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Preliminary phytochemical screening and anticancer potential of *Kohautia aspera* (Heyne Ex Roth) Bremek

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ABSTRACT

Phytochemical screening followed by anticancer potential evaluation of *Kohautia aspera* (Heyne ex Roth) Bremek was the purpose of the study. Successive extraction was performed with four solvents of different polarity as well as hot percolation method for the aqueous extract. The anticancer potential of the plant was investigated by standard MTT assay against (MCF-7) human breast adenocarcinoma cells. The extracts showed many phytoconstituents. *In vitro* cytotoxic activity of plant increased with increasing concentration of extracts. The presence of important phytoconstituents in plants may provide protection against a number of diseases. The investigation concludes that *Kohautia aspera* (Heyne ex Roth) Bremek possesses significant anticancer potential.

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INTRODUCTION

Secondary metabolites important for human life are synthesized by plants (Bhargav *et al.*, 2012) . Alkaloids, tannins, flavanoids and phenolic compounds are some important bioactive constituents (Rahman *et al.*, 2014). Research in recent years is more oriented towards folk medicine, searching for the development of better drugs. Traditional medicines also cope with the relentless rise of noncommunicable diseases (World Health Organization, 2013). Affordability of many traditional medicines makes them more attractive. Thus these traditional medicines soared health care costs and got universal austerity (Tiwari, 2017).

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 2mm 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 (Lewis, 1965; Fosberg, 1956).

Malignant diseases which may affect various parts of the body are generally called by the term cancer (Soni *et al.*, 2017). Uncontrolled and rapid abnormal cell formation is the characteristics of these malignant diseases. These cells mass together and form a tumor. This proliferates through the body by initiating abnormal cell growth at other sites. It

may progress and leads to the death of an organism. Plants and their products for the treatment of diseases have been extensively used by humans for many years (Suppakul *et al.*, 2003; Arsu *et al.*, 2012). Without causing toxicity, they maintain the vitality and health of individuals and also cure diseases even including cancer. More than 50% of modern drugs in clinical use are of natural products. Many of them also have the ability to control cancer cells (Meyer *et al.*, 1996). Literature 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 the anticancer activity in certain regions of Tamilnadu and Kerala, and hence the study was proposed.

MTT assay, by Mossman, is improved by others (Mosmann, 1983; Nikš and Otto, 1990; Jin *et al.*, 2011) . This assay is one of the most reliable and accepted methods to access cell proliferation. This colorimetric and quantitative assay measures the activation, proliferation and viability of cells. Enzyme cellular mitochondrial dehydrogenase reduces the yellow water-soluble substrate 3-(4, 5-dimethyl thiazol-2yl)-2, 5 diphenyl tetrazolium bromide (MTT) into dark blue, purple formazon product in living cells. The formed product is insoluble in water. In a range of cell lines, formazon product is directly proportional to the cell number (Gerlier and Thomasset, 1986; Minu *et al.*, 2014). These results found to be consistent with the results obtained from other assays like 3H-thymidine uptake assay (Vega-Avila and Pugsley, 2011). The MTT assays have greater acceptability. It detects cells which are not dividing but which are still metabolically active. Thus this assay is used to differentiate proliferation and also cell activation. The procedure is applicable in monolayer or suspended cell preparations. Cells die during the test making it difficult to conduct follow up assessments of cell culture. 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 (Wang *et al.*, 2002; Henriksson *et al.*, 2006; Al-Nasiry *et al.*, 2007).

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 the 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 labelled and stored thereafter in the refrigerator until further use (Evans, 2002; Kokate and Purohit, 2010).

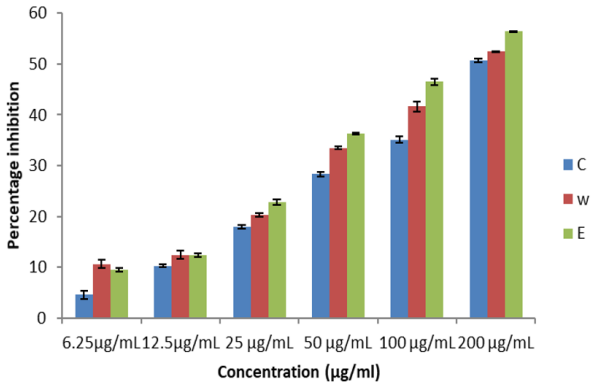

Figure 1: Growth inhibition of Chloroform, Water and Ethanolic extracts of *Kohautia aspera* (Heyne ex Roth) Bremek on MCF-7 cancer cells. (C- chloroform extract. W-water extract. E-ethanol extract)

Preliminary Phytochemical Screening of Plant Extracts

Qualitative tests on every extract of *Kohautia aspera* (Heyne ex Roth) Bremek performed for phytoconstituents present (Harborne, 1973; Mukherjee, 2002; Pvman, 1931).

Based on the presence of phytoconstituents, choroform, ethanol and water extracts were selected for the investigation of cytotoxic activity.

Cell culture

The cell culture used was MCF-7 human breast adenocarcinoma cancer cells, from the National Centre for Cell Sciences, Pune. These cancer cells were maintained in Dulbecco□s modified eagles media (with 10%FBS) and grown at 37°C in 5% carbon dioxide in a humidified atmosphere.

In-vitro cytotoxic activity of *Kohautia aspera* (Heyne ex Roth) Bremek

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.

Table 1: Preliminary qualitative tests for phytochemicals in *Kohautia aspera* (Heyne ex Roth) Bremek

S.No.	Test	Petroleum ether	Chloroform	Ethyl acetate	Ethanol	Water
1.	Alkaloids	-	+	+	+	-
2.	Carbohydrates	-	+	+	-	+
3.	Glycosides	+	-	-	+	-
4.	Terpenoids	-	+	-	+	-
5.	Proteins	+	+	+	-	-
6.	Amino acids	+	+	+	-	-
7.	Steroids	+	+	-	+	-
8.	Flavonoids	-	-	-	+	+
9.	Phenols	-	+	-	+	+
10.	Tannins	-	+	-	+	+
11.	Quinones	-	-	+	-	-
12	Anthraquinones	-	-	-	-	-
13	Saponins	-	-	-	+	+

Table 2: Cytotoxic activity of Chloroform extract by MCF-7 cancer cells

Concentration (μg/ml)	% Inhibition
6.25	4.61 ± 0.82
12.5	10.24 ± 0.25
25	17.93 ± 0.41
50	28.32 ± 0.46
100	35.11 ± 0.65
200	50.66 ± 0.32

Table 3: Cytotoxic activity of Water extractby MCF-7 cancer cells

Concentration(μg/ml)	% Inhibition
6.25	10.64 ± 0.76
12.5	12.43 ± 0.80
25	20.29 ± 0.32
50	33.42 ± 0.28
100	41.67 ± 0.98
200	52.39 ± 0.16

Table 4: Cytotoxic- the activity of Ethanolic extract by MCF-7 cancer cells

Concentration(μg/ml)	% Inhibition
6.25	9.45 ± 0.34
12.5	12.35 ± 0.40
25	22.77 ± 0.55
50	36.29 ± 0.18
100	46.46 ± 0.60
200	56.35 ± 0.09

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 the 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 $\pm$ SD.

RESULTS AND DISCUSSION

Preliminary phytochemical screening of different extracts of *Kohautia aspera* (Heyne ex Roth) Bremek exhibited the presence of glycosides, proteins, aminoacids and steroids in petroleum ether extract, alkaloids, carbohydrates, proteins, aminoacids, steroids, phenols, terpenoids and tannins in chloroform extract, alkaloids carbohydrates, glycosides, proteins, aminoacids and quinones in ethyl acetate extract, alkaloids, glycosides, terpenoids, flavanoids, steroids, phenols, tannins and saponins in ethanolic extract and carbohydrates, phenols, tannins, flavanoids and saponins in water extract (Middletone, 1952; Khandalwal and Sethi, 1999; Paech and Tracey, 1955; Shellard, 1957). The results are presented in Table 1.

The *in vitro* cytotoxic activity using MTT assay on MCF-7 cancer cells was conducted. Control and three extracts (chloroform, ethanolic and water) were used. The results are presented in Tables 2 and 3 andTable 4. Different concentrations of the extract were used to determine the 50% growth inhibition (IC50). Results of different extracts of a plant from 6.25-200 μg/ml are represented in Figure 1. This assay on three extracts of *Kohautia aspera* (Heyne ex Roth) Bremek showed a significant effect on MCF-7 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 181.99 μg/ml (chloroform extract), 167.02 μg/ml (water extract) and 148.09 μg/ml (ethanolic extract).

CONCLUSIONS

Therefore 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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