



## Phytochemical, Antimicrobial & Antidiabetic Evaluation of *Sphagneticola trilobata* Leaves Extract

Krishnasamy L.<sup>1\*</sup>, Praisay Joy Bell I.,<sup>2</sup> Ramesh T.<sup>3</sup> Aditya Mohan Bharadwaj<sup>4</sup>  
and Chandramughi V.P.<sup>5</sup>

<sup>1</sup>PG & Research Department of Biotechnology, Hindustan College of Arts & Science, Padur,  
Kelambakkam – 603103

<sup>2</sup>Department of Biotechnology, School of Bio-Sciences and Technology, VIT University, Vellore –  
632014

<sup>3</sup>G.S. Gill Research Institute, A Unit of Guru Nanak Educational Society, Affiliated to University of  
Madras, Guru Nanak Salai, Velachery – 600 042

<sup>4</sup>School of Bio-Sciences and Technology, VIT University, Vellore – 632014

<sup>5</sup>Department of Pharmacology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical  
and Technical Sciences (SIMATS), Chennai – 600077

\*Corresponding author: lksamy2004@gmail.com

### ABSTRACT

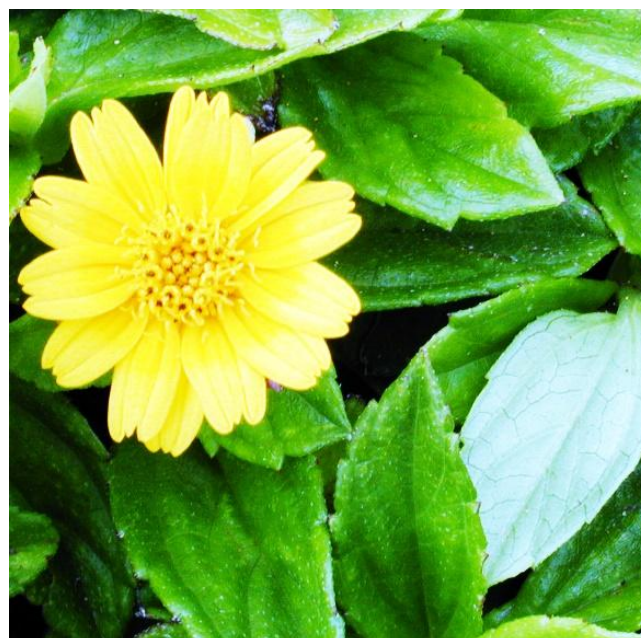
*Plant sources are used as medicines for treating disease and disorder since from ancient times. They are widely used due to their safe and effective active principles and as they are clinically effective and less toxic. They produce an enormous amount of secondary metabolites (phytochemicals) with medicinal and antimicrobial properties. It has been documented that medicinal plants are used to cure diseases in the history of civilization. Sphagneticola trilobata is a plant species of family Asteraceae. It's also called as Wedelia chinensis or Wedelia trilobata. It is a highly resistant, creeping perennial plant, with succulent stems. It has been found that Sphagneticola trilobata is used to treat various kinds of ailments including fever, bites and stings, cold, wounds, kidney dysfunction and dysmenorrhea. This research aims to study the phyto constituents, biological and therapeutic activities of Sphagneticola trilobata. This plant is effectively studied for its antimicrobial, anticancer, anti-inflammatory and anti-diabetic properties. The GC-MS analysis of Sphagneticola trilobata reveals that there are 15 bioactive compounds present in the methanolic leaf extract. Among those the first six bioactive compounds are evaluated for its antidiabetic properties.*

**Keywords:** *Phytochemicals, antimicrobial, anticancer*

## Introduction

Plants remain as an effective therapeutics as they are safe and cause less side effects. Plants possess many bioactive compounds called phytochemicals. Phytochemicals are known to be secondary metabolites which on modified synthetic pathways from primary metabolites. These phytochemicals, which are present in plants are generally referred to be as storehouse of chemo therapeutants. Natural products provide the basis for the development of medicines for serious human diseases like cancer. Despite the availability of numerous treatments derived from natural sources, quest for further anti-cancer compounds is ongoing in the hopes of expanding treatment options and identifying less harmful but more effective medications [5]. Effective novel anticancer drugs may often be found in molecular extracts of herbal medicines. These plants are devoid of pollutants of chemical or biological origin, as well as being rich with organic chemicals & tiny molecules that are more stable and also have intriguing biological functions. This is age of the herbal medicines that are trying to inflict the same effect that antibodies have been doing without the danger of resistant microorganism [1]. Plants are a house to a number of natural compounds that may have the ability to stop the growth of microorganisms. Local plants have been used to extract compounds that have good antimicrobial activity [6].

**Figure 1:** A photograph of *Sphagneticola trilobata*



*Sphagneticola trilobata* is a perennial plant that forms a low mat and may extend to a length of two meters or more. It can reach a height of thirty centimeters. Flowering parts are

upright, stems are spherical and solid with few hairs, and they root at the nodes [7]. The leaves are stalkless or borne on very short stalks; they are simple, opposite, succulent, shiny, dark green on top & lighter upon underside; they are irregularly toothed or serrated; & they typically have two lateral lobes. The inflorescences resemble daisies and are borne individually on tall stalks. They have a vivid yellow to orange color. The fruit is an achene with four angular faces and uneven scales [2].

## **MATERIALS AND METHODS**

### **Plant collection & sample preparation**

Fresh & healthy leaves of *Sphagneticola trilobata* were collected from the herbal garden of Hindustan College of Arts & Science, Chennai. The collected leaves sample were sent for authentication and the macroscopic structure and scientific classification of the plant was studied. Leaves being cut in smaller pieces & kept in hot air for 48hrs. After, leaves were grained into powder form with the help of a mixer. 100gm of *Sphagneticola trilobata* leaves powder was mixed with 300ml of methanol and it was kept in a shaking incubator for 48 hours. Initially, the sample was filtered with the help of a double-layered muslin cloth. Finally filtered with Whatman filter paper for twice. The leaves sample were extracted and poured into a conical flask and covered with muslin cloth for evaporation (48hrs). The extract kept into water bath at 100°C for 15mintues [2, 3, 8].

### **Phytochemical analysis**

Standard methods were used to detect presence of secondary metabolites like test for tannin. The powdered leaf extract sample weighed 0.30 grams, and it was cooked for 10min in water bath of 30 cubic centimeters. After boiling, filtration of mix was done by Whatman filter paper No. 42 (125mm) to remove any remaining solids. Three drops of 0.1% ferric chloride was applied to 5cm<sup>3</sup> of filtrate. Positive tests manifested as brown green or blue-black hue [2, 3, 9].

### **GC-MS analysis**

The leaf sample weight equivalent of 10mg is soaked in 100 ml Ethanol. After 5 days the solvent was filtered and allowed to evaporate. The yield of 2 mg was dissolved in 20 ml ethanol used to run gas chromatography - mass spectrometry with ethanol as column solvent. GC-MS analysis of root extract had been researched SHIMADZU GC-MS QP 2010 with CARBOWAX capillary column & helium as carrier gas. Ethanolic extract of was injected in

to the column. The column was fused with silica 50m x0.25mm. Research carried were 20 minutes. Carrier gas was helium, & split ratio was 5:4 at 100 ° C, 235 ° C for column temperature after 3 minutes, & 240 ° C for injecting temperature.

A 1µl sample were vaporized in split-less injector at 300°C for 22 minutes. Gas chromatography-mass spectrometry analysis utilizing NIST08 library helped pinpoint bioactive chemical [5, 10].

### **Antimicrobial activity**

In vitro antibacterial activity of *Sphagneticola trilobata* (L) extract was evaluated by the agar well diffusion method. The result had been deliberated by measuring mean diameter of zone of inhibition against the tested bacteria. The bacteria culture was purchased from the department of Microbiology, HCAS. Strains of *E.coli*, *Pseudomonas*, *S. typhi* & *S. aureus* were used to determine the microbial activity [7].

### **Antidiabetic activity**

#### **Inhibition of alpha amylase**

Various samples and the reference medication were collected at various concentrations. After incubating the tubes at 25°C for 30 minutes, 1ml of α-amylase into 0.2M sodium phosphate buffer is added to every tube. Every tube was then given 1 ml of a 1% starch solution in a 0.2M sodium phosphate buffer. After that, we let the reaction mixtures sit at 25 degrees for 3 minutes. 1 milliliter (3,5 dinitrosalicylic acid) was used to halt the process. We diluted the reactants with 9ml of distilled water. At 540nm absorbance was observed [4, 11].

OD sample – OD control

% of alpha amylase inhibition =  $\frac{\text{OD sample} - \text{OD control}}{\text{OD sample}}$  x 100

OD sample

## **RESULTS AND DISCUSSION**

The phytochemicals present in *Sphagneticola trilobata* were Phenol, Flavonoids, Steroids, Saponins, Tannins, Diterpenes, Carbohydrate, Protein, Glycosides etc (Table 1).

**Table 1:** Results of phytochemical analysis

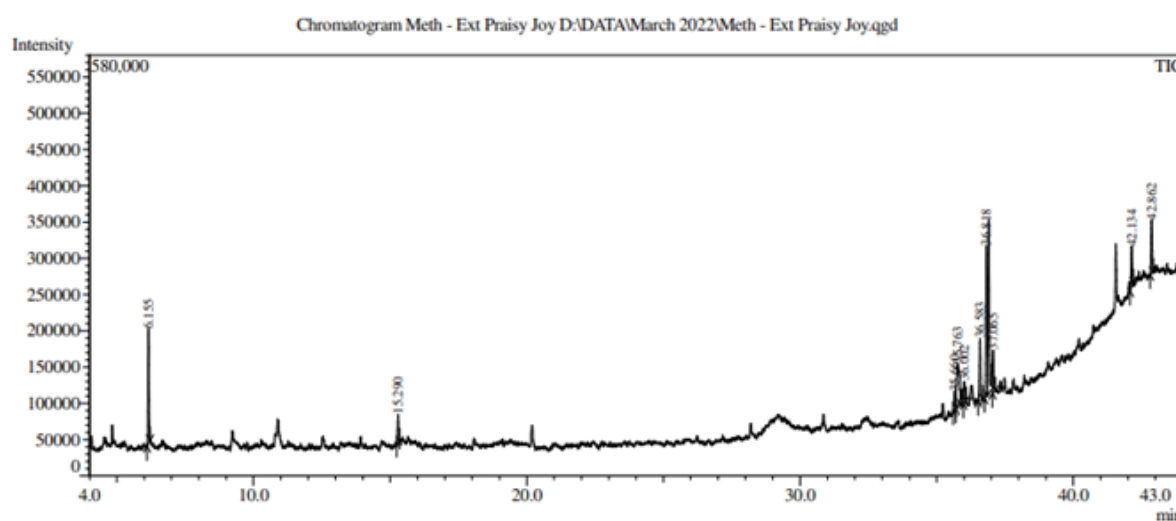
S. No	Phytochemicals	Presence	Absence
1	Phenol	+	
2	Flavonoids	+	
3	Steroids	+	
4	Terpenoids		-
5	Alkaloids		-
6	Saponins	+	
7	Tannins	+	
8	Diterpenes	+	
9	Carbohydrate	+	
10	Protein	+	
11	Glycosides	+	
	a) Liebermann	+	
	b) Keller Kiani	+	
	c) Salkowski test	+	

(+) Presence or (-) Absence of Phytochemicals in the root sample of *Sphagneticola trilobata*

The presence of secondary metabolites exhibits various pharmacological actions such as Anti-viral, Anti-bacterial, Anti-diabetic, Anti-cancer, Anti-obesity, Anti-coagulant, Anti-tubercular, Anti-oxidant, Anti-hypersensitive, Immuno-modulatory properties.

GC-MS analysis of *Sphagneticola trilobata*, leaf extract revealed presence of 10 bioactive compounds. Among the 10 compounds, 7 peak compounds were analyzed for the presence of anti-inflammatory, anticancer and antidiabetic activity. Bioactive compounds obtained through GC-MS analysis of *Sphagneticola trilobata* leaf extract (Table 2 & Figure 2).

**Figure 2:** Peak obtained through GC-MS analysis of *Sphagneticola trilobata* leaf extract



**Table 2:** Compounds obtained in GC-MS

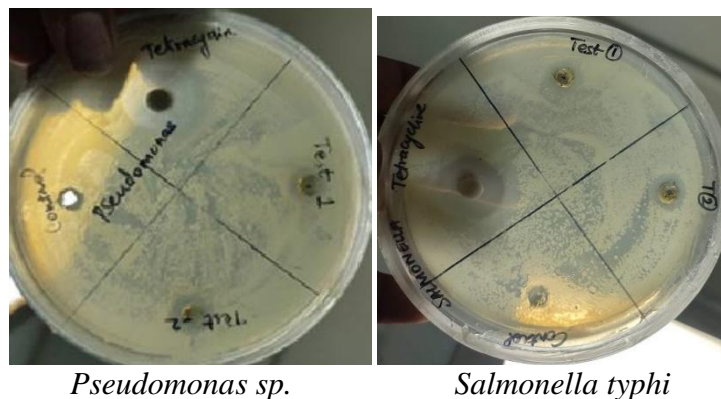
Peak Report TIC				
Peak#	R.Time	Area	Area%	Name
1	6.155	402976	15.01	5-Hydroxymethylfurfural
2	15.290	113231	4.22	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-
3	35.660	116652	4.34	(7R,8R)-CIS-ANTI-CIS-TRICYCLO[7.3.0.0.0.0.0]
4	35.763	439363	16.36	9-Octadecenoic acid (Z)-, oxiranylmethyl ester
5	36.002	110417	4.11	2,2,4,4,6,6,8,8,10,10,12,12,14,14,16,16,18,18-
6	36.583	270863	10.09	2-(7-Hydroxymethyl-3,11-dimethyl-dodeca-2-
7	36.818	628463	23.40	7B,9,9A-TRIHYDROXY-3-(HYDROXYMETHYL)-
8	37.065	168462	6.27	BICYCLO[4.1.0]HEPTANE-7-METHANOL
9	42.134	184910	6.89	TRICYCLO[4.4.0.0(2,7)]DECANE, 1-METHYL-
10	42.862	250176	9.32	Benzenesulfonamide, 4-fluoro-N-[2-(5-fluoro-
		2685513	100.00	

The 10 bioactive compounds identified possess various medicinal properties; 9- octadecanoic acid(z) is an Anti-oxidant, Anti- inflammatory, Anti- androgenic, Neuroprotective, pesticide, nematocidal and hemolytic. Antioxidant 5 hydroxymethylfurfural also inhibits immune-mediated allergic responses, reduces xanthine oxidase activity, and helps avoid sickle haemoglobin. 1H-Cycloprop[e] Most of the volatile component is a decahydro-1,1,7-trimethyl-4-methylene azulene-7-ol (Spathulenol), which has many pharmacological effects including immunoinhibitory, anti-inflammatory, anti-cancer, & apoptosis induction.

**Table 3:** Results of antibacterial activity

S. No	Microorganisms	50 µl	100 µl	Control (ciprofloxacin)
1	<i>Pseudomonas sp.</i>	5 mm	12 mm	15 mm
2	<i>Salmonella typhi</i>	5 mm	7 mm	15 mm
3	<i>Staphylococcus aureus</i>	5 mm	9 mm	15 mm
4	<i>E. coli</i>	7 mm	11 mm	15 mm

**Figure 3:** Photographs of antibacterial assay



*Pseudomonas sp.*

*Salmonella typhi*





*Staphylococcus aureus*

*E. coli*

The methanolic extract of *Sphagneticola trilobata* showed good inhibitory effect against the pathogenic bacteria (Table 3 & Figure 3). The leaf extract exhibited antidiabetic activity (Table 4 & Figure 4).

**Table 4:** Results of antidiabetic activity

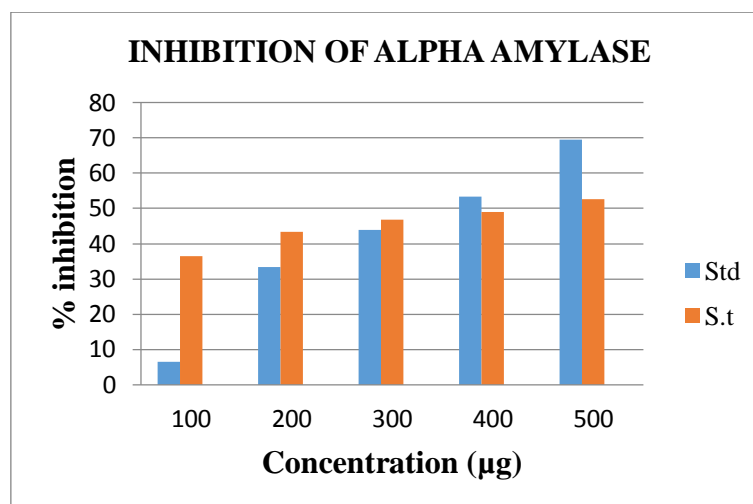
**Control - 0.14**

Concentration (µg)	100	200	300	400	500
Standard O.D.	0.15	0.21	0.25	0.30	0.46
% of inhibition	6.6	33.3	44.0	53.3	69.5

**Blank - 0.26**

Concentration (µg)	100	200	300	400	500
Standard O.D.	0.41	0.46	0.49	0.51	0.55
% of inhibition	36.5	43.4	46.9	49.0	52.7

**Figure 4:** A graph showing percentage of inhibition of alpha amylase



### Conclusion

*Sphagneticola trilobata* is a plant species of family Asteraceae. It has been found that *Sphagneticola trilobata* is used to treat various kinds of ailments including fever, bites and stings, cold, wounds, kidney dysfunction and dysmenorrhea. This study confirm that the phyto-constituents such as Phenol, Flavonoids, Steroids, Saponins, Tannins, Diterpenes, Carbohydrate, Protein and Glycosides are present in the leaf extract. Leaves of this plant is effectively against the bacterial species like, *Pseudomonas sp.*, *E. coli*, *Staphylococcus aureus* and *Salmonella typhi*. The GC-MS analysis of *Sphagneticola trilobata* reveals that there are 15 bioactive compounds present in the methanolic leaf extract. The crude extract of this plant exhibited antidiabetic properties. The further study should be required to evaluate its other pharmacological activity, anti-cancer property, cytotoxic properties etc.

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