



In vitro determination of anti-cancer, anti-Inflammatory and anti-dibetic potential among selected species of *Spilanthes* Jacq.

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Abstract

Many medicinal plants were with a long history of use in medicine against a variety of diseases. *Spilanthes* (Asteraceae) an important medicinal plant grown in tropics and subtropics, well-known indigenous medicine, has been used for the treatment of many disease such cancer, diabetes mellitus and skin infections. The aim of this study was to focused the biological activities of four *Spilanthes* spp. against anti-inflammatory, antidiabetic and anticancer activities and to evaluate the pharmacognostic activity of the plants. *S. vazhachalensis* Sheela, *S. ghoshinis*, *S. calva* DC, *S. radicans* Jacq plants showed good anticancer activity against DLA cell lines using the assays. Ethanolic extract of *S. ghoshinis* showed potent cytotoxic activity with cytotoxic percentage (10%-57%) and *S. calva* showed least activity (2%-11%). Anti-inflammatory studies and antidiabetic property of the *Spilanthes* spp. showed better result and thus it has immense role ethnomedicine and preparation of new drug formulation in near future.

Keywords: *Spilanthes*, Pharmacognostic, Asteraceae, Ethnomedicine.

Introduction

Asteraceae family comprises of genus *Spilanthes* has got over 60 species. *Spilanthes acmella* is also known as the tooth ache plant. It grows well in full sun to partial shade reaching a height of 14 to 15 inches with a spread of up to 30 inches ache plant¹.

The taste and impression we get from *Spilanthes* we can consider it for use with various infections such as cold or flu and common fungal infections such as ringworm, athlete's foot and jock itch². Other uses for *Spilanthes* might include viral outbreaks of cold sores and herpes and conditions associated with parasitic infections. *Spilanthes* as its also beneficial for toothaches as well as abscesses of the gums and sore throats³.

The genus *Spilanthes* includes many plant species that are being used for the treatment of various diorders including life-threatening diseases. Researchers have done different in vivo and in vitro pharmacological screenings to authenticate the traditonal uses like anti-inflammatory, anticancer, antidiuretic properties⁴.

Spilanthes calva DC is distributed all throughout the Indian Peninsula. The common habitat of this Indian medicinal plant is in moist habitats of the plains and lower hill regions. This plant has been credited as medicinal pant for its curative properties. The medicinal plant, *Spilanthes calva* DC is an annual herb which is up to 60 centimeter tall. The stems are erect or decumbent at base and more or less hairy. Leaves in this Indian medicinal plant grow in the opposite direction. The leaves are triangular ovate or lanceolate in shape. Each leaf margins

dentate or almost entire and is available as sparsely pubescent beneath. Flower heads of the *Spilanthes calva* D Care ovoid and pale yellow or white in colour. The flowers mature as long-peduncled, solitary or in terminal panicles. Achenes of this medicinal herb are obovate to trigonous in shape with ciliate edges Erect herbs; stem terete, minutely pubescent. Leaves opposite, ovate, obtuse at base, serrulate at margin, acute at apex, glabrous, 5 – 7 pairs; petiole 1 cm long, hairy; puberulous. Head white, discoid, conlike, axillary and terminal, 8 – 10 mm across; peduncle 4 – 7 cm long; peduncle 4 – 7 cm long. Receptacle glabrous; Involucral bracts oblong, subentire at margin, subulate-obtuse at apex, 3- nerved at base, minutely hairy beneath⁵.

Spilanthes ghoshinis Sheela mostly prostrate, stem terete, minutely pubescent, greenish. leaves opposite, 2.5-5 to 2-3.5 mm, ovate-acute, base rounded or sub-cordate, margins distantly serrate, glabrous, petioles 0.5-1.2 cm, pubescent. heads discoid, axillary, solitary, 6-7.5 mm across, subglobose, turning conical, florets yellowish orange, peduncle 3.5-8.5 mm. involucral bracts 1-2 seriate, 3.25-5.5 to 1.75-3 mm. oblong, subentire, obtuse or subacute at apex, 3-nerved from base, minutely pubescent, chaffy, receptacle glabrous, paleaceous. palea 3-3.25 mm, oblong, boat-shaped, concave, apex obtuse, glabrous, margins scarious. heads discoid disc florets many bisexual corolla-tube 1.75-2 mm. dilated above lobes 5, stamens 5, anthers brownish⁶.

Spilanthes radicans Jacq is erect herbs, stem terete, minutely pubescent, leaves petiole 1 cm long, hairy, lamina 7-4 cm, puberulous, ovate-acute, base obtuse, margin minutely serrate,

heads 8 mm. across, white discoid, axillary, solitary, peduncle 4-7 cm long. receptacle glabrous, involucre bracts oblong, obtuse or subulate, subtentire, 3-nerved from base, minutely hairy outside, chaffy, paleas 4 mm long, concave, boat-shaped, glabrous, keeled along the back. 4-5 lobed papillose⁷.

Spilanthes vazhachalensis Sheela is Mostly prostrate; rarely erect herbs. Stem are Terete, minutely pubescent, greenish, leaf 6.5X-7X3.5-4cm. ovate, base rounded or sub-cordate, Heads heterogamous, axillary, usually solitary, rarely 2-3 in each axil. 6-7 mm across, sub globose, turning conical, pale yellow, peduncle 4-8 cm long. corolla is 3 lobed⁸. These are the characters of plants selected for the study, and pharmacological aspects like anti-inflammatory, anticancer, antidiabetic properties have been evaluated in the study.

The pharmaceutical interest in plants as a source of medicines as relatively small amounts of plant material are needed to conduct the screening for bioactivity which is the basis of many contemporary drug development strategies by Gerard Bodeker. Plant drugs are generally less toxic and free from side effects than the synthetic ones. Literature survey proposed that it has multiple pharmacological actions, which include antifungal, antipyretic, local anaesthetic, diuretic, vasorelaxant, anti-human immunodeficiency virus, toothache relieve and anti-inflammatory⁹. Thus four selected plants of *Spilanthes* Jacq. were studied and pharmacological aspects like anti-inflammatory, anticancer, antidiabetic properties have been evaluated in the study.

Materials and methods

The whole plants of four species of *Spilanthes* Jacq. collected from different localities of Kerala. The materials were cleaned, weighed, oven dried and powdered. Samples were kept in polythene bags sealed and stored. The ethanol extract of *S. vazhachalensis* Sheela, *S. ghoshinis*, *S. calva* DC, *S. radicans* Jacq. were taken by cold extraction method and was used for studying the pharmacological activity.

Anti-inflammatory activity was examined through anti platelet activity. Anticancer activity examined by cyto-toxicity evaluation (DLA cell line assay) and Anti-diabetic activity were also assessed.

Anti-diabetic activity: Different concentration of extract was taken into different test tubes. (Stock-10mg/ml) Make the volume to 0.5ml with phosphate buffer of pH 6.9, Blank was measured by taking 1 ml of phosphate buffer. Control was measured by taking 0.5ml of phosphate buffer. The solution was then treated with 0.5ml of alpha amylase (0.5mg/ml). The solution was incubated at 25°C for 10 minutes. Added 0.5ml of 1% starch solution in 0.02 M sodium phosphate buffer of pH 6.9 to all the tubes, and then incubate at 25°C for 10 minutes. The reaction was stopped by adding 1.0 ml of DNS and the reaction mixture was kept in boiling water bath for 5 minutes, cooled to room temperature. The solution was mixed with 8 ml distilled

water. Read the absorbance of the solution in calorimeter at 570 nm against blank solution¹⁰.

Percentage Inhibition = $(\text{Optical density of control} - \text{Optical Density of test}) / \text{Optical density of control} \times 100$

Anti-inflammatory activity: Fresh whole human blood (10 ml) was collected and transferred to the heparinized centrifuged tubes. The tubes were centrifuged at 3000 rpm for 10 min and were washed three times with equal volume of normal saline. The volume of the blood was measured and reconstituted as 10% v/v suspension with normal saline.

The reaction mixture consisted of different concentration of test (Stock-10mg/ml) and 1ml of 10% RBCs suspension, instead of test, saline was added to the control test tube. All the centrifuge tubes containing reaction mixture were incubated in a water bath at 56°C for 30 min. At the end of the incubation, the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5min and the absorbance of the supernatants was taken at 560nm¹¹.

Percentage Inhibition = $(\text{optical density of control} - \text{optical Density of test}) / \text{optical density of control} \times 100$

Anti-cancer activity: In-vitro cytotoxicity study: Using Dalton's lymphoma ascites cells (DLA), the test compounds were monitored for a short term in vitro cyto-toxicity. The tumour cells, aspirated from the peritoneal cavity of tumour bearing mice, were washed thrice with PBS or normal saline. The Trypan blue exclusion method was used to determine Cell viability. Viable cell suspension was added to the tubes containing various concentrations of the test compounds. Volume was made up to 1ml using phosphate buffered saline (PBS) control tube which contained only cell suspension. These assay mixture were incubated for 3 hours at a temperature of 37°C. Further cell suspension was mixed with 0.1 ml of 1% trypan blue and kept for 2-3 minutes and loaded on a haemocytometer. Dead cells took up the blue colour of trypan blue while live cells did not take up the dye. The numbers of stained and unstained cells were counted separately⁹.

% cytotoxicity = $\text{No. of dead cells} / \text{No. of live cell} + \text{no. of dead cell} \times 100$

Results and discussion

Anti-diabetic activity: Pharmacological aspects basically deals with drug analysis to check the biological activity among the selected medicinal plants of Genus *Spilanthes* Jacq. The present investigation on the selected plants of *Spilanthes* showed the anti-diabetic activity. The result showed that the *Spilanthes* species showed good antidiabetic activity among the species, *Spilanthes radicans* showed percentage of inhibition (concentration varying from 25 µl to 100µl) with a range of 46%-73%, similarly *Spilanthes ghoshinis* showed inhibition

percentage as 10%-66% while *Spilanthes calva* showed 13%-43% of inhibition¹⁷. The *Spilanthes vazhachalensis* has got high percentage of inhibition when compared other selected species (46%-90%). Figure-1 showed the Anti-diabetic percentage of inhibition among *Spilanthes* Jacq. A similar report on Antidiabetic and thrombolytic effects of ethanolic extract of *Spilanthes paniculata* leaves showed reduction in glucose level from 23-17% from the initial levels of studies conducted¹².

Anti-cancer activity: Drug concentration and Percent cell death (DLA) of *Spilanthes* are shown in Table-1, with different concentration of (10µg to 200µg) were done. Different percentage of inhibition is shown with respect to the four species of *Spilanthes* Jacq. *Spilanthes ghoshinis* Sheela showed high percentage anti-cancer inhibition value the other three species. The value range from 10% to 57% and least were shown by *S. calva* (2% -11%). Another work showed that extracts, fractions and isolates of *S. acmella* Murr. were evaluated for antimicrobial, antioxidant and cytotoxic activities against the KB and HuCCA-1 cell lines, results showed that all the extracts exhibited ED50 value⁶.

Table-1: Anti-cancer activity of selected species of *Spilanthes* Jacq.

Conc. µg/ml	<i>S. calva</i>	<i>S. radicans</i>	<i>S. ghoshinis</i>	<i>S. vazhachalensis</i>
200µg	11%	34%	57%	15%
100µg	6%	20%	40%	8%
50µg	2%	11%	32%	4%
20µg	0	5%	18%	0
10µg	0	0	10%	0

Anti-inflammatory activity: The present work showed that among the *Spilanthes calva* showed maximum percentage of inhibition that is 27.27%¹ and least were seen in *Spilanthes vazhachalensis* with inhibition rang (9-18%). similar report showed that ethanol extract of *Spilanthes acmella* showed significant anti-inflammatory activity (62.9%) was observed in a dose dependent manner¹³.

¹⁴*Spilanthes* spp. are an important source of anti-inflammatory compounds, and there are numerous studies that validate their use in ethnomedicine, and other painful conditions. *Spilanthes* species has got many medicinal properties as they contain different phytochemicals, which are necessary for curing different ailments. Each secondary metabolites have specific role in medicinal field for treating diverse diseases. This investigations can support or replace the synthetic drug to a natural one in the near future. Diverse pharmacological activities of this plant species were previously reported^{15,4}.

Conclusion

Valuable annual herb multifunctional roles as indigenous medicine for therapeutics in health care, beauty care and cosmetics as well as health food or supplements enriched with numerous antioxidants. From the present work, it can be concluded majority of the *Spilanthes* species possess anti-diabetic, anti-inflammatory, anti-cancer activity. *Spilanthes ghoshinis* Sheela showed good anticancerous effect, whereas *Spilanthes calva* showed better anti-inflammatory activity, while *Spilanthes vazhachalensis* Sheela species showed anti-diabetic effect when compared to other species of *Spilanthes*. Thus a new formulation can be derived from this medicinal plants, that need to be used for the future world and thus finally find out a solution for treating dreadful diseases.

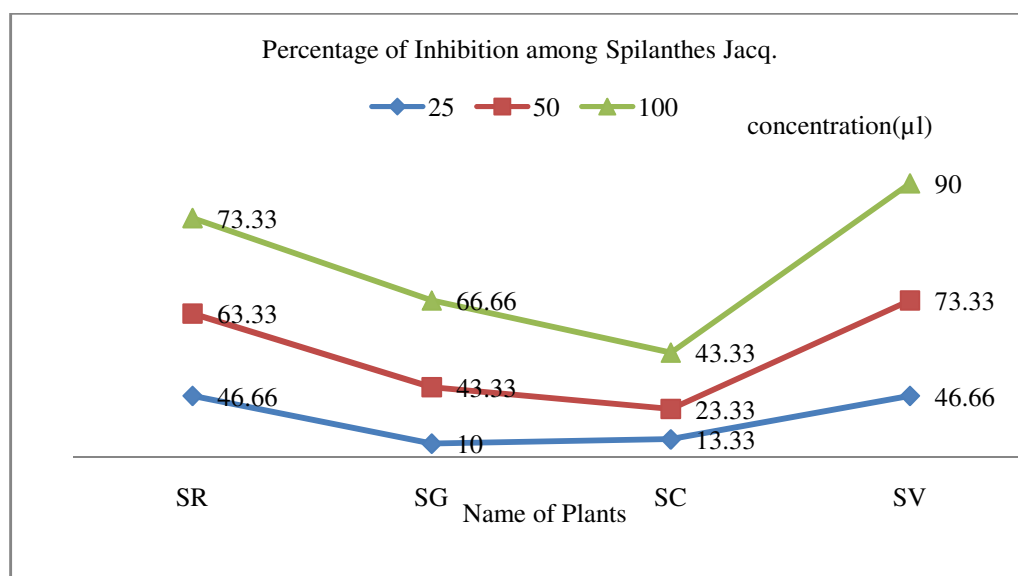


Figure-1: Anti-diabetic percentage of inhibition among *Spilanthes* Jacq.

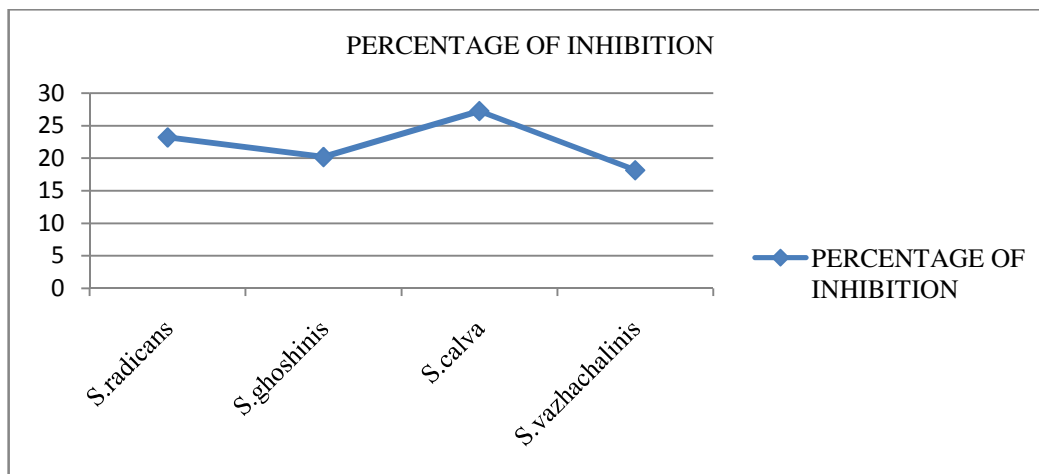


Figure-2: Anti-inflammatory activity of selected species of *Spilanthes* Jacq.

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