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Bioactive Profiling and Antibacterial Study of *Emilia sonchifolia.*

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ABSTRACT

Emilia sonchifolia, a member of the family Asteraceae, has been reported for its potential applications in the ayurvedic system of medicine for treating various diseases. The core objective of this study is to compare the phytochemical and pharmaceutical activities of aqueous and methanolic extracts of the aerial plant parts. Phytochemical screening was performed to identify the presence of protein and a myriad of bioactive secondary metabolites like alkaloids, terpenoids, tannins, saponins and flavonoids. Assays like total antioxidant, hydrogen-peroxide and Ferric Reducing Antioxidant Power, antidiabetic, minimum inhibitory concentration and agar well diffusion method were performed in both extracts. Thin layer chromatography and UV-Vis analysis were performed for prospective studies.

Keywords: Phytochemicals, Phytoconstituents, Antioxidants, Antibacterial, TLC, UV-Visible.

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INTRODUCTION

Plants play a vital role as a source of food and vegetables, aesthetics and medicine, feeding livestock, and trees as furniture. Since plants has many economic values, they are also used for maintaining good health, sustenance or satisfaction [1]. Apart from other uses, the plants that we use as food also act as medicine and pharmaceuticals, helping attenuate the disease and mortality rate. Cancer, cardiovascular disease and cerebrovascular diseases, now the primary cause of mortality, are alleviated by the antioxidant activity of the plant parts [2].

Over 50% of the marketed drugs so far, are made up of natural products, mainly from plants. Different plant parts like stem, leaves, flowers and roots are used in the development of pharmaceutical products. Adopting this as the main strategy, plant-based drugs constitute the finest replacement for synthetic drugs in this modern pharmaceutical world [1].

Emilia sonchifolia (L.) DC. commonly known as red tassel flower, lilac tassel flower, cupid's shaving brush, purple sow thistle. The native of the plant is India, China and Southeast Asia. It is found to be distributed around most parts of the world like Africa, America, also across several tropical region. *Emilia sonchifolia* is classified under the family Asteraceae. It is an annual herbwith a stem erect, hairy stem, growing to a height of 8-24inches tall [8].

Emilia sonchifolia as a medicinal plant, used for many pharmacological activities like antimicrobial, antiviral, anti-inflammatory, analgesic, anticancer, antioxidant, anti-diabetic, hepatoprotective, anti-cataract and anticonvulsant activities. This plant produces secondary compounds in which some are very effective against pathogens, parasites and microbes like bacteria and fungi [5].

Figure 1: *Emilia sonchifolia* plant

In addition to phytochemicals, the plant also harboured chemical constituents like quercetin 3-O-β-D-glucopyranoside, lupeol, linoleic acid, methylparaben, isovanillic acid and much more different kind of compounds[3]. In traditional medicine, the leaves extracts are used for the treatment of fever, dysentery, cuts and wounds, eye inflammations, night blindness, infantile tympanites and bowel complaints[7]. Given the relative effectiveness of *Emilia sonchifolia* as a pharmaceutical plant, a decisive study is required to support the research evidence for the plant's bioactive profile.

MATERIAL AND METHODS

Plant Material

Emilia sonchifolia used for the study was collected from the Botanical garden of Centre for Bioscience and Nanoscience Research, Eachanari, Coimbatore 21. The aerial parts including leaves and stem were collected, washed and air dried.

Preparations of plant extracts

Fresh leaves and stem of the *Emilia sonchifolia* plants were harvested and washed to remove dirt. The Aqueous and Methanolic extract of the plants were prepared by using 1.5g plant parts, crushed using motor and pestle. 20ml of distilled water and methanol was added for aqueous and methanolic extract. The mixtures was placed in orbital shaking incubator at 60-70rpm for 24hours at 40°C. Filtered using Whatman No.1 filter paper. The filtrate is stored in stock vials[4].

Experimental

Phytochemical Analysis

The tests were conducted for the analysis of various phytochemical constituents present in *Emilia sonchifolia* by using the standard procedures [13][9]. The phytochemical analysis was done for both aqueous and methanolic extracts of the plant.

Alkaloid Test

The detection of alkaloids in the extracts was identified by Mayer's test. For this test 1ml of Mayer's reagent (0.113g of mercuric chloride dissolved in 5ml distilled water) and a few drops of iodine solution was added to the 1ml of plant extracts. Formation of yellow colour indicates alkaloid presence.

Terpenoid Test

Equal volume of concentrated H_2SO_4 and extracts were mixed and water bath it for 2-4min. Formation of greyish colouration indicated the presence of terpenoids.

Tannin Test

To 1ml of extracts, 1ml of 2% $FeCl_3$ was added. The presence of tannin in the extracts is observed by formation of blue green colour.

Saponin Test

Distilled water and the plant extracts were added and shaken vigorously for the formation of foam layer at the top of mixture, indicating the saponin presence.

Flavonoid Test

Few drops of 5% NaOH was added to the extracts, the formation of yellow colouration indicated the presence of flavonoids in the extracts.

Protein Test

Plant extracts and a few drops of HNO_3 were added, for identifying the protein presence indicated by yellow colour.

Antioxidant Activity

Total Antioxidant Assay

The total antioxidant of the extracts were investigated by phosphomolybdenum method, which involves the addiction of 0.5ml total antioxidant reaction mixture (0.6M H2SO4, 28mM sodium phosphate, 4mM ammonium molybdate) to the extracts (methanolic and aqueous). The mixtureswere allowed to incubate at 50°C for 90min. The Optical Density of the extracts was measured in the UV visible spectrophotometer at the wavelength of 695nm. The spectrophotometer used for the analysis was Labtronics LT-291. The mg/g of total antioxidant was calculated by using ascorbic acid as a standard. The principle of this assay is based on the reduction of the Phosphate-Molybdenum (VI) to Phosphate-Molybdenum (V)[10].

Hydrogen peroxide Assay

The Hydrogen Peroxide assay for antioxidant was based on the principle of measuring the hydrogen peroxide reduction in an incubation system with H2O2[6]. To the extract (methanolic and aqueous) of 0.5ml, 2ml of 20mM hydrogen peroxide was added and mixed, followed by the addiction of 0.9ml ethanol. After a period of 10-15min, the mixture was determined at 230nm using a spectrophotometer(Labtronics LT-291) [11].

FRAP Assay

Ferric Reducing Antioxidant Power assay method involves the inclusion of equal volume of the phosphate buffer to the extracts (methanolic and aqueous), to which 0.1% potassium ferricyanide was added and incubated for 20 min at a temperature of 50°C. To this mixture, 1ml of 10% Trichloro acetic acid was added and mixed well. 1ml of distilled water and 0.5ml of 0.1% ferric chloride was added. Using a spectrophotometer(Labtronics LT-291) the OD was measured for the extracts at a wavelength of 700 nm [12].

Characterisation of Plant Compounds

UV-VIS study

The characterisation of compounds in the aqueous and methanol extracts sample were performed by UV-Visible study by using spectrophotometer (Labtronics LT-291) which is studied in the nanometre of 200-800nm for methanol and 200-600nm for aqueous.

Thin Layer Chromatography

Thin layer chromatography is a technique were the test samples are loaded at the one end of the stationary phase usually made up of an absorbent material like silica gel coated on an aluminium sheet. Solvent acts as a mobile phase. By mixing distilled water, methanol, chloroform, acetic acid and formic acid in the ratio of 1:2:1.5:1:1.5, the solvent was prepared. The loaded TLC plate was placed in the solvent until the solvent moves more than 75%. Then the plate was taken out and allowed to air dry. A violet colour spot can be visualized when viewed under UV light [15].

Antidiabetic Activity

α-Amylase assay

The α-amylase assay was performed by adding 0.5ml of 0.1% starch solution in 16mM of sodium acetate buffer to the plant extracts. To which 0.2ml of α-amylase solution(27.5g α-amylase in 100ml distilled water) was added, followed by the addition of 0.5ml of solution mixture made using sodium potassium tartarate and 96mM 3,5-dinitrosalicylic acid. The solution mixture were incubated for 10min at 25°C in alkaline condition. The principle of this assay was the reduction of 3,5di-nitrosalicylic acid to 3-amino-5-nitrosalicylic acid. The OD value of the extracts are analysed in spectrophotometer at 540nm. The spectrophotometer used is Labtronics LT-291 [12].

α-Glucosidase assay

In α-glucosidase test, 1ml of 2% starch solution was added to the extracts, followed by the addition of 1ml 0.2M tris buffer, which was incubated for 15-30min at a temperature of 37°C. By adding 0.2ml of α-glucosidase the reaction begins, where the α-glucosidase catalyses the disaccharide to monosaccharide. The extract mixture was allowed to incubate at 35°C for 45min. 2ml of 6N hydrochloric acid was added toterminate the reaction. Now the solution mixture was measured for the optical density in spectrophotometer at 540nm [12].

$$\text{Percentage of inhibition} = (C - T/C) \times 100$$

Where, C stands for Control OD and T stands for Sample OD.

The above formula was used to calculate the percentage of inhibition for both the α-amylase and α-glucosidase tests.

Antibacterial Activity

Minimum Inhibition Concentration Assay

The MIC assay was performed to identify the percentage of inhibition of the plant extracts by turbidity method. For this assay Luria-Bertani broth was prepared under sterilized condition. To 1ml of Luria-Bertani broth 10µL of E.coli bacterial culture was added. The plant extracts (aqueous and methanol) were added in a varying concentration of 5mg, 10mg, 15mg. Cultures were incubated for 24hrs at 37°C. Now the optical density value for both extracts (aqueous and methanol) were measured using spectrophotometer(Labtronics LT-291) at a wavelength of 600nm [15]. The percentage of inhibition was calculated by using the formula:

$$\text{Percentage of inhibition} = (C - T/C) \times 100$$

Where, C stands for Control OD and T stands for Sample OD.

Agar Well Diffusion Method

The method applied for the study of antibacterial assay was agar well diffusion method, and the medium used for this study was Muller Hinton Agar medium. The procedure has to be done in sterilised condition. The agar medium prepared was sterilised and poured in the petri dishes and allow to solidify. The bacteria *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Salmonella typhi* were uniformly distributed by swab method. 10µL of the aqueous extract, methanol extract, DMSO were loaded into the well made using the cork borer. An antibiotic disc, Cefazolin(Cz30) was placed which was used as positive control. The inoculated petri dishes were allowed to incubate at a temperature of 37°C for 24hrs in the incubator. By measuring the zone of inhibition the antibacterial activity was determined [13].

RESULT

Phytochemical Analysis

The phytochemical analysis tests performed for both the aqueous and methanol extracts of aerial parts, includes the stem and leaf of *Emilia sonchifolia*. The tests conducted were to identify the presence and absence of the phytochemical such as Alkaloids, Terpenoids, Tannin, Saponin, Flavonoids and Protein. The results of the phytochemical screening of both the aqueous and methanol plant extract were listed below in Table1.

Table1: Phytochemical Analysis

Phytochemical constituent	Extracts	
	Aqueous	Methanol
Alkaloids	+	+
Terpenoids	-	+
Tannins	+	+
Saponins	+	-
Flavonoids	+	-
Proteins	+	-

Note*: + present, - absent

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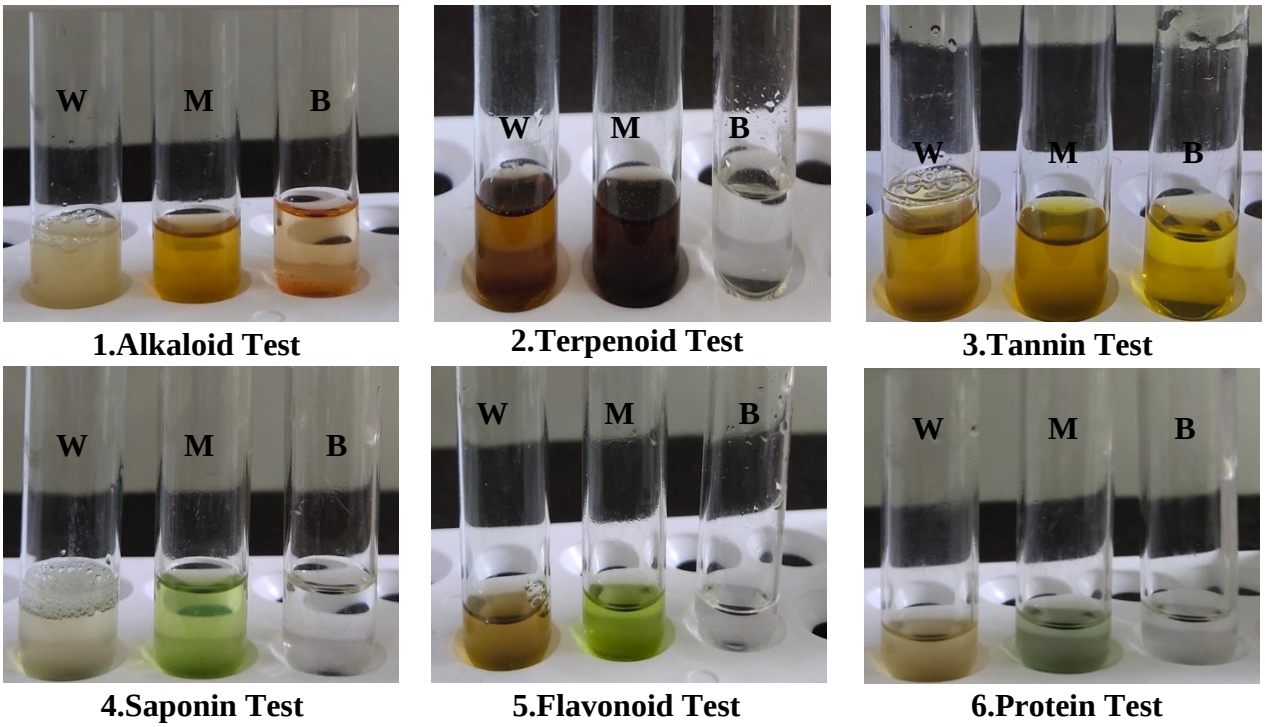
Figure 2: Phytochemical Analysis



Note*: W-Aqueous extract, M-Methanol extract, B-Blank

Antioxidant Activity

The tests performed to check the antioxidant activity were Total Antioxidant assay, Hydrogen peroxide assay, FRAP assay, conducted for both aqueous and methanol extracts of the plant and analysed in spectrophotometer. The result for the total antioxidant and FRAP were analysed in mg/g, result were tabulated in the Table2. The hydrogen peroxide assay result was calculated in percentage and recorded in the Table 3.

Assay	mg/g	
	Aqueous extract	Methanol extract
Total Antioxidant	0.668	0.646
FRAP	0.761	1.105

Table 2: Antioxidant Activity- Total Antioxidant and FRAP Assay

Assay	% of inhibition	
	Aqueous	Methanol
Hydrogen peroxide	51.5%	18.3%

Table 3: Antioxidant Activity- Hydrogen peroxide Assay

Thin Layer Chromatography

The thin layer chromatography viewed under the UV light showed a visible colour spots, which indicates the movement of compounds in the methanol and aqueous extracts which had moved along with the solvent.

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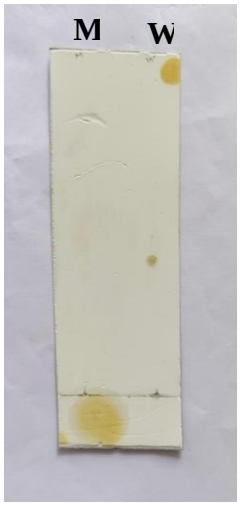

Figure 3: Thin Layer Chromatography

Note*: W-Aqueous extract, M-Methanol extract

Antidiabetic Activity

The tests performed for the antidiabetic activity were α-amylase and α-glucosidase assay. Which was observed in the spectrophotometer and the optical densitywas measured at 540nm. Their results were tabulated below in the Table4.

Assay	% of inhibition	
	Methanol extract	Aqueous extract
α-amylase	71.9%	31.8%
α-glucosidase	90.0%	36.5%

Table 4: Antidiabetic Activity

Antibacterial Activity

Minimum Inhibition Concentration Assay

The antibacterial activity of the extract(methanol and aqueous) was identified by turbidity method by using extract of various concentrations (5mg, 10mg, 15mg) and the bacterium used for the culture was E.coli. By measuring the OD, the percentage of inhibition can be calculated. The results of the methanol extract was tabled in Table5, and for aqueous extract as follow in Table6.

Result	Control	Extract(methanol) concentration (mg)		
		5	10	15
% of inhibition	0%	36.8%	57.8%	81.2%

Table 5: Antibacterial Activity-MIC Assay for Methanol Extract

Result	Control	Extract(aqueous) concentration (mg)		
		5	10	15
% of inhibition		30.5%	42.9%	57.1%

Table 6: Antibacterial Activity-MIC Assay for Aqueous Extract

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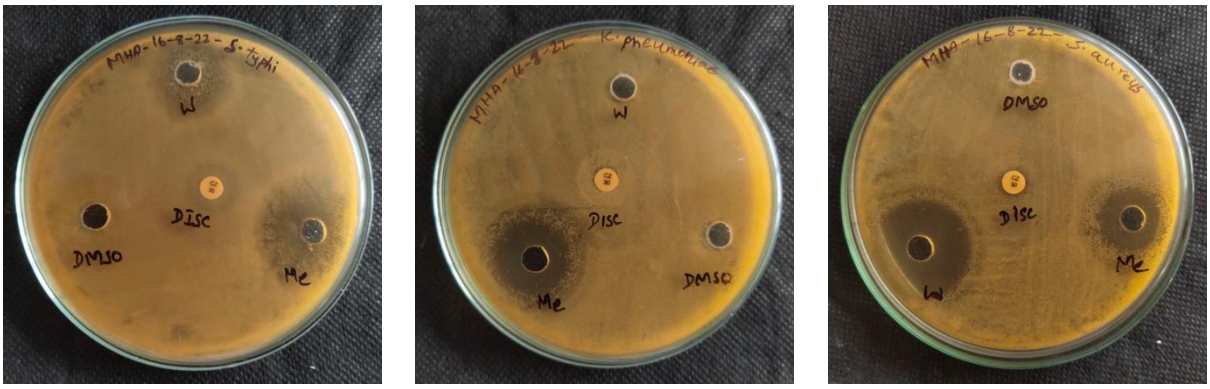
Agar Well Diffusion Method

The antimicrobial activity exhibited by the aqueous and methanol extract of the plant were identified by measuring the zone of inhibition. The zone of inhibition for each type of the bacteria were tabulated below in the Table7. The result shows that the methanol extract shows more effective zone of inhibition for the bacteria *Klebsiella pneumoniae* and *Salmonella typhi*, the aqueous extract shows effective zone of inhibition for the bacterium *Staphylococcus aureus.*

Bacteria used	Zone of inhibition (mm)			
	Methanol	Aqueous	DMSO	Disc (Cz30)
Klebsiella pneumoniae	9	1	0	1
Salmonella typhi	8	5	0	3
Staphylococcus aureus	7	10	0	1

Table 7: Antibacterial Activity-Agar Well Diffusion Method



Klebsiella pneumoniae ***Salmonella typhi*** ***Staphylococcus aureus***

Figure4: Antibacterial Activity-Agar Well Diffusion Method

Note*: W-Aqueous extract, Me-Methanol extract

UV-Vis study

UV-Visible spectroscopy analysis

UV-Visible spectroscopy analysis of both the extracts was scanned, methanol extract scanned between 200-800nm showed five peaks at the wavelength 760nm, 660nm, 520nm, 415nm and 385nm which can be seen in the Figure5. In the case of aqueous extract scanned between the wavelength of 200-600nm visualised eleven peaks in 395nm, 375nm, 355nm, 340nm, 320nm,275nm, 270nm, 250nm, 230nm, 225nm, 220nm as seen in the Figure6.

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Figure 5: UV-Vis Study – Methanol Extract

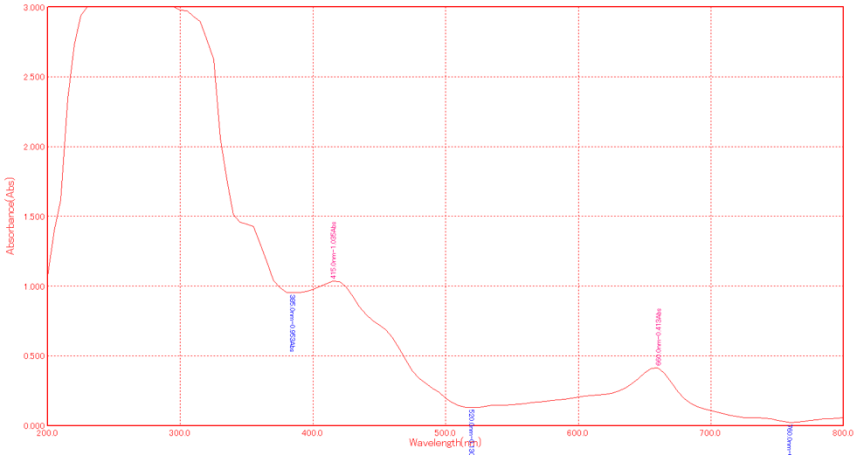
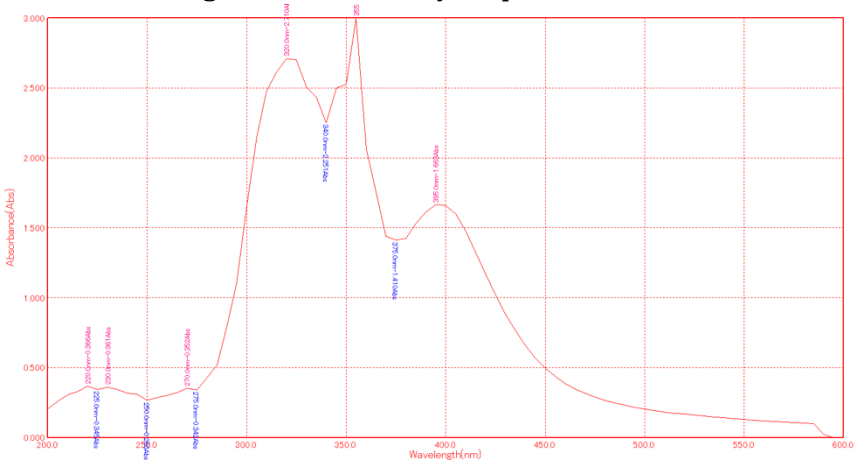


Figure 6: UV-Vis Study – Aqueous Extract



DISCUSSION

The present study of the phytochemical activity had showed the presence of alkaloid and tannins in both extracts, whereas saponins, flavonoids and proteins was present in aqueous extract but they were absent in methanol extract, instead terpenoids is present in it and absent in aqueous extract. Neethu Vijayakumar et al., studies showed the presence of alkaloid, terpenoid, flavonoid, tannin and carotenoid in the methanolic extract of this plant [4]. The antioxidant activity performed were total antioxidant, FRAP and hydrogen peroxide using the plant extracts. The hydrogen peroxide assay showed antioxidant activity of 51.5% for aqueous extract and 18.3% for methanol extract. Insufficient phenolic property may a reason for low antioxidant property of methanolic extract [4]. The current antibacterial assay showed that the methanol extract had higher antibacterial activity as compared to the aqueous extract. Methanol extract shows high resistance in accordance to their concentration. Dash Gouri Kumar et al., had studied the antimicrobial activity of *Emilia sonchifolia*, which shows that the methanolic extract of the plant have showed various microbial resistance against 20 species of the bacteria like E. coli, Staphylococcus spp., Bacillus spp., Enterobacter spp., Pseudomonas spp., etc., and 12 filamentous fungi and 3 yeast species [5]. In addition to higher antibacterial activity in the methanol extract, it also shows higher antidiabetic activity as compared to the aqueous extract.

The bioactive components separated from the thin layer chromatography were future analysed in UV-visible analysis using a UV-spectrophotometer.

CONCLUSION

This research evaluates the efficacy of *Emilia sonchifolia* has many pharmaceutical activities like antioxidant, antidiabetic and antibacterial agent. The result shows that this plant extract can be employed and used in the formulation of drug discovery. Most commonly, they can be used in the medicinal field like siddha and ayurveda. Since the plant is consider as an edible. It can also be used in our daily food diet. Due to its pharmaceutical activities it has the ability of lowering blood sugar level, prevent bacterial diseases like diarrhoea, typhoid and other like illness like cold and fever.

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