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Antibacterial evaluation of ethanolic extract of *Cedrus deodara* wood

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Abstract

Cedrus deodara plant posses various pharmacological properties like anti inflammatory,antispasmolytic, anticancer, analgesic, immunomodulatory and insecticidal activity. In present study, phytochemical screening of ethanolic extract of Cedrus deodara was carried out. Result indicated that the plant contain alkaloids glycosides flavonoids, triterpenoids, tannins, proteins, and fixed oil. Antibacterial evaluation of the plant was carried out against set of three gram positive and three gram negative micro organism and c.deodara found to have a good antibacterial action.

Key words: *Cedrus deodara*, Ethanolic extract, Antibacterial evaluation

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INTRODUCTION

Cedrus deodara is an evergreen conifer tree reaching unto 85 m in height with almost rough black, furrowed bark and spreading branches, shoots dimorphic, leaves 2-5,-5-8 cm needle like triquetrous, sharp, pointed, flowers usually monoecious, but some trees or branches habitually bear flowers of one sex [1].

All parts are bitter, hot, slightly pungent, oleaginous, useful in inflammations, dyspepsia, insomnia, cough, fever, urinary discharges, ozoena, bronchitis, itching, elephantiasis, tuberculous glands, leucoderma, opthalmia, plies, disorders of the mind, diseases of the skin and of the blood [2-5].The leaves lessen inflammation applied in tuberculous glands. The wood is bitter, diuretic, carminative, expectorant, and useful in rheumatism, piles, palsy, epilepsy, stones in the kidney and bladder, useful in fever, costiveness, piles pulmonary complaints, and prolapsus recti [6, 7]. The oil is analgestic and alexipharmic, useful for bruises and injuries to joints, boils, tubercular glands, skin diseases (Yunani), as a diaphoretic, in skin diseases [8-11]. It is considered to possess diaphoretic, diuretic and carminative properties and to be useful in fevers, flatulence and urinary disorders[4]. A decoction of this drug was administered to cases of chronic fevers and the result was unsatisfactory [12-14].Wood is carminative; bark is powerfully astringent and febrifuge. Leaves have mild terebinthinate properties [15, 16]. On the basis of literature review it is found that there is no specific scientific reports on the antibacterial activity of the Plant extract against all microorganism but there is some report on antibacterial activity of essential oil of C.deodara against only *E.coli*.

Figure 1. Plant of *Cedrus deodara*

MATERIALS AND METHODS

Plant material

The *Cedrus deodara* wood was purchased from the botanical garden of BK Mody Govt Pharmacy College Rajkot. The plant material was taxonomically identified from institutional committee of RBPMPC Atkot.

Preparation of Extract

The dried powder material of *Cedrus deodara* wood were defatted with petroleum ether (60-80^{o}C) and further extracted with ethanol. The solvent was removed by distillation under low pressure by rotary evaporator. The resulting semisolid mass was dried and used for Phytochemical analysis and antibacterial activity.

Phytochemical screening

The ethanol extract of *Cedrus deodara (Clarke)* subjected to various color reaction to identify the nature of the components. [17, 18]

Test for alkaloids

The small portion of ethanol extracts were stored separately with a few drops of dilute hydrochloric acid and filtered. The filtrate was tested with various alkaloidal agents, such as Mayer's reagent (cream precipitate) Dragendorffs reagent (orange brown precipitate).

Test for carbohydrates and glycosides

Small quantities of ethanolic extract were dissolved separately in 5 ml of distilled water and filtered. The filtrate may be subjected to Molisch's test to defect the absence of carbohydrates. Another small portion of extract was hydrolyzed with dilute hydrochloric acid for few hours in water-bath and was subjected to Liebermann- Burchard's, legal and Borntrager's test to defect absence of different glycosides.

Test for flavonoids
5 ml of dilute ammonia solution were added to a portion of aqueous filtrate of plant extract followed by addition of concentrated H_2SO_4. A yellow coloration absorbed in extract indicated presence of flavonoids. The yellow coloration disappeared on standing.

Test for steroids
(2ml) Two ml of acetic anhydride was added to 0.5 g ethanolic extract with 2ml H_2SO_4. The color changed from violet to blue or green in samples indicated presence of steroid.

Test for terpenoids (salkowski test)
Five ml of extract was mixed in 2 ml of chloroform, and concentrated H_2SO_4 (3ml), was carefully added to form a layer. A reddish brown coloration of the interface was formed indicated presence of terpenoids.

Test for saponin
About 1 ml of alcoholic and agrees extract was diluted with distilled water to 20ml and shaken in graduated cylinder for 15 minutes. One cm layer of foam indicated presence of saponin.

Test for tannin Vanillin-hydrochloric acid test
When a drug is treated with vanillin-hydrochloric acid reagent, pink or red color is formed due to formation of phloroglucinol.

Test for protein
Mellon's reaction: Million's reagent (mercuric nitrate in nitric acid containing a trace of nitrous acid) usually yields a white precipitate on addition to a protein solution which turns red on heating. This reaction is characteristic of phenols (e.g. the phenolic amino acid tyrosine).

Test for volatile oil
Place a thick section of drug on glass slide. Add a drop of Sudan red 3rd reagent and after two minute wash with 50% alcohol mount in glycerin. In microscope, oil globule appear red color.

Antibacterial Activity
In the present research work, the antibacterial activity spectrum of ethanolic extract of *Cedrus deodara* was analyzed.[13-16] Three Gram positive bacteria, *Staphylococcus aureus* (MTCC 737)**,** *Enterococcus faecalis* (MTCC 439), *Bacillus cereus* (MTCC 430) and three Gram negative bacteria *Klebsiella pneumoniae* (MTCC 109), *Pseudomonas aeruginosa* (MTCC 2642), *Escherichia coli* (MTCC 1687) were used. Inoculum size was adjusted to 1 to 2 × 10^7 CFU (Colony Forming Units)/ml by serial dilution with sterilized nutrient broth media. Nutrient agar (pH 7.2-7.4) was used for routine susceptibility testing of nonfastidious bacteria. Stock solution of 10000µg/ml was prepared in 20 % v/v water in DMSO. Using the stock solution, 6000µg/ml, 4000µg/ml, 2000µg/ml and 1500µg/ml solutions were prepared from which 100 µl solution was taken for assay. Ciprofloxacin was used as a standard. 20 % v/v WFI in DMSO was used as a control. Antibacterial assay was carried out by agar Well Diffusion Method [19-23]. After 16 to 18 hours of incubation, each plate is examined.

RESULTS AND DISCUSSION

Phytochemical Study
The phytochemical studies revealed the presence of alkaloids, glycosides flavonoids, triterpenoid, tannins, proteins and fixed oil.(Table 1)

The result of in vitro antibacterial activity indicate that *C.deodara* was more effective against all gram positive microorganism used in the study as compare to gram negative bacteria. In both strain C.deodara exhibited considerable antibacterial activity. Hence it sis concluded that C.deodara has potent antibacterial activity. So, further investigation of the plant in search of active constituent, responsible for antibacterial activity is on going.

Table 1. Qualitative Phytochemical analysis of ethanolic extract of *Cedrus deodara* wood

Phytochemical constituents	Ethanolic extract
Alkaloids	+
Saponins	-
Glycosides	+
Carbohydrates	-
Tannins & Phenolic Compounds	+
Triterpenoids	+
Proteins and Amino acids	-
Fixed Oils Fats	+
Flavonoids	+

(+) Present,
(-) Absent

Figure 2. Graphical presentation of zone of inhibition against test microorganism

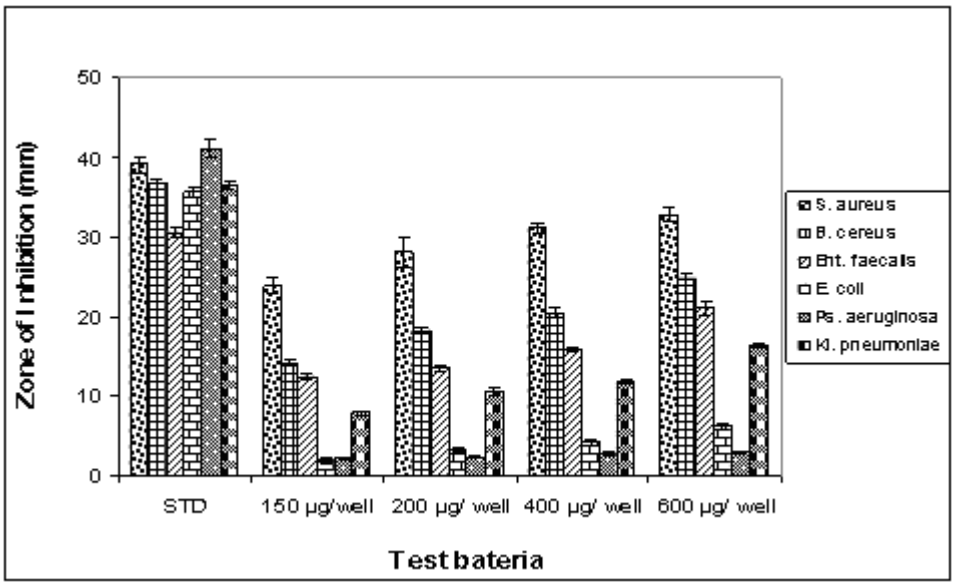

Table 2. Zone of inhibition of ethanolic extract of *Cedrus deodara*

	S. aureus	***B. cereus***	***Ent. faecalis***	***E. coli***	***Ps. aeruginosa***	***Kl. pneumoniae***
STD	39.10 ±0.95	36.67 ± 0.61	30.67± 0.61	35.60 ± 0.53	41.07± 1.01	36.53 ± 0.61
150 µg/well	23.93 ± 1.03	14.13 ± 0.41	12.47 ± 0.42	2.00 ± 0.40	2.20 ± 0.20	7.93 ± 0.31
200 µg/ well	28.23± 1.86	18.33 ± 0.31	13.60 ± 0.35	3.33 ± 0.15	2.47 ± 0.12	10.60 ± 0.60
400 µg/ well	31.07 ± 0.72	20.53 ± 0.61	15.80 ± 0.20	4.27 ± 0.31	2.80 ± 0.20	11.87 ± 0.31
600 µg/ well	32.90 ± 0.95	24.80 ± 0.80	21.00 ± 0.87	6.30 ± 0.26	3.00 ± 0.20	16.33 ± 0.31

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