether and methanolic extract of *Holoptelea integrifolia* leaves showed the prominent presence of steroids, triterpenoids, glycosides, saponins, flavonoids, proteins, tannins and carbohydrates. The previous scientific studies have shown that these secondary plant metabolites are mainly responsible for the pharmacological actions and thus thereby it supported the traditional uses.[18, 19] Which may also be responsible for various actions of *Holoptelea integrifolia*. This gives a green signal towards further exploration of this plant for the validation of traditional claims for various complaints for which there is either no or very limited satisfactory pharmacotherapy.

As the plant has shown its potential effectiveness in treating various disorders for which the most common mechanism may be through its antioxidant potential. Also, *Holoptelea integrifolia* has been traditionally claimed to possess antioxidant properties. So in order to assess its efficacy as a potent antioxidant agent the plant was investigated using two in vitro models namely hydroxyl radical scavenging activity and total reducing ability models.

Hydroxyl radical is the most reactive oxygen species among all reactive oxygen species owing to its strong ability to react with various biomolecules. Hydroxyl radical reacts with several biological materials oxidatively by hydrogen withdrawal, double-bond addition, electron transfer and radical formation, and initiates autoxidation, polymerization, and fragmentation. Hydroxyl radicals are highly reactive biological molecules and its scavenging may provide an important therapeutic approach against oxidative stress induced ailments.[20] HI extracts showed dose dependant increase in percent inhibition i.e. hydroxyl radical scavenging activity. Total reducing ability is considered as the ability of Fe3+–Fe2+ transformation. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity.[21,22] The HI extracts showed dose dependant total reduction capacity by dose dependent increase in absorbance indicating total reducing ability in turn potential antioxidant activity. These results indicate its usefulness in various disorders associated with oxidative stress.

CONCLUSION

The results indicate that petroleum ether and methanol extracts contained such phyto-chemial compounds which are active in case of antioxidant activity using Hydroxyl Radical Scavenging Activity and Total Reduction capability models. The petroleum ether extract of Holoptelea integrifolia leaves is more potent for the observed antioxidant activity than methanol extract. The observed antioxidant activity may be due to the presence of phytosterols and flavonoids in both the extracts, which support the ethnomedicinal application of the plant as an antioxidant agent. Further studies are required to find and isolate active principles and determine the mechanism of their antioxidant action. Also our study suggests the application of Holoptelea integrifolia leaves as an antioxidant.

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