

IN VITRO ANTIMICROBIAL ACTIVITY OF HIMALAYAN MEDICINAL PLANT
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ABSTRACT

The antimicrobial activity of the all extracts of *Senecio chrysanthemoides* were studied against five (gram negative and gram positive bacteria) and three fungal stain. The results showed that the minimum inhibitory concentration (MIC) of *Senecio chrysanthemoides* extract was 50µg/ml against *Salmonella entericatypm*. The ethye acetate extract of *Senecio chrysanthemoides* showed significant activity 19±1mm, 17±1mm and 13±1mm against *E. coli*, *Salmonella entericatypm* and *Klebsiella pneumonia*, against food poisoning bacteria and the order of the species based on total antibacterial activity is as follows: *Escherichia coli* > *Salmonella entericatypm* > *Klebsiella pneumonia* and phytochemical screening for the presence of glycosides, alkaloids, phenols and tannins.

KEYWORDS: Antibacterial, Antifungal and Phytochemical screening.

INTRODUCTION

India has great wealth of medicinal plants and their traditional uses. The use of traditional medicinal plants as a source for relief from illness. Herbal medicine is the oldest form of health care known to mankind. Herbs have been used by all cultures throughout the history and they constitute an integral part of the development of modern civilization. Medicinal and aromatic plants and their derived are rich in antibacterial compounds which could be an alternate way to combat bacterial diseases even against some bacteria which are becoming resistant to certain synthetic medicines. The genus *Senecio* belongs to the tribe Senecioneae, is the largest and most complex genus in the family Asteraceae, which includes more than 1000 species with a worldwide distribution. It is commonly found throughout the Himalayan region at an altitude of 3500-4000m tall glabrous thickherbs, leaves large reniform, petiole winged, heads yellow whole plants aromatic [Naithani, et al., 1984]. About 1000 species are found in world out of which only 43 species found in India [Gaur, et al., 1999]. The plants of this genus have been studied extensively because of the traditional medicinal. The leaves, stems and flowers are used mostly in folk medicine for the treatment of various ailments [E.Uzun, et al 2004].

In the third world countries, 20,000 plants species are believed to be used medicinally (T.K. Mukherjee, et al 2004). At present, the pharmaceutical sector in India is making use of 280 medicinal plant species, of which 175 are found in the IHR (U. Dhar, et al 2000).

The plants of the genus *Senecio* are used traditionally used for treatment of dysentery, conjunctivitis, infections, rheumatism, cancer, cough suppressant, asthma, bronchitis, eczema and inflammation. *Senecio aryunensis* is used in traditional Chinese medicine in northwestern China to treat dysentery, conjunctivitis and tumefaction [The Encyclopedia et al 1977]. In traditional medicine, the use of *Senecio* species for treatment of asthma, coughs, bronchitis, eczema and wound healing have also been reported [E.Burgueno et al 2006; A. A. Bolzan et al 2007; E.Uzun, et al 2004]. *Senecio tenuifolius* is poisonous to livestock, but the leaves of the plant are used topically as remedy for skin diseases to reduce swelling and pain [D. S. Bhakuni et al 1982].

1. MATERIALS AND METHODS

2.1 Plant Material

Whole plants of *Senecio chrysanthemoides* were collected from the Tungnath (Chopta), Rudraprayag Uttarakhand India in October 2014. The plant was identified from Department of Botany, HNB Garhwal University Srinagar Uttarakhand. A Voucher Specimen (GUH-3354) was deposited in the Department of Botany.

2.2 Preparation of plant Extract

The plant material was separated into its selected part air dried ground to moderately fine powder and soxhlet extracted with increasing polarity solvent (petroleum ether, chloroform, ethyl acetate, acetone, methanolic, ethanolic and water) [Lin J et al., 1999]. Each extract was evaporated to dryness under reduce pressure using

rotary evaporator. The coarse powder of tuber were subjected to successive hot continuous extraction with various solvent each time before extracting with next solvent the powdered material will be air dried (weight of crude extract 500gm). The various concentrated extracts were stored in air tight container for further studies.

2.3 Media

Nutrient broth, Nutrient agar, Muller Hinton agar, Malt extract broth and Sabouraud dextrose agar, Alcohol, Hydrochloric acid, alcohol, and sulphuric acid, Distilled water etc all product of Himedia Laboratories Mumbai (India) were used in this study.

2.4 Bacterial Strains

Ten bacterial strains were used namely *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *salmonella entericatypim*, The bacterial strains were supplied by the Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh, India, Customer no, 4351.

2.5 Fungal Strains

Three fungal strains were used namely *Candida albicans*, *Aspergillus flavus* and *Aspergillus parasiticus*, The fungal strains were supplied by the Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh, India.

2.6 Antibacterial assay

The disc diffusion assay methods were used to determine the growth inhibition of bacteria by plant extracts [Iennette E.H. et al., 1985] Diluted bacterial culture (100µl) was spread over nutrient agar plates with a sterile glass L-rod. 10mg/ml and 50mg/ml of the each extracts were applied to each filter paper disc (Whatman No. 1, 5 mm diam.) and allowed to dry before being placed on the agar plate. Each extract was tested in triplicate (3 discs/plate) and the plates were inoculated at 37°C for 24 h. After incubation, the diameter of inhibition zones was measured with a caliper.

2.7 Antifungal assay

The antifungal activity was tested by disc diffusion method [Taylor, et al., 1995; Espinel et al 2002]. The Sabouraud dextrose agar plates were each similarly seeded with each fungal strain The 24 hrs. both culture of each bacterium and 7 days inoculated fungus culture were used to seed sterile Sabouraud dextrose agar at 45°C respectively, and fungal plates were incubated at 25-28°C for 7 days after which diameter of zones of inhibition were measured. Each disc filled with extract.

2.8 Phytochemical analysis

The qualitative phytochemical properties of the dried powdered sample were determined using standard methods [Kokate et al., 2005].

2. RESULT AND DISCUSSION

Plants are important source of potentially bioactive constituents for the development of new chemotherapeutic agents. The first step towards this goal is the in vitro antimicrobial activity. The results of antibacterial, antifungal and phytochemical screening activity table 1, 2 and 3 reveals that antibacterial, antifungal and phytochemical screening activity of tubers of *Senecio chrysanthemoides* was evaluated against ten bacterial and three fungal human pathogenic strains.

3.1 Antibacterial and antifungal activity

The ethyl acetate extract of *Senecio chrysanthemoides* showed significant activity 19±1mm, 17±1mm and 13±1mm against *E. coli*, *Salmonella entericatypm* and *Klebsiella pneumonia* against food poisoning bacteria and the order of the species based on total antibacterial activity is as follows: *Escherichia coli* > *Salmonella entericatypm* > *Klebsiella pneumonia*.

3.2 Phytochemical screening

The phytochemical screening of plant for the presence of glycosides, flavonoids, phenols, resins and tannins, however alkaloids were minor or absent.

Table 1 Antibacterial activity of five bacterial strains against *Senecio chrysanthemoides* plant tubers extract, Disc size, 5 mm, Inhibitory zone size ±1 mm, mm means (millimetres) and – indicate (NIZ) No inhibitory zone.

Bacterial Name		Erythromycin	Petroleum ether Extract		Ethyl acetate Extract		Methanol Extract	
Genus /Species /Subspecies	MTCC (Code)	10 Mg/ml	10 Mg/ml	50 Mg/ml	10 Mg/ml	50 Mg/ml	10 Mg/ml	50 Mg/ml
<i>Klebsiella pneumonia</i>	443	12	6	-	12	17	8	6
<i>Pseudomonas aeruginosa</i>	854	10	13	-	10	9	7	8
<i>Escherichia coli</i>	432	7	8	10	08	19	10	10
<i>Salmonella entericatypm</i>	1255	11	-	-	9	13	12	9
<i>Staphylococcus aureus</i>	737	9	4	6	11	13	12	9

Table 2 Fungal activities of three fungal strains against *Senecio chrysanthemoides* plant extract, Disc size, 5 mm, Inhibitory zone size ± 1 mm, mm means (millimetres) and – indicate (NIZ) No inhibitory zone.

Fungal Name		Ketoconazole	Petroleum ether Extract		Ethyl acetate Extract		Methanol Extract	
Genus /Species /Subspecies	MTCC (Code)	10 Mg/ml	10 Mg/ml	50 Mg/ml	10 Mg/ml	50 Mg/ml	10 Mg/ml	50 Mg/ml
<i>Aspergillus parasiticus</i>		7	6	8	4	8	8	3
<i>Aspergillus flavus</i>		7	5	6	-	8	-	8
<i>Candida albicans</i>		9	-	9	-	-	-	9

Table: 3 Phytochemical screening of *Senecio chrysanthemoides* different extract, (+) – Present, (-) – Absent.

Test	Water Extract	Methanolic Extract	Ethyl acetate Extract	Pt. ether Extract
Alkaloid				
Mayer's test	(-)	(+)	(+)	(+)
Dragondroff test	(-)	(+)	(+)	(+)
Phenolics compound				
Ferric chloride	(-)	(+)	(-)	(-)
Nitric acid	(-)	(+)	(-)	(+)
Carbohydrates/ glycosides				
Molish test	(+)	(-)	(+)	(+)
Fehling test	(+)	(-)	(+)	(+)
Benedict test	(+)	(-)	(+)	(+)
Flavonoids				
Shinoda/pew	(-)	(-)	(-)	(-)
Ammonia	(-)	(-)	(-)	(-)
Saponins	(-)	(-)	(-)	(-)
Tannins				
Pyrogall & catechol	(-)	(+)	(+)	(+)
Gallic acid	(-)	(+)	(+)	(+)
Resin	(-)	(-)	(-)	(-)

3. CONCLUSION

The present study results focused on antimicrobial activity and phytochemical screening of *Senecio chrysanthemoides* this investigation revealed that antimicrobial and antifungal activity against selected bacterial and fungal strains. Which encourage developing a novel broad spectrum antimicrobial formulation in future. Now our research will be directed to develop a broad spectrum antimicrobial herbal formulation with this plant. Even at low concentrations, these plant species contained potent antimicrobial and antifungal activity nearly equal to that of the commercial fungicide used as a positive control.

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