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PHYTOCHEMICAL AND ANTIMICROBIAL ACTIVITY OF *SPILANTHES ACMELLA* LINN.

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ABSTRACT

Spilanthes acmella Linn. (Asteraceae) is a common annual spreading herb found in a rice field throughout in India; five crude extracts were prepared from the whole plant *Spilanthes acmella* using different solvents by Soxhlet method. The extracts were subjected to screening to detect Preliminary phytochemical analysis and potential antimicrobial activity against Escherichia coli, *Staphylococcus aureus, Klebsiella pneumonia, Salmonella typhi* and fungal strain *Candida albicans* as standard by agar well diffusion method. The aim of our present study was to find out the Preliminary phytochemical analysis and antimicrobial activity of the different extracts of entire plant including flower heads of *Spilanthes acmella*. The different extracts such as Hexane, Methanol, Ethanol, petroleum ether and aqueous extracts exhibits comparable antimicrobial activity with the control.

KEYWORDS: *Spilanthes acmella*, phytochemical activity and Antimicrobial activity.

INTRODUCTION

Spilanthes acmella Linn belongs to family *Asteraceae*. The plant is commonly known as 'Akarkara'. *Spilanthes* is a genus comprising of over 60 species that are widely distributed in tropical and subtropical regions of the world, such as Africa, America, Borneo, India, Sri Lanka and Asia (Sahu et al., 2011; Tiwari et al.,2011). *S. acmella* is native to Brazil and is cultivated throughout the year as ornamental or medicinal plant. It is an annual or short-lived herb that is 40-60 centimeters tall. It is grown in damp area (Tiwari et al.,2011; Wongsawatkul et al., 2008) and has low rate of germination or poor vegetative propagation (Tiwari et al., 2011). Its flowers and leaves have pungent taste and when touched it is

accompanied by tingling sensation and numbness (Wongsawatkul et al., 2008). The plant species has been used commonly as a folk remedy,e.g. for toothache, rheumatic and fever (Wongsawatkul et al., 2008), as fresh vegetable (Tiwari et al., 2011) as well as spice for Japanese appetizer (Leng et al.,2011).

S. acmella had previously been reported. It constitutes a diverse group of compounds. Major isolates were lipophilic alkylamides or alkamides bearing different number of unsaturated hydrocarbons (alkenes and alkynes), such as spilanthol (1) or affinin *(2E,6Z,8E)-N*-isobutyl-2,6,8- decatrienamide (Gokhale and Bhide, 1945; Ramsewak et al., 1999) and amide derivatives. In general, when alkamides are chewed, a pungent taste is released and causes itch and salivation (Rios, 2012). Alkamides are structurally related to animal endocannabinoids and is highly active in the central nervous system. Particularly, anandamide (*N*arachidonoylethanolamine) is an endogenous cannabinoid cerebral neurotransmitter. Spilanthol was first isolated in 1945 from the flower head ethanol Extract of S. acmella. In early 1903, it was first obtained from the different plant species, *S. acmella* L.var. *oleracea* Clarke (Gokhale and Bhide, 1945). Aside from being found in *S. acmella*, spilanthol was also found in other plant species (Rios, 2012). The synthesis of spilanthol was reported in multistep and afforded low overall yields. However, an efficient synthetic method had been developed (Wang et al., 1998). Thus far, the spilanthol is commercially available in form of alcoholic (65 % ethanol) extract Vogel *Spilanthes*. In The local and tribal peoples used the flower heads are chewed to relieve the toothache and other mouth related troubles. Leaves are used externally in treatment of skin diseases. Root decoction is used as purgative. Leaf decoction is used as diuretic. Whole plant is used in treatment of dysentery, phytosterols (e.g. *β*-sitosterol, stigmasterol, *α*- and *β*-amyrins), oils (e.g. limonene and *β*- caryophyllene), sesquiterpenes, *α*- and *β*- bisabolenes and cadinenes, flavonoid glucoside and a mixture of long chain hydrocarbons (C22-C35) were reported (Sahu etal., 2011; Tiwari et al., 2011).

MATERIALS AND METHODS

A. Collection, identification and processing of plant material

Fresh plant material was collected from Ajaneri village paddy field and nearby area of Nashik. Plant was correctly identified with the help of Flora of Maharashtra and Flora of Nashik District. Plant material washed under running tap water dried under shade. It was then homogenized to fine powder with electric blender and stored in airtight bottles. This sample was used for extraction of organic compound.

B. Extraction of organic crude materials from Sphaeranthus *indicus* Linn.

50 gm of whole plant powder sample weighted and used for soxlation.

Solvent Used:- Depending on polarity the following solvent selected

1. Distilled water 2. Ethyl alcohol 3. Hexane 4. Petroleum ether 5. Methanol.

a) Phytochemical analysis of plant extract: The phytochemical are essential to metabolism and chemical processes of plant body. The phytochemical are studied are alkaloids terpenoids & steroids, flavonoids, glycosides, tannins & saponin.

IDENTIFICATION TEST: The test were done to find the presence of active chemical such as alkaloids, glycosides, terpenoids, steroid, flavonoids, saponin, tannin by the following procedure.

Alkaloids

Detection of alkaloids (Evans, 2002): solvent free extract, 50 mg is stirred with ml of dilute hydrochloric Acid and filtered. The filtered is tested carefully with various alkaloid reagents as follows.

a. Mayer's test: To a few ml of filtrate, a drop or two of Mayer's reagent are added by the side of test tube. A white or creamy precipitate indicates the test as positive.

Mayer's Reagent: Mercuric chloride (1.358gm) is dissolved in 60 ml of water and potassium iodide (5.0 g) is dissolved in 10 ml of water. The two solutions are mixed and up to 100 ml with water.

b. Wagner's (Wanger, 2004): To a few ml of filtrate, few drops of Wagner's reagent are added by the side of the test tube. A reddish-brown precipitate confirms the test as positive.

Detection of Carbohydrates and Glycosides: The extract (100gm) is dissolved in 5 ml of water and filtered. The filtered is subjected to the following tests.

a. Mayer's test: to 2 ml of filtered, two drops of alcoholic solution of a-naphthol are added, the mixture is shaken well and 1 ml of concentrated sulphuric acid is added slowly along the sides of the test tube and allowed to stand. A violet ring indicates the presence of carbohydrates.

b. Barrfoed's: To 1 ml of filtered, 1 ml of Barfoed's reagent is added and heated on a boiling water bath for 2 min. Red precipitate indicates presence of sugar.

Barfoed's reagent:- Copper acetate, 30.5gm is dissolved in 1.8 ml of glacial acid.

c. Benedict's test: To 0.5 ml of filtrate, 0.5 ml of Benedict's reagent is added. The mixture is heated on a boiling water bath for 2 min. A characteristic colored precipitate indicates the presence of sugar.

d. To 3 ml of the aqueous extract was added about 1 ml of Iodine solution. A purple color at the interphase indicates the presence of carbohydrate.

e. Keller Kiliani test

2 ml of extract was dissolved in 2 ml of glacial acetic acid containing one drop of ferric chloride solution. The mixture was then poured into the test tube containing 1 ml of concentrated sulphuric acid. A brown ring at the interphase indicates the presence of deoxe sugar, characteristics of cardenolides.

Detection of Saponin: The extract (50mg) is diluted with distilled water and made up to 20 ml. The suspension is shaken in a graduated cylinder for 15 min. A two cm layer of form indicates the presence of saponin.

Detection of proteins and Amino acids: The extract (100 mg) is dissolved in 10 ml of distilled water and filtered through Whitman No. 1 filter paper and the filtrate is subjected to tests for proteins and amino acid.

a. Million's test: To 2 ml of filtrate, few drops of Million's reagent are added. A white precipitate indicates the presence of proteins.

b. Ninhydrin test: Two drops of ninhydrine solution (10 mg of ninhydrine in 200 ml of acetone) are added to two ml of aqueous filtrate. A characteristic purple color indicates the presence of amino acids.

Detection of Phenolic compounds and Tannins

a. Ferric chloride test: The extract (50 mg) is dissolved in 5 ml of distilled water. To this, few drops of neutral 5% ferric chloride solution are added. A dark green color indicates the presence of Phenolic compounds.

Detection of Gum and Mucilage: The extract (100 mg) is dissolved in 10 ml of distilled water and to this 25 ml of absolute alcohol is added with constant stirring. White or cloudy precipitate indicates the presence of gums and mucilage.

Glycoside: Glycosides are compounds which upon hydrolysis give rise to one more sugars (glycones) and a compound which is not a sugar (aglycone or genine). To the solution of the extract in glacial acetic acid, few drops of ferric chloride and concentrated sulphuric acid and observed for a reddish brown coloration at the junction of two layers and the bluish green color in the upper layer.

Terpenoid and steroids: Four milligrams of extract was treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then concentrated solution of sulphuric acid was added slowly and red violet color for steroids (Siddique and Ali,1997).

Flavonoids: Four ml of extract solution was treated with 1.5 ml of 50 % methanol solution. The solution was warmed and metal magnesium was added. To this solution,5-6 drops of concentrated hydrochloric acid was added and red color for flavones.

Tannins: To 0.5 ml of extract solution 1 ml of water and 1-2 drops of ferric chloride solution was added. Blue color was observed for Gallic tannins and green black for catecholic tannins.

Fixed oils and fats: a) A small Quantity if extract is pressed between two filter papers. Oil stain on the paper indicates the presence of fixed oil.

Saponification test

A few drops of 0.5 N alcoholic potassium hydroxide solutions are added to a small quantity of extract along with a drop of phenolphthalein. The mixture is heated on water bath for two hours. Formation of soup r partial neutralization of alkali indicates the presence of fixed oils and fats.

C. Antimicrobial Activity

Inoculum: The microorganism isolated and incubated at 35+2 C for 4 hrs. The turbidity of the resulting bacterial adjusted to turbidity equivalent to 1 McFarland turbidity standard. This level of turbidity is equivalent to approximately 3.0 X 108 CFU/ml.

Bacterial strain used

To study antimicrobial activity following four bacterial strain and One fungal strain *Candida albicans* used.

1. *Escherichia coli (ATCC25922)*
2. *Klebsiella pneumonia (ATCC 25922)*
3. *Salmonella paratyphi*
4. *Staphylococcus aureus (ATCC25923)*
5. *Candida albicans*

Agar well diffusion method

The modified agar well diffusion method was employed Muller-Hinton agar plates were inoculated by streaking the swab over the entire sterile over the entire sterile agar surface. This procedure was repeated by streaking two more times, rotating the plate approximately 60^0 each time to ensure even distribution of the inoculums. As a final step the rim of the agar was also swabbed. After allowing the inoculums to dry at room temperature, 6-mm-diameter wells were bored in the agar.

Each extract was check for antimicrobial activity by introducing 100ul of 4000µg /ml concentration into triplicate well simultaneously and the dilution medium for the positive control was respective solvents. The plates were allowed to stand at room temperature for 1 hour for extract to diffuse into the agar and then they were incubated at 35± 2 0 C for 24 hr. solvent extract were showed area of inhibition that solvent extract further analyzed for find out minimum inhibition concentration (MIC) by using 25µl, 50µl, 75 µl, 100µl, and 125 µl against positive control used pure solvent 100 µl. The plates were allowed to stand at room temperature for 1 hour for extract to diffuse into the agar and then they were incubated at 35± 2 0 C for 24 hr. zone of inhibition measured with scale and observations were noted in notebook.

RESULT AND DISCUSSION

The plant material was subjected to successive extraction with Hexane, Methanol, Ethanol, petroleum ether and distilled water. Result of physiochemical properties are showed in table - 1. Phytochemical studies of different crude extract reveled presence of alkaloids, carbohydrates, steroids, saponin, tannins and phenols etc.(in table no 2). All the plant extracts detected presence of alkaloids in ethanol extract. Carbohydrates are present strongly present in methanol extract. Saponins are only present in distilled water and ethanol extract.

Phenolic compounds are present in ethanol and tannins present in hexane and distilled water, presence in hexane extract, fixed oil and volatile oils are strongly present in all extract except distilled water. The phytochemical compounds identified in the presence study this are bioactive and it shows various pharmacological activities like Rheumatism, fever Diuretics Flu, cough, rabies diseases, Tuberculosis, antimalarials, Antibacterials activity showed by leaves and flowers extracts (Bunyapraphatsara and Chokechareunporn, 1999; Farnsworth and Bunyapraphatsara, 1992 Yadav and Singh, 2010, Haw and Keng, 2003) Antifungal, skin diseases, Immunomodulatory, Antiscorbutic, Local anesthetics Digestive leaves (Tiwari et al., 2011 Sahu et al., 2011 Leng et al., 2011;) Obesity control, (lipase inhibitor) flowers (Yuliana et al., 2011) Snake bite whole plant (Tiwari et al., 2011) Dental Toothache leaves, flower (Haw and Keng, 2003; Tiwari et al., 2011).

Spilanthes acmella Linn whole plant five extract Distilled water, hexane, methanol, ethanol, and petroleum ether) tested against human pathogenic bacteria and fungi. Out of this five extracts, hexane, showed high in vitro antibacterial activity against *S. aureus*, hexane extract showed strong antibacterial activity against *E. coli*, *S aureus. C. albicans* fungal pathogen strongly inhibited by ethanol and hexane extract and none of antimicrobial activity found against bacteria *K. pneumonia*, D.W water extract and pure solvent used as control not showed any Activity against micro organism (Table -3). Further studies carried out isolation and purification of medicinally important compounds is useful for various pharmacological activities and it's also help curing the various diseases.

Table:- 01 Physicochemical Characteristics of Different Extracts of *Spilanthes acmella* Linn.

Solvent	Initial weight of powder (gm)	Final weight of powder (gm)	Weight of crude extract (gm)	Color of Extract
Distilled water	50	46.65	1.33	Dark brown
Ethanol	50	48.64	1.29	Dark Green
Methanol	50	47.78	1.22	Light green
Hexane	50	48.57	1.37	Green
Pet. Ether	50	48.85	1.10	Pale green

Table:- 02 Preliminary phytochemical analysis of crude extract of *Spilanthes acmella* Linn.

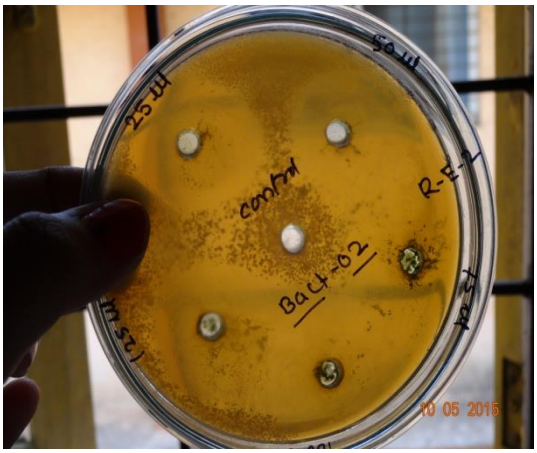
Sr.No	Phytochemical test	Distilled water	Ethanol	Methanol	Hexane	Pet. Ether
1.	Alkaloid					

	a. Mayer's reagent	+	++	++	+	+
	b. Wagner's reagent	+	++	–	–	–
2.	Carbohydrate					
	a. Molish's test	+	_	-	+	+
	b. Barfoed's test	_	_	+++	++	+
	c. Benedict's test	_	+	+++	++	_
	d. Keller-Kiliani	_	+	++	+	+
3.	Saponins					
	a. Foam test	+++	+	+	_	+
4.	Proteins and Amino acids					
	a. Million's test	_	+	++	++	_
	b. Ninhydrin test	_	_	+	+	_
5.	Phenolic Compounds					
	a. Ferric test	_	++	_	+	_
6.	Tannins					
	a. Gelatin test	++	+	_	+++	+
7.	Gum and Mucilage					
	a. 95% alcohol	+	+++	+	+	+
8.	Fixed oil and fats					
	a. Spot test	_	++	++	++	+++
	b. Saponification test	_	++	++	+	++
09	Volatile oils					
	a. steam distillation	_	++	+	++	+++
10.	Flavonoid	+	+	++	_	+
11.	Glycosides	+	_	++	+++	+

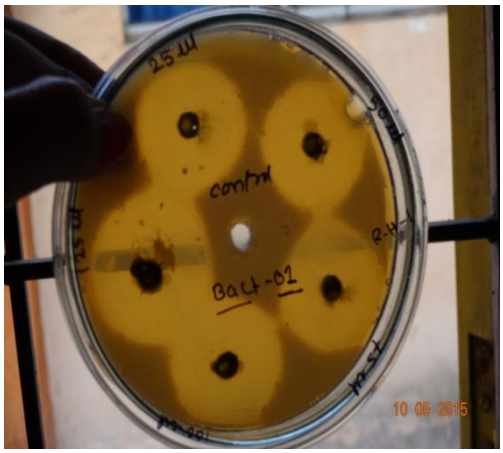
Table No: 3 Antimicrobial Activity of *Spilanthes acmella* Linn.

Sr. no	**Microorganism strain**		**Extract of *Spilanthes acmella***					
		Zone of inhibition in cm						**Control**
1.	*E. coli*	Conc. Of Extract in µl	25	50	75	100	125	
		Distilled water	Nil	Nil	Nil	Nil	Nil	Nil
		Ethanol	Nil	Nil	Nil	Nil	Nil	Nil
		Methanol	Nil	Nil	Nil	Nil	Nil	Nil
		Hexane	**1.6**	**1.9**	**2.4**	**2.8**	**3.7**	Nil
		Pet. Ether	Nil	Nil	Nil	Nil	Nil	Nil
2.	*S. aureus*							
		Distilled water	Nil	Nil	Nil	Nil	Nil	Nil
		Ethanol	Nil	Nil	Nil	Nil	Nil	Nil
		Methanol	Nil	Nil	Nil	Nil	Nil	Nil
		Hexane	**2.5**	**2.9**	**3.0**	**3.6**	**4.8**	Nil
		Pet. Ether	**2.4**	**2.9**	**3.2**	**3.4**	**4.2**	Nil
3.	*K. pneumonia*							
		Distilled	Nil	Nil	Nil	Nil	Nil	Nil

		water						
		Ethanol	Nil	Nil	Nil	Nil	Nil	Nil
		Methanol	Nil	Nil	Nil	Nil	Nil	Nil
		Hexane	Nil	Nil	Nil	Nil	Nil	Nil
		Pet. Ether	Nil	Nil	Nil	Nil	Nil	Nil
4.	*S. paratyphi*							
		Distilled water	Nil	Nil	Nil	Nil	Nil	Nil
		Ethanol	Nil	Nil	Nil	Nil	Nil	Nil
		Methanol	Nil	Nil	Nil	Nil	Nil	Nil
		Hexane	Nil	Nil	Nil	Nil	Nil	Nil
		Pet. Ether	Nil	Nil	Nil	Nil	Nil	Nil
5.	*C. albicans*							
		Distilled water	Nil	Nil	Nil	Nil	Nil	Nil
		Ethanol	**0.6**	**0.9**	**1.2**	**1.6**	**2.1**	**Nil**
		Methanol	Nil	Nil	Nil	Nil	Nil	Nil
		Hexane	Nil	Nil	Nil	Nil	Nil	Nil
		Pet. Ether	**2.2**	**2.7**	**2.8**	**3.1**	**3.6**	**Nil**



(MIC of Hexane Exrtact on *S. aureus)*



(MIC of Ehanol Exrtact on *E. Coli)*

(MIC of Hexane Exrtact on *C. albicans)*

(MIC of Pet.Ether Exrtact on *C. albicans)*

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