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Research Article

Antibacterial and Antifungal Activity of *Centratherum anthelminticum* seeds Asteraceae (Compositae)

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ABSTRACT

Centratherum anthelminticum Asteraceae (Compositae) is a (Wild) Kuntze has been obtained from the north of India Uttarakhand state. The antibacterial and antifungal effects of chloroform of plant seeds were tested against the different bacteria and fungus eg. *Stalophylococous aureous* ATCC-29737, *Escherischia coli* ATCC-14169, *Pseudomonas aerugenosa* ATCC-9027, *Bacillus Subtilis* ATCC-6633 Fungus *Colletotrichum gloeosporioides*, *Phomopsis dalbergiae*, *Trichoderma piluliferum***.** By disc diffusion method or microdilution technique *in-vitro*. The growth of *E-coli*, Pseudomonas *aerugenosa* the gram-negative bacteria and Fungus, have been inhibited by the chloroform extracts of the seeds of the *Centratherum anthelminticum* the extracts did not prevent the growth of the other test organism. This improves the existence of the antimicrobial and antifungal activity of the plant. The results showed that the seeds extract of *Centratherum anthelminticum* had the strong antibacterial activity of 0.0020 µg/ml against the *E.coli*, 0.006 µg/ml against the *Pseudomonas aerugenosa*, 0.0025 µg/ml against fungus used.

1. Introduction

India has a rater rich flora due to its geological and morphological structure and climatic features. The recent study have shown flora of India has about 50,000 plant species including 20,000 fungi, over 20,000 vascular plants, 600 pteridophyts, 2700 bryophytes, 5000 algae besides over 20000 microorganisms**[1]**. About 500 herbs are still employed within conventional medicine, although whole plants are rarely used[2]. Nearly 50% of the drug used in medicine is of plant origin**[3]**. The value of these plants products is estimated to be the range of $ 500-800 billion annually.

India's biodiversity constitutes 5% of the World biodiversity on 2% of the Earth surface Number of plant species in India is estimated to be over 45,000 representing about 7% of the world flora. This includes over 15,000 flowering plants of which 4900 species are endemic to the country**[4]**. Out of 18 'hotspots' 2 hotspots are in India - the Eastern Himalayas and Western Ghats**[5]**. India is one among the 12 mega diversity nations in the world medicinal plants and modern medicine research on medicinal plants carried out so far reveals that, as a primary source, medicinal plants have played an important role in developing a number of modern drugs. These drugs include the curare alkaloids, aspirin, antibiotics, cortisone, reserpine, vincoleucoblastine, podophyllotoxin etc**[6]**.

The northern part of India has a great diversity of medicinal plants because of the majestic Himalaya range. 1748 species are known as medicinal plants**[7]**. About 800 species of angiosperms, 44 species of gymnosperms and 600 species of pteridophytes have been reported in the India Himalaya**[8]**. Out of the no of higher plants from India, approximately 46% are endemic of the Himalaya, 59 of the total medicinal plant species, 62 species of medicinal plants are endemic to the Himalaya and 208 extend their distribution to the adjacent area, and are therefore classified as near endemic. Of the total 675 species of Himalaya wild edibles, 171 are used for the treatment of the disease**[9]**. Because of this interesting physical geography and historical reason *Centratherum anthelminticumis* rather rich about endemic taxon today. The plant is native to America. It is one of the 13 pan tropical tribes of the family Asteraceae comprising of more than 1500 species**[10-11]**.

A genus of 20 species of herbs distributed in Asia, America and Australia 9 species are present in India. It also found in Ceylor, Afghanistan, Malaysia ascending to 5500 ft in Himalaya and Kashi Mountain**[12]**. In India 58 species are reported to occur out of which 45 species are known to occur I peninsular India of these about 20 are indigenous to south India. Most the south Indian species are confirmed to higher ranges of Western Ghats where grow wild as herbs, shrubs and under shrubs**.**

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Scientifically only two species i.e. *V. cinerea* and *V. anthelmintica* willd have been studies. Natural hybridization occurs and is recorded in U.S.A although hybridization occurs by various mechanisms viz. ecological, seasonal, geographical, hybrid in viability or sterility is later generation preserve essential integrity of the species.

The two species of African origin i.e *V. galamensis* and V. luscious plantation were tried to raised at RRL, Jammu to ascertain their ecological suitability, adaptability and yield potential under agro climatic conditions of Jammu. Both these species have now been successfully domesticated and sustained efforts have been made to develop them as cheap alternate and renewable source of epoxy oil. *Centratherum anthelminticum* is an erect, robust, leafy annual, 3-5 ft high, leaves petioled about 3 to 8 inches long, florets, pale violet, about 3/16 inches in diameter; achenes (single seeded fruit, referred to as seed), greenish-brown, 3/6 to 1/4 inch long and marked with about 10 lighter colored longitudinal ridges

The plant *Centratherum anthelminticum* (Willd) Kuntze. Called as 'Kalijiri' in Hindi is reported to be a medicinally important plant, this species has a wide variety of application in traditional medicine, especially for treatment of fever, cough, and diarrhoea. The various part of *Centratherum anthelminticum* are documented to possess medicinal properties such as:-

Seeds are reported to be astringent and cure intestinal colic, ulcers cough, flatulence and skin disease. 50 % methanolic extract seeds showed antiviral activity against Ranikhet virus disease besides being spermicidal. The seed oil contains 25% oil and 70% vernolic acid the oil has all the epoxy acid in the form of trivernoline as seeds are anthelmintic alexiphamac and effective against thread and round worms.

The aerial part of the plant shows anthelmintic, mild laxative smooth muscle relaxant and mild hypotensive affects of an aqueous alcoholic decoction. Flowers are purgative and used for treatment of asthma, kidney trouble and inflammatory swellings, fruits and tonic, diuretic and antiseptic**[13-15]**.

Because of the resistance acquires and genetic transmitting ability of bacteria to drugs which are utilized as therapeutic agents, antibacterial and antifungal drugs have gain great impotence in drug industry. Although new synthetic chemical antibiotic have been produced last three decades, resistance to these drugs by micro- organisms has increased**[16]**. The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments by studies conducted in different countries**[17-23]**. The microbial trait of many plats comes from the secondary metabolism of plants known as phenolic compounds which are parts of the essential oils**[24]** as well as tannins**[25]**.

In this study chloroform extract prepared from the seeds of *Centratherum anthelminticum* plant were evaluated for the first time for antibacterial and antifungal activities.

2. Experimental

Plant specimen taken from the north of India Uttarakhand state district 2010 and authenticated by Dr. S. K. Srivastava, Scientist-D/H.O.D., Botanical Survey of India, Northern regional centre, Dehradun (Accession No.113273), Voucher specimen have been deposited in the Herbarium of the above cited Department as *Centratherum anthelminticum* (L.) O. Kuntze (DSN 01).

Preparation of plant extracts

The shade dried (40gm) seeds of plant were grounded then extracted with chloroform (250 ml) for 6 hrs by using soxhlet equipment. The extract was filtering using whatman filter paper (No.1) and then concentrated in vacuo at 55-60 ^{0}C. For the bioassay, the extracts were suspended in chloroform at a concentration of 15 mg/mL.

Microorganisms

All organism were obtained from the Gurukul Kangri University Faculty of ayurved and medical science, Department of Pharmaceutical Sciences, Haridwar India and include: *Stalophylococous aureous* (ATCC-29737), *Escherischia coli (*ATCC-141690, *Pseudomonas aerugenosa* (ATCC-9027), *Bacillus Subtilis* (ATCC-6633) Fungus *Colletotrichum gloeosporioides, Phomopsis dalbergiae, Trichoderma piluliferum***.** Gentamycine and clotrimazole were used as standard antibacterial and antifungal agent, respectively.

Antimicrobial and antifungal activity

The antimicrobial activities of chloroform extract of the seeds were evaluated in vitro against an assortment of two gram positive and two gram negative bacteria and three funguses. The bacteria were grown in nutrient agar at 37 ^{0}C and maintained at 4^{0}C.

The minimum inhibitory concentration (MIC_S) of chloroform extract of the seeds and reference antibiotics were determined by microdilution techniques in nutrient broth media for bacteria and Potato Dextrose Agar (PDA) medium for fungus**[26]**. The extracts was first dissolved at a concentration of 20 mg/100 µL in dimethyl sulphoxide (%10 v/v) containing Tween 80 (% 5 v/v)**[27]**. Reference antibiotic initially tested using a concentration of 0.40 mg/mL for gentamycine in distilled water and 0.50 mg/mL for clotrimazloe in ethanol. Then the two fold dilutions of each compound were performed. Inoculla for assay were prepared from activated culture in broth media by dilution in growth medium to give a final viable cell count of 4.0-5.5 x 10^5 CFU/mL. each drug solution (25 µL) inoculums of microorganism (25 µL) were added in to each well of a flat-bottom, 96-well microtiter plate prefilled with 200 µL of medium to give a total volume of 250 µL. micrititer plate were incubated at 37 ^{0}C for 24 hrs for bacteria and 48-72 hrs for fungus. The solvent dimethyl sulphoxide and ethanol were used as the negative control for all experiments.

After incubation, MIC value was detected by adding 50 µL of 0.5% triphenyl tetrazolium chloride aqueous solution[28-29]. MIC was defined as the lowest concentration of extracts that inhibit visible growth as indicating by the TTC reduction. In the presence of bacterial growth by reduction reactions, TTC change the color of microbial cells from colorless to red. This provides clearly defined and easily readable end points. All tests were repeated three times to confirm the results.

3. Result and discussion

The antimicrobial activity of *C. anthelminticum* seeds was given in table-1. As can clearly be seen from this table-1, the extracts provided from the seeds of *C. anthelminticum* were found to be effective against E-coli ATCC-14169, *Pseudomonas aerugenosa* ATCC-9027, *Bacillus Subtilis* ATCC-6633 as gram-negative bacteria and Fungus, showing MIC values 0.0020 µg/ml against the *E.coli*, 0.006 µg/ml against the *Pseudomonas aerugenosa*, 0.0025 µg/ml against fungus used. However *C. anthelminticum* was not effective against *Stalophylococous aureous* ATCC-29737 and *Bacillus Subtilis* ATCC-6633 as gram-positive bacteria.

Table 1: Antimicrobial effect of *Centratherum anthelminticum* (Willd) Kuntze

Microorganisms	**Minimum inhibitory concentration (MIC)**		
	Extracts (µg/mL)	**Gentamycine (µg/mL)**	**Clotrimazole (µg/mL)**
Stalophylococous aureous ATCC-29737	-	1.25	n.t*
Escherischia coli ATCC-14169	0.0020	1.25	n.t
Pseudomonas aerugenosa ATCC-9027	*0.006*	1.25	n.t
Bacillus Subtilis ATCC-6633	-	1.25	n.t
Colletotrichum gloeosporioides	*0.025*	n.t	0.78
Phomopsis dalbergiae.	*0.025*	n.t	0.78
Trichoderma piluliferum	*0.025*	n.t	0.78

*: not tested.

The microorganism *Escherischia coli* which is already known to be multi- resistant to drugs had its growth inhibited by the extracts of *C. anthelminticum*. On the other hand *Pseudomonas aerugenosawhich*[30] is also resistance to different antibiotics had its growth inhibited also by *c anthelminticum* extracts. Such results are interesting because the control of these bacteria was noticed to be very difficult by therapeutic mean[31]. While the control of resistance bacteria is becoming a threat to human health, the studies regarding the mode of action of these compounds in the bacteria cell should be done. The synergetic effect of *C. anthelminticum* extract from the association of antibiotic against resistance bacteria will lead to new choice for the treatment of infectious diseases. This effect enables the use of respective antibiotic when it is no longer effective by itself during therapeutic treatment.

Many plant phenols are reported as fungi-toxic agents. Yeast and antifungal activity evaluated together and tannins can be toxic to filamentous fungi, yeast and bacteria[32]. Condense tannins have been determined to bind cell walls of ruminal bacteria, preventing growth and protease activity[33].

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