ISSN: 0975 -8542

Journal of Global Pharma Technology

Available Online at: www.jgpt.co.in

RESEARCH ARTICLE

Evaluation of Antioxidant and Anticancer Activity of *Kohautia aspera* (Heyne ex Roth) Bremek

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Abstract

The purpose of present study was to investigate the antioxidant and anticancer activities of selected extracts of *Kohautia aspera* (Heyne ex Roth) Bremek. The chloroform, water and ethanolic extracts which are rich in phyto constituents were subjected to DPPH photometric assay and standard MTT assay. The percentage of growth inhibition in antioxidant study increased with increasing concentration of extracts. The ethanolic extract showed better antioxidant activity followed by other extracts. The anticancer potential of plant was investigated by standard MTT assay against SK-MEL 28 skin cancer cells. *In vitro* cytotoxic activity of plant increased with increasing concentration of extracts. The results clearly indicated that the extracts of the study species has potential inhibition of free radicals and powerful anticancer activity against skin cancer cells.

Keywords: *DPPH photometric assay, Kohautia aspera (Heyne ex Roth) Bremek, MTT assay.*

Introduction

Species of independent existence which contains unpaired electrons are said to be free radicals. Free radical species containing unpaired electrons reacts with other molecules by giving or taking electrons. They can react with carbohydrates, proteins, lipids and DNA [1, 2]. They may be nitrogen derived or oxygen derived. Examples of oxygen derived ones are superoxide, hydroxyl, hydroperoxyl, peroxyl and alkoxyl radicals. Hydrogen peroxide oxygen is a non-radical species. Nitrogen derived species are nitric oxide, peroxynitrate, nitrogen dioxide and dinitrogentrioxide [3, 4].Main feature of antioxidants is to trap free radicals. Living systems produces free radicals in normal process of metabolism. They may cause many pathological conditions including ageing process [5, 6].Radical scavenging activity of antioxidants prevents this pathological condition due to free radicals [7, 8].Antioxidants safe guard our body from various diseases. Experimental study datas confirmed that plants are the major source of antioxidants. Some of them are flavanoids, flavones, isoflavones, lignins, anthocyanins, catechins, coumarins and isocatechins. Serious side effects like damage of liver and carcinogenesis are reported from some antioxidants. Example; butylated hydroxy anisole, propyl gallate, butylated hydroxy toluene and butylated hydroquinone [9]. Therefore it is better to replace synthetic antioxidants having more side effects with natural antioxidants having fewer side effects. Bioactive secondary metabolites such as alkaloids, tannins, flavanoids and phenolic compounds important for life are produced by plants [10, 11].In recent years, research in folk medicine search for the development of better drugs. Traditional medicines also cope with relentless rise of noncommunicable diseases. Many traditional medicines are more attractive as they soared the health care costs and got universal austerity [12]. *Kohautia aspera* (Heyne ex Roth) Bremek is an annual herb, family Rubiaceae. It is up to 40 cm tall and leaves linear to narrowly elliptic. Stems scabrid, 20-40 x 1-4 mm, acute, scabrid particularly along margins;

stipule sheath. 1 mm long with 2 up to two mm long fimbriae. Flowers in lax cymes with commonly 2 subsessile flowers at one node. Calyx-lobes narrowly triangular, 1-1.5 millimeter long. Corolla mostly white or sometimes bluish, brownish or pinkish; lobes 1-1.5 mm long; tube 2.5-3.5 mm long. Style with 2-lobed stigma and 2 mm long. Capsule diameter 2-4 mm ± sparsely papillose. Seeds 0.4-0.6 mm long and pale brown. The plant is distributed in Eritrea, Ethiopia, Arabian Peninsula, Pakistan and India. Malignant diseases affecting body are called cancer [13].

The characteristics of this are rapid abnormal cell formations. These cells accumulate and form a tumor [14].This proliferates the body and leads to the death of an organism. Plants and their products have been extensively used by humans for many years [15]. They maintain the health of individuals without causing toxicity and also cure diseases even including cancer.

Natural products comprise more than 50% in clinical use. Many have the ability to control cancer cells. Literatures did not provide evidence which is scientific to prove the antitumor activity of *Kohautia aspera* (Heyne ex Roth) Bremek. But the plant is used for anticancer activity in certain regions of Kerala as well as Tamilnadu and hence the study was proposed.

MTT assay, by Mossman is an accepted method to access cell proliferation [16, 17]. This colorimetric and quantitative assay measures the formazan product formation in living cells which is proportional to cell number [18, 19]. 3H-thymidine uptake assay results found to be consistent with the results obtained from MTT assays. In a cancer cell line, the concentration of an anticancer drug which kills half of the cells is the IC50 value and the value calculated by regression analysis [20].

Materials and Methods

Plant Material and Preparation of Extracts

Fresh plants of. *Kohautia aspera* (Heyne ex Roth) Bremek were collected from Thirunelveli District, Tamilnadu, India. Mr. Chelladurai, Research officer, Central council for research in Sidha and Ayurveda, Government of India, identified and authenticated the plant. The parts of the plant were gabled for elimination of contaminants, shade dried and then powdered. About 300g of the powdered plant was successively extracted with Petroleum ether, Chloroform, Ethyl acetate and Ethanol using soxhelet extractor. Method of hot percolation was followed for water for 48hours. Rotary evaporator used for concentrating the extracts, weighed, properly labeled and stored there after in refrigerator until further use [21]. Based on the presence of phytoconstituents, choroform, ethanol and water extracts were selected for the investigation of antioxidant and cytotoxic activity.

Antioxidant Assay

DPPH Photometric Assay

Measurement of absorption of DPPH solution carried out at 517nm after addition of antioxidant. The absorption was decreased and reference used was ascorbic acid (10mg/ml DMSO).Stable free radical-1, 1-diphenyl-2-picryl hydrazyl is red in colour and turns yellow on scavenging [22]. This character of DPPH assay is used to show free radical scavenging activity. The scavenging between (H-A) antioxidant and (DPPH) is written as, [H-A] + DPPH→ (A) + DPPH-H. The absorbance decreases as a consequence of reaction of antioxidants with DPPH.

Scavenging potential of extracts or antioxidants in terms of their hydrogen donating ability is indicated by the degree of discoloration. Extracts of volumes (1.25 to10µl) were made to 40µl using DMSO and then 2.96ml DPPH (0.1mM) was added. Incubation of reaction mixtures carried out at room temperature in dark condition. The absorbance of mixture after 20 minutes was observed at 517nm. DPPH (3ml) taken as control [23].

In Vitro cytotoxic Activity of Kohautia aspera (Heyne ex Roth) Bremek

Cell Culture

The cell culture used was SK-MEL 28 skin cancer cells, from National Centre for Cell Sciences, Pune. These cancer cells were maintained in Dulbecco's modified eagle's media (with 10%FBS) and grown at 37°C in 5% carbon dioxide in humidified atmosphere. The cells were trypsinized for two minutes and transferred to T flasks in complete aseptic conditions.

Extracts were added to grown cells at different concentrations from a stock of 10mg/ml in 0.1% DMSO and incubated for 24 hours.

MTT Assay

Percentage difference in the viability determined using standard MTT assay after incubation of 24 hours. Suspension of cell culture was washed with 1 X PBS (phosphate buffer saline) and added 200µl solution of MTT to the culture flask (5 mg/volume MTT dissolved in PBS and then filtered through 0.2 µm filters).

Three hours incubated at 37°C, MTT solution completely removed and then washed with 1 x PBS. DMSO (300µl) was added to every culture flask, 30 minutes incubated at room temperature, all cells get lyses and color obtained was homogenous. Solution transferred to centrifuge tubes and 2 minutes centrifuged at top speed to precipitate all cell debris. Optical density was measured at 540nm with DMSO blank. Percentage viability calculated using following formula.

% viability= (OD of Test/OD of Control) X 100

Statistical Analysis

Measurements of all analysis were replicated, three times. Experimental results were expressed as mean ± SD. The IC50 values were calculated from linear regression analysis.

Results and Discussion

Preliminary phytochemical screening of different extracts of *Kohautia aspera* (Heyne ex Roth) Bremek exhibited the presence of many important phytoconstituents like glycosides, proteins, aminoacids, steroids, alkaloids, carbohydrates, phenols, quinines, saponins, flavanoids and tannins [24]. The *in vitro* antioxidant and anticancer activities of selected extracts such as chloroform, ethanol and water were carried out as these extracts are rich in phytoconstituents [25].

Antioxidant Activities of Selected Extracts of *Kohautia aspera* (Heyne ex Roth) Bremek

Based on the evidenced phytochemical constituent's of *Kohautia aspera* (Heyne ex Roth) Bremek, it is vital to evaluate the antioxidant potential [26, 27]. Plants due to their therapeutic principles are considered as an important source of remedy since ancient era. Hence it is important to determine the free radical scavenging activity. The *in vitro* antioxidant activity of chloroform, water and ethanol extracts by DPPH method was conducted as per the procedures mentioned. Different concentrations (31.25-1000µg/ ml) of plant extracts were used to determine the antioxidant activity and 50% growth inhibition (IC50).

The results showed that, with increasing concentration of extracts the percentage of growth inhibition is also increasing. Results are given in Table 1 and graphically in Figure 1. A stable free radical that has been commonly utilized to prove the antioxidant activity of various natural products is DPPH. The more antioxidants present in the extract the more DPPH reduction will occur.

Present investigation infers that the plant has considerable DPPH radical scavenging activity and its maximum was elicited in ethanolic extract. The antioxidant effect of extracts may be due to neutralization of DPPH free radicals, either by transfer of electrons or by transfer of hydrogen atom [28]. The IC50 value of chloroform, water as well as ethanolic extracts by DPPH assay method was found to be 557.37µg/ml, 428.38µg/ml and 114.19µg/ml respectively.

Table 1: Percentage inhibition of extracts of *Kohautia aspera* (Heyne ex Roth) Bremek -DPPH method

Concentration (µg/ml)	Percentage inhibition		
	Ethanol	Water	Chloroform
31.25	30.15 ± 0.69	7.56 ± 0.58	10.26 ± 0.46
62.5	45.8 ± 0.85	17.58 ± 0.84	19.28 ± 0.89
125	55.62 ± 0.74	22.8 ± 0.91	28.23 ± 0.48
250	65.92 ± 0.54	49.26 ± 0.64	39.56 ± 0.67
500	84.69 ± 0.35	69.59 ± 0.14	54.85 ± 0.97
1000	97.85 ± 0.87	88.86 ± 0.73	69.65 ± 0.44

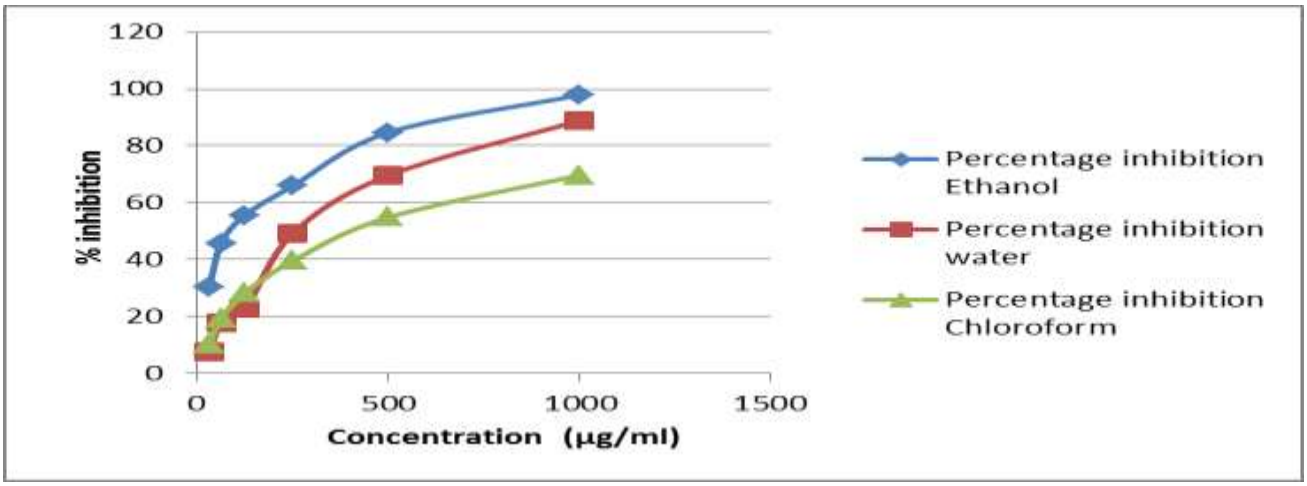

Figure 1: Comparative study of % Inhibition of different extracts of *Kohautia aspera* (Heyne ex Roth) Bremek- DPPH assay method

Anticancer Activity of Selected Extracts of *Kohautia aspera* (Heyne ex Roth) Bremek

The *in vitro* cytotoxic activity using MTT assay on SK MEL cancer cells was conducted. Control and three extracts (chloroform, ethanolic and water) were used. The results are presented in Table.2. Different concentrations of the extract were used to determine the 50% growth inhibition (IC50). Results of different extracts of plant from 25-400µg/ml are represented in Figure.2.

This assay on three extracts of *Kohautia aspera* (Heyne ex Roth) Bremek showed significant effect on SKMEL skin cancer cells at microgram levels. The results makes clear that, with increasing microgram concentration of different extracts the growth inhibition in percentage also increased. MTT assay demonstrated that, all the three extracts exhibit good anticancer activity and satisfactory IC50 values of 255.63µg/ml (chloroform extract), 233.48µg/ml (water extract) and 165.79 µg/ml (ethanolic extract).

Table 2: Cytotoxic activity of extracts of *Kohautia aspera* (Heyne ex Roth) Bremek by SKMEL skin cancer cells

Concentration (µg/ml)	Percentage inhibition		
	Chloroform	Water	Ethanol
25	2.56 ± 0.58	5.41 ± 0.64	19.45 ± 0.53
50	8.63 ± 0.36	11.32 ± 0.46	29.08 ± 0.68
100	21.89 ± 0.57	23.63 ± 0.76	34.53± 0.81
200	50.54 ± 0.87	51.87 ± 0.28	67.25 ± 0.93
400	72.3 ± 0.82	79.54 ± 0.77	89.53± 0.67

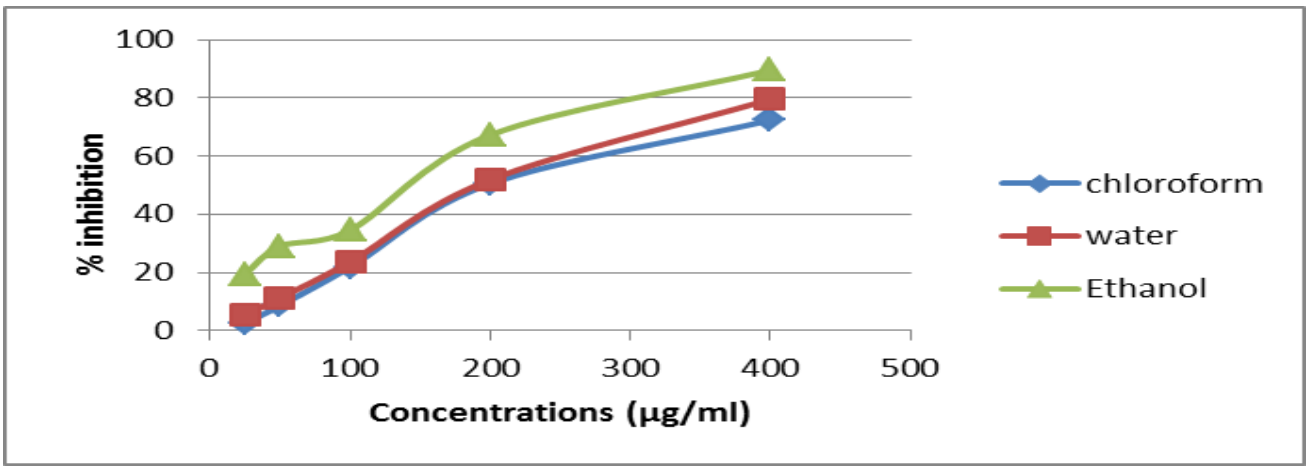

Fig.2: Growth inhibition of Chloroform, water and Ethanolic extracts of *Kohautia aspera* (Heyne ex Roth) Bremek on SKMEL skin cancer cells

Conclusion

The study concludes that the chloroform, water as well as ethanolic extracts of the plant posses antioxidant activity. The ethanolic extract of the plant was having good antioxidant activity when compared to others. The extracts of *Kohautia aspera* (Heyne ex Roth) Bremek can be considered as potential sources for anticancer activity and further studies are to be

conducted for isolation of biologically active substances and their identification.

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