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Evaluation of nitric oxide scavenging activity of steam distilled oil and detection of class of compounds present in the non-polar extract of cedrus

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ABSTRACT

The current investigation subjected for the extraction of volatile oil from the heart wood of cedrus using the Clevenger apparatus. The non-polar extract was then subjected to preliminary phytochemical screening for the detection of the class of compounds. Even though the yield of the oil was very low, it possesses significant anti-oxidant activity.

INTRODUCTION

Cedrus deodara (deodar cedar, Himalayan cedar, or deodar) is a species of cedarnative to the western Himalayas in eastern Afghanistan, northern Pakistan, northern India ,south western most Tibet and western Nepal, occurring at 1,500–3,200 m (4,921–10,499 ft) altitude. It is a large evergreen coniferous tree reaching 40–50 m (131–164 ft) tall, exceptionally 60 m (197 ft) with a trunk up to 3 m (10 ft) in diameter. It has a conic crown with level branches and drooping branchlets[1]. The leaves are needle-like, mostly 2.5–5 cm long, occasionally up to 7 cm long, slender (1 m thick), borne singly on long shoots, and in dense clusters of 20-30 on short shoots; they vary from bright green to glaucous blue-green in color. The female cones are barrel-shaped, 7–13 cm long and 5–9 cm broad, and disintegrate when mature (in 12 months) to release the winged seeds. The male cones are 4–6 cm long, and shed their pollen in autumn.[1] The heartwood is carminative, diaphoretic, diuretic and expectorant[2]. A decoction of the wood is used in the treatment of fevers, flatulence, pulmonary and urinary disorders, rheumatism, piles, kidney stones, insomnia, diabetes etc[3]. The plant yields a medicinal essential oil by distillation of the wood, it is used in the treatment of phthisis, bronchitis, blennorrhagia and skin eruptions[4].

MATERIALS AND METHODS

COLLECTION AND AUTHENTIFICATION OF SAMPLE

The samples were collected from natural resources from the Malappuram District and authenticated from the Taxonomy Department of Uwin Life Science, Malappuram. The sample specimen was stored in Uwin Life Science, Malappuram. The collected specimens were then coarsely powdered[5].



STEAM DISTILLATION

The steam distillation was carried out using Clevenger apparatus. The oil was passed through unhydrous sodium sulphate for the removal of aqueous matter. It was then subjected to Nitric oxide assay for the evaluation of free radical activity scavenging.



EXTRACTION

The dried and powdered samples were extracted separately by using petroleum ether. The extraction was carried out by refluxing method and can be used to check antioxidant activity of the samples[6].

PRELIMINARY PHYTOCHEMICAL SCREENING

1. Test for Alkaloids

a) Dragendroff's test

8g of Bi(NO3)3.5H2O was dissolved in 20 ml of HNO3 and 2.72g of KI in 400ml of water. These were mixed and allow to stand when KNO3 crystals out. The supernatant was decanted off and made up to 100ml with distilled water. The alkaloids were regenerated from the precipitate by treating with sodium carbonate followed by extraction of the liberated base with ether. To 50ml of alcoholic solution of extract was added to 2ml of HCl. To this acidic medium 1ml of reagent was added. An orange red precipitate produces immediately indicates the presence of alkaloids.

b)Wagner's test (Iodine-Potassium iodide solution)

1.2 gm of Iodine and 2gm of H2SO4 and the solution was diluted to 100ml. 10ml of alcoholic extract was acidified by adding 1.5% v/v of HCl and a few drops of Wagner's reagent. Formation of yellow or brown precipitate confirmed the presence of alkaloids.

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2. Test for Glycosides

A small amount of alcoholic extract was dissolved in 1ml of water and the aqueous NaOH solution was dissolved in 1ml of water and the aqueous NaOH solution was added. Formation of yellow color indicates the presence of glycosides.

4. Test for Flavanoids

In a test tube containing 0.5ml of alcoholic extract, 5-10 drops of dilute HCl and small piece of ZnCl or magnesium were added and the solution was boiled for a few minutes. In the presence of flavanoids, reddish pink or dirty brown color was produced.

6.Test for Steroid

Salkowski test

To 2ml of chloroform extract, 1ml of concentrated sulphuric acid was added carefully along the sides of the test tubes. A red color was produced in the chloroform layer in the presence of steroids[7].

ANTI OXIDANT STUDY

Nitric oxide radical scavenging assay

Sodium nitro prusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with O2 to produce nitrite ions, which can be measured at 540nm spectrophotometrically in the presence of Griess reagent.

Procedure: 5mg of extract was dissolved and mad up to 10ml with methanol. The sample was completely soluble. $50\mu l$ of 10mM sodium nitro prusside and $50\mu l$ test solution of various concentrations are illuminated using fluorescence light at room temperature for 150 minutes. Following incubation, $125\mu l$ of Griess reagent was added and incubated for 30 minutes at room temperature. The absorbance was measured at 546nm. (Griess reagent: 1% sulphanilic acid, 2% phosphoric acid and 0.1% N-1- napthyl ethylene diaminedihydrochloride)[8].

RESULTS AND DISCUSSION

TESTS	RESULTS
Alkaloids	+
Flavanoids	+
Glycosides	_
Steroids	_
Carbohydrates	+
Amino acids	

ANTIOXIDANT STUDY



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CONCLUSION

Cedrus oil is being used commonly for various oilments. From the present study it was concluded that the steam distilled oil from the heart wood of cedrus possess significant nitric oxide scavenging activity. The non-polar extract contains a variety of class of compounds which are promising. Thus the scientific investigation ends with a validation for the validation for the usuage of cedrus oil.

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