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EVALUATION OF ANTIOXIDANT ACTIVITY OF ISOLATED PHYTOSTEROL FROM LEAVES OF HOLOPTELEA INTEGRIFOLIA (ROXB.) PLANCH

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ABSTRACT

Holoptelea integrifolia (Roxb.) Planch has been used from long time in traditional medicine. The main objective of the work was to evaluate the antioxidant activity of Holoptelea integrifolia isolated Phytosterol *(HIIP). The antioxidant activity of HIIP isolated from petroleum ether extract of leaves was evaluated using Hydroxyl Radical Scavenging Activity. Preliminary Phytochemical investigation of the petroleum etherextract (PEHI Scavenging Activity and thereby showed antioxidant activity. Scavenging Activity and thereby showed antioxidant activity*. The results confirms that HIIP from petroleum ether extract also shows antioxidant activity using Hydroxyl Radical Scavenging Activity, which suggests the application of the plant as an antioxidant agent.

KEYWORDS: Holoptelea integrifoila isolated phytosterol, Antioxidant, Hydroxyl Radical Scavenging Activity.

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, rheumatism.[11] In our previous studies the antioxidant activity of petroleum ether and methanol extract of leaf of Holoptelea integrifolia was evaluated and it was found that astringent, acrid, thermogenic, antiinflammatory, digestive, carminative, laxative, anthelminti c, 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 petroleum ether extract (PEHI) has more significant antioxidant activity than methanolic extract (MHI)[12] in the similar lines in this present study a phytosterol (HIIP) was isolated from petroleum ether extract and it was studied for antiarthritic activity. Hence in the current dissertation the antioxidant activity of Isolated phytosterol from petroleum ether 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 it's medicinal properties. The mucilaginous bark is boiled and the juice squeezed out and applied to rheumatic swellings.[13] 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 integ rifolia* were authenticated and confirmed from Dr.V.Chelladurai, Research Officer, Botany, C.C.R.A.S. (Retired), Govt. of India by compairing 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.

High Performance Thin Layer Chromatography (HPTLC) Instrumentation

HPTLC system of CAMAG, Muttenz, Switzerland, Anchrom Enterprises (I) Pvt. Ltd, Mumbai, consisting of sample applicator (Linomat 5), Twin trough chamber with lid (20×10 cm, CAMAG, Muttenz, Switzerland), UV cabinet (Aetron, Mumbai) with dual wavelength (254/366 nm) and the HPTLC photo documentation (Aetron, Mumbai) were used for study. UV spectra was recorded using CAMAG TLC Scanner - IV, LC/MS was recorded using SHIMADZU- LC/MS 2020, IR spectra was recorded using SHIMADZU-IR PRESTIGE-21, NMR spectra was recorded using Mercury plus 300 MHZ NMR Spectrometer.

Plant Material, Extraction and Isolation of *Holoptelea integrifolia* phytosterol (HIIP) from petroleum ether extract by preparative TLC

The dried and powdered leaves (1kg) of *Holoptelea integrifolia* was extracted with petroleum ether (b.p. 60-80ºC) for three times. After evaporation of the solvent under reduced pressure, the yield obtained was 4.8%w/w.

The petroleum ether extract was prepared in petroleum ether as a sample solution applied on Precoated silica gel aluminium plates 60F254, 20 cm x 10 cm with 250 µm thickness with CAMAG Linomat V (Switzerland) was used. The plates were washed by methanol and

activated at 120^0 C for 20 min before the start of chromatography. The sample solution was applied by using CAMAG microlitre syringe on the plates. The distance between the 2 bands was 5 mm with constant application rate of 1.0 µl/s was applied.

The compostion of mobile phase used for isolation of phytosterol was Chloroform: Ethyl acetate, in the volume ratio of 4:6 (v/v) and 20 ml of mobile phase was used per chromatography.

The plates were developed in 20 cm x 10 cm twin trough glass chamber saturated with filter paper Whatmann No.1 in mobile phase for 20 min at room temperature, and length of chromatogram run was 8.0 cm.

TLC plates were dried with the help of air dryer. Later on, densitometric scanning was performed with CAMAG TLC Scanner IV at 540 nm. The TLC Plate was dipped in Anisaldehyde Sulphuric acid reagent and then dried in oven at 110 ^{o}C. Concentration of the compound was then determined.

The yield of HIIP obtained was 6 mg for a total of 40 preparative TLC Plates. In order to get sufficient quantity of HIIP, TLC plate of 1mm thickness was used. 20 gm of PEHI has given 228 mg, HIIP yield by using this method.[14-22]

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.

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

1. Preliminary photochemical screening
2. Antioxidant activity

Preliminary phytochemical screening of petroleum ether extract

The extract was subjected to follow chemical tests to detect the phytochemical constituents present in it. 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.[23]

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 deionized 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

% 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.[24,25]

RESULTS

Hydroxyl Radical Scavenging Activity of HIIP

HIIP showed dose dependant increase in percent inhibition i.e. hydroxyl radical scavenging activity and thereby showed antioxidant activity. The observations are given in table no.1.

Table No. 1: Hydroxyl Radical Scavenging Activity of HIIP At Different Concentrations

Concentration	Control (Blank)	HIIP (% inhibition)	Vitamin- C (% inhibition)
20 µg/ml	0.1011	0.0878 (13.15)	0.0905 (10.39)
40 µg/ml	0.1029	0.0884 (14.09)	0.0862 (21.16)
60 µg/ml	0.1021	0.0835 (18.21)	0.0771 (30.45)
80 µg/ml	0.1030	0.0833 (19.12)	0.0755 (28.80)
100 µg/ml	0.1050	0.0808 (23.04)	0.0770 (49.67)

Where HIIP: *Holoptelea integrifolia* Isolated Phytosterol, Vitamin- C was used as the positive control

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 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[26,27] Which may also be responsible for the 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 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 in vitro model namely hydroxyl radical scavenging activity.

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.[28] The HIIP showed dose dependant increase in percent inhibition i.e. hydroxyl radical scavenging activity which serve as a significant indicator of its potential antioxidant activity. These results indicate its usefulness in various disorders associated with oxidative stress.

CONCLUSION

Majority of scientific documentation suggested prominent role of phytosterols towards anti oxidant activity. Hence, HIIP from petroleum ether extract of leaves of HI have naturally exhibited significant antioxidant activity using Hydroxyl Radical Scavenging activity, which support the ethnomedicinal application of the plant as an antioxidantagent, also our studies confirm the application of Holoptelea integrifolia leaves as an antioxidant.

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