ANTIBACTERIAL ACTION OF GRADIENT EXTRACTS OF *SPILANTHES* CALVA DC.

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ABSTRACT

Spilanthes calva has been used as medication for a number of diseases in ancient system of medicine. In the present study, *Spilanthes* calva evaluated for its antibacterial potential and phytochemical contents in various solvent extracts of the plant in increasing polarity towards pathogenic bacterial species involved in dermatological diseases in human being. Antibacterial activity was initially evaluated by disc diffusion method. Petroleum ether extract was found to be active against Pseudomonas aeruginosa. The results indicated that the plant exhibited antibacterial activity against more than one pathogen. Phytochemical analysis was also done to evaluate the nature of active principles. Flavonoids were detected in petroleum ether and ethanol extracts.

KEYWORDS : Spilanthes calva; Asteraceae; Antibacterial; disc diffusion; MIC

INTRODUCTION

Spilanthescalva DC belongs to the family Asteraceae commonly known as toothache plant, akalkada (Sanskrit), kuppamanjel (Malayalam)(sasidharan2004). The plant occurs throughout India, ascending to 1700m often occurs as a weed in rice fields, an erect usually pubescent annual herb with ovate crenate leaves, 1-2 inch long. The achenes are normally non ciliate on the margins, rough on the faces and with a pappus are absent. Leaves opposite, simple, petiolate. Heads homogamous and disciform, involucre short, bracts subseriate, unequal. Receptacle elongate, anhter bases truncate, style arms long truncate(gamble js 1921). The raw leaves of *Spilanthes calva* are used as flavouring for salads, soups and meats in Brazil and India. It is grown widely as an ornamental because of the attractive colourful heads. *Spilanthes calva* has been used as a traditional medicine for toothache, rheumatism and fever. The flower heads are chewed to relieve the toothache and other mouth related troubles. Leaves are used externally in treatment of skin diseases (Agharkar SP1991). Bombay presidency., Root decoction is used as purgative. Leaf decoction is used as diuretic and lithotriptic. Whole plant is used in treatment of dysentery (verma DM 1993)Bioactive N-isobutylamides, including spilanthol isolated from hexane extract of dried flower buds of Spilanthes acmella. These compounds possessed antilarvicidal activity (rameshwak 1999)Present study evaluates antibacterial potential of the whole plant in various extracts. The extracts are extracted in different solvents of increasing polarity towards pathogenic microorganisms involved various in skin diseases. *Spilanthes calva* has been used as medication for a number of diseases in ancient system of medicine. In the present study, the whole plant of *Spilanthes calva* evaluated for its antibacterial potential and phytochemical contents in various solvent extracts of the plant in increasing polarity towards pathogenic bacterial species involved in dermatological diseases in human being.

MATERIALS AND METHODOLOGY

ANTIMICROBIAL ACTIVITY OF WHOLE PLANT EXTRACTS

Preparation of plant extract Fresh specimens were collected in the month of December 2016 from munnar,idukki District of Kerala State, India. A voucher specimen was deposited at the herbarium ofst.teresas college ernakulam. The air-dried whole plant of material (100g) was ground and utilised for preparing extracts. Extracts of, acetone, ethanol,methanol,chloroform and water were made successively .(ragavendra MP2006)

MICROORGANISMS USED

Test organisms were collected from the culture collection of the st xaviers college aluva,kerala These include Staphylococcus aureus ,Escherichia coli ,Bacillus, Pseudomonas aeruginosa and Klebsiella pneumoniae subsp pneumoniae . The bacteria were sub cultured on nutrient agar slants, incubated at 37oC for 24 hours and stored at 4oC in the refrigerator to maintain the stock culture.

IN VITRO ANTIBACTERIAL ASSAY

The disc diffusion method as illustrated by Bauer et al. (1966) was used to determine the growth inhibition of bacteria by plant extracts. Sterile Agar media (pH 7.4 2) was poured into sterile petridish and after solidification, the bacteria (1 ml broth of approximately 105 CFU) were swabbed with a sterile needle under aseptic conditions. Sterile discs prepared using Whatman No. 4 Filter Paper, of 5-mm diameter were used in the study. The original solvent in which the extract prepared was used as a control. Test materials were dissolved in the respective solvent to obtain a stock solution of concentration of 100 mg/ml. 10 µL of the solution was loaded per disc to attain a concentration of 1 mg/disc. The discs (including control) were used after drying them in an incubator at 50°C to remove any trace of solvent. Discs were introduced onto the surface of the medium. The plates were incubated overnight at appropriate incubation temperatures. Microbial growth was determined by measuring the diameter of zone of inhibition. Experiments were conducted in more than three replicates and average inhibitory zone diameter was determined along with standard deviation.

MINIMUM BACTERICIDAL CONCENTRATION (MBC)

Samples from the tubes used in the MIC assays, which did not show any visible growth after a period of incubation were subcultured onto a freshly prepared nutrient medium(harborn). The minimum bactericidal concentrationwas taken as the lowest concentration of the extract that did not yield a single colony on the nutrient agar plate after 24 hours incubation period.

PRELIMINARY DETECTION OF PHYTOCHEMICALS

The crude samples were subjected to phytochemical screening for the detection of alkaloid, phenolics, Triterpenoids, flavonoids using the method of Harborne (1973)

RESULT AND DISCUSSION

Antibacterial activity was initially evaluated by disc diffusion method. ethanolextract was found to be active against staphylococus. The results indicated that the plant exhibited antibacterial activity against more than one pathogen. Phytochemical analysis was also done to evaluate the nature of active principles. Flavonoids were detected in petroleum ether and ethanol extracts. Alkaloids were detected in petroleum ether, acetone and ethanol extracts and phenolics in acetone, ethanol and water extracts.

Table 1. Antibacterial activity of *Spilanthes calva* in gradient extraction

Tragia involucrata extracted in solvents	Inhibition zone (mm) Value = Mean ± SD				
	pseudomonas	bacillus	ecoli	staphylococcus	klebsiella
chloroform	-	-	0.9	-	-
Acetone	0.4	1.0	0.9	0.9	0.7
Ethanol	0.5	1.0	1.0	1.7	0.9
Water	0.6	-	0.5	-	-
methanol	-	0.7	0.8	0.7	0.5

Table 2. Phytochemicals detected in various extracts of *Spilanthes calva*

Extract used	Phytochemicals detected in various extracts			
	Flavonoids	Alkaloids	Triterpenoids	Phenolics
Petroleum Ether	+	+	–	–
Acetone	-	+	+	+
Ethanol	+	+	–	+
Water	–	–	–	+

(Values: + present; - absent)

Antibacterial activity observed at its maximum in acetone ethanol towards staphylococcus reported that the plants are chewed to relieve the toothache and other mouth related troubles and plants are used externally in treatment of skin diseases. The present investigation supported the antibacterial property of the plant towards tested pathogens involved in various skin diseases. The phytochemical evaluation of *Spilanthes calva* showed that alkaloids, triterpenoids and phenolics were present in active acetone extract. Petroleum ether extract showed the presence of alkaloids and flavonoids. Ethanol extract showed the occurrence of alkaloids, flavonoids and phenolics.. The present result supported the ethnobotanical role of the plant in controlling skin diseases as reported by Agharkar (1991) and Verma et al., (1993) a detailed study of toxicity and pharmacological effects of the compounds are necessary prior to its medicinal application.

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