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Research Article

Estimation of flavonoids and phenols, and antioxidant capacity of *Spilanthes ghoshinis* Sheela

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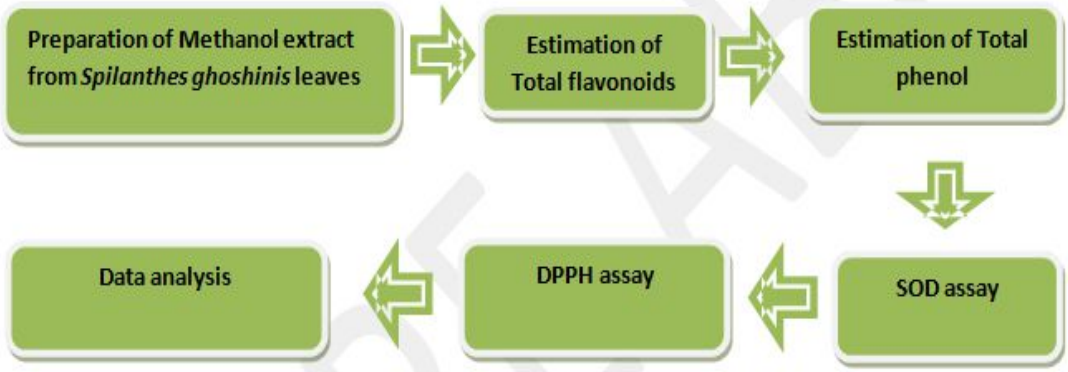
Objective: The present study was undertaken to investigate the total flavonoids, phenols and antioxidant effect of *Spilanthes ghoshinis* Sheela.

Methodology:



Duration taken for the research: 3 months

Conclusion: Findings of the study indicate that *Spilanthes ghoshinis* extract contains detectable amounts of phenolic and flavonoids compounds and exhibits good antioxidant and free radical scavenging activities. Assays show that *Spilanthes ghoshinis* extract is a significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses.

Applicable Industries: Pharmaceutical industry

Expected outcome: Herbal therapeutic drug ingredient

Research Article

Estimation of flavonoids and phenols, and antioxidant capacity of *Spilanthes ghoshinis* Sheela

Durga and Sheela

Abstract

Spilanthes ghoshinis is a medicinal, folklore plant used for variety of ailment across the country. The present work was carried to evaluate the secondary metabolite like flavonoids and phenol. Antioxidant capacity was calculated using DPPH and SOD assay. The present result showed that *Spilanthes ghoshinis* have better flavonoids and phenol content, while IC50 value of SOD is 248.03µg/µl and IC50 value is148.38 µg/µl for DPPH.

This study can provide a better insight for the ayurvedic system of medicine.

Keywords: DPPH, flavonoid, *ghoshinis,* phenols, SOD, *Spilanthes*

Introduction

Spilanthes ghoshinis Sheela belongs to the genus *Spilanthes*, family Asteraceae (Compositae). It is an herb found all around the world and widely distributed throughout the tropics and subtropics and is grown as an ornamental (and as a medicinal) plant in various parts of the globe. Commonly it is known as Tooth ache plant or Paracress or Eyeball plant. The active constituent spilanthol chiefly present in leaves and flower heads, and produce analgesic activity that can numb toothache. The whole plants can be used in the treatment of dysentery and rheumatism. A decoction of the plant can be taken internally as a diuretic and able to resolve stones in the bladder, while a decoction of the roots can be used as a purgative. It is also used as a defensive medicine for scurvy and stimulates digestion. Besides these medicinal uses, the flower heads have been used as a spice for appetizers by the Japanese (Chakarabotty *et al*., 2002; Dubey *et al*., 2013).

Flavonoids consist of a large group of polyphenolic compounds having a benzo-γ-pyrone structure and are ubiquitously present in plants. They are synthesized by phenyl propanoid pathway. Available reports tend to show that secondary metabolites of phenolic nature including flavonoids are responsible for the variety of pharmacological activities. The flavonoids are mostly used for the health benefits due to antioxidant properties. The hydroxyl group present in the flavonoids regulates the antioxidant activity by scavenging free radicals and/or by chelating metal ions (Gangwar *et al*., 2014; Kenwat *et al*., 2014). The chelation of metals could be crucial in the prevention of radical generation which damage target biomolecules. The various studies reported protective effects of flavonoids against many infectious (bacterial and viral diseases) and degenerative diseases such as cardiovascular diseases, cancers, and other age-related diseases (Kumar, 2013; Pandey, 2007)

Plant polyphenols are natural antioxidants and most of their pharmacological properties are due to their antioxidant action. This is generally considered to reflect their ability to scavenge endogenously generated oxygen radicals or those radicals formed by various xenobiotics, radiation etc. Many studies suggest that a phytochemical rich diet which includes colorful fruits and vegetables may reduce the risk of human cancer diseases. Phenolic compounds (commonly referred to as flavonoids or polyphenols) are ubiquitous phytochemicals present in plant foods with numerous biological activities including antioxidant properties. Phenolics exert antioxidant properties through various mechanisms of action including the scavenging of free radicals during the course of normal cell metabolism thereby preventing damage to lipid proteins and nucleic acids eventually cell damage and death. Dietary intake of natural Phenolic antioxidant has been suggested to contribute to the prevention of heart disease and cancer. Antioxidant and radical scavenging properties of the plant extracts will be evaluated using different antioxidant tests including. 1) Total phenolic and flavonoid content, 2) Total antioxidant capacity.

Materials and Methods

Plant collection

The whole plant of *Spilanthes ghoshinis* were collected from Aluva Ernakulam district of Kerala, India during the month of July 2016. Collected plant material was washed thoroughly in running tap water, rinsed in distilled water and shade dried in open air and grinded into powder for further use.

Sample preparation

The powder sample was weighted and phenolic and flavonoid compound was extracted with 50 mL of 80% aqueous methanol on an ultrasonic bath for 20 minute. An aliquot (2mL) of the extract was centrifuged.

Preliminary Phytochemical analysis (Yadav, 2011)

Test for flavonoids

(a) The drug in alcoholic and aqueous solution with few mL of ammonia is seen in U.V. and visible light, formation of fluorescence indicates the presence of flavonoids.

(b) Little quantity of extract is treated with amyl alcohol, sodium acetate and ferric chloride. A yellow colour solution formed, disappears on addition of an acid indicates the presence of flavonoids

(c) Shinoda's test: The alcoholic extract of powder treated with magnesium foil and concentrated HCl give intense cherry red colour indicates the presence of flavonones or orange red colour indicates the presence of flavonols.

(d) The extract is treated with sodium hydroxide; formation of yellow colour indicates the presence of flavones.

(e) The extract is treated with concentrated H_2SO_4, formation of yellow or orange colour indicates flavones.

Test for phenolic compounds

(a) Take the little quantity of test solution and mixed with basic lead acetate solution. Formation of white precipitates indicates the presence of tannins.

(b) To 1mL of the extract, add ferric chloride solution, formation of a dark blue or greenish black colour product shows the presence of tannins.

(c) The little quantity of test extract is treated with Potassium ferric cyanide and ammonia solution. A deep red colour indicates the presence of tannins.

Total flavonoid content

Flavones and flavonols contents were analyzed by the colorimetric method. 9.8 mLof the prepared extract was mixed with a 10% solution of aluminum chloride (200 µL). After 30 min, absorption was measured at a 425 nm wavelength. The amount was calculated using quercetin calibration curve. The results were expressed as the quercetin equivalent (QE) mg per 100 mL of the sample (Chang *et al.,* 2002)

Total phenol content

Total phenol content was determined using colorimetric method. 2.0 mL of the prepared extract was oxidized using Folin - Ciocalteu reagent (400 µL), and sodium carbonate solution (75 g/L) was then added to the reaction mixture to reach a 10.0 mL volume. After 2 h, the suspension was centrifuged for 10 min at 5000 rpm, and absorption was measured at a 760 nm wavelength. The amount was calculated using the gallic acid calibration curve. The results were expressed as gallic acid equivalent (GAE) mg per 100 mL of the sample (Omorugi BE *et al*., 2011)

Antioxidant activity

Superoxide scavenging activity

Superoxide scavenging (SOD) was carried out by using alkaline Dimethyl sulfoxide (DMSO). Solid potassium superoxide was allowed to stand in contact with dry DMSO for at least 24 h and the solution was filtered immediately before use. Filtrate (200 mL) was added to 2.8mL of an aqueous solution containing Nitrobluetetrazolium (56 mM), EDTA (10 mM) and potassium phophate buffer

(10 mM, pH 7.4). Sample extract (1 mL) at various concentrations (100-500 µg/mL) in water was added and the absorbance was recorded at 560 nm against a control in which pure DMSO has been added instead of alkaline DMSO (Rice-evan *et al*., 1995, Roy *et al.,* 2014).

DPPH Assay

This assay was used in many studies for testing antioxidant activity. 2,2-diphenyl-1-picryl-hydrazil stable radical (DPPH) evidently offers a convenient and accurate method for titrating the oxidizable groups of natural and synthetic antioxidants. This assay was based on the reduction of a methanolic solution of the colored free radical DPPH by free radical scavenger. The degradation of DPPH was evaluated by comparison with a control sample without hydrogen-donating compounds. The decrease in absorbance of DPPH at its absorbance maximum of 517 nm was proportional to the concentration of free radical scavenger added to DPPH reagent solution. Lower absorbance of reaction mixture indicated higher antioxidant activity.

In this study, methanolic solution of DPPH (100 mM, 2.95 mL), 0.05 mL of extracts dissolved in methanol was added at different concentrations (100-500 µg/mL). Reaction mixture was shaken and after 30 min at room temperature, the absorbance values were measured at 517 nm and converted into percentage of antioxidant activity (% AA). Ascorbic acid was used as standard. The degree of discoloration indicates the scavenging efficacy of the extract, was calculated by the following equation (Sahu, 2011, Sellappan *et al*., 2007)

$$\% AA = 100 - \{[(Abs_{sample} - Abs_{blank}) \times 100] / Abs_{DPPH}\}$$

Results and Discussion

Preliminary phytochemical screening showed the presence of phenol, flavonoids in methanol extract of *Spilanthesghoshinis plant* in Table 1. Total favanoid and phenol in *Spilanthes ghoshinis* Sheela have been estimated as 1.001±0.05 and 7.542±0.24 mg/gm respectively (Table 2). It was supported by earlier reports for the presences of phenols and flavonoids in the *Spilanthes acmella* leaves. Flavonoids and phenols exhibit a wide range of biological activities, one of which is they have the properties of antioxidant activity. Being plant secondary metabolites, the phenolics or polyphenols are very important judging from the virtue of their antioxidant activities by chelating redox-active metal ions, inactivating lipid free radical chains, and avoiding the hydroperoxide conversions into reactive oxyradicals.

Table 1: Preliminary phytochemical screening of methanol extract of *S. ghoshinis*

Metabolite	*S. ghoshinis extract*
Flavonoid	
U.V.	+
Feric chloride	+
shinoda"s test	+
sodium hydroxide test	+
concentrated sulphuric acid	+
Phenol	
lead acetate	+
ferric chloride	+
potassium ferric cyanide	+

+ indicates presence

Table 2: Estimation of Total favanoid and phenol in *Spilanthes ghoshinis* Sheela.

plant extract	(mg/gm)
Total flavonoids	1.001±0.05
Phenol	7.542±0.24

The plant extract was able to reduce the stable free radical of DPPH to the yellow coloured diphenylpicrylhydrazine. This evidences that the *Spilanthes ghoshinis* extract contains some active constituents that are capable of donating hydrogen to a free radical in order to remove odd electron which is responsible for radical's reactivity. DPPH radical scavenging method has been proven to be good because its results are not affected by substrate polarity. Scavenging ability of the *S. ghoshinis* extract shows the potential in SOD assay than DPPH assay (Figure 1) while comparing methanolic extract of *S.ghoshinis*. From the similar study it starts that estimation of total flavonoids and antioxidant activity of *Spilanthes acmella* leaves with the lowest IC50 - value for DPPH and Superoxide scavenging (Nabi and Shrivastava, 2016). From this study IC50 value of SOD is 248.03µg/µl and IC50 value is148.38 µg/µl for DPPH.

Superoxide anion radical is one of the strongest reactive oxygen species among the free radicals and also very harmful to cellular components. It has been reported that flavonoids are found to be most effective antioxidants mainly because they can easily scavenge superoxide anions. The results suggest that radical scavenging effect of extract is significant and further analysis need to be carried out to analyse the antioxidant capacity by *in vivo* models for getting further clarity in the study

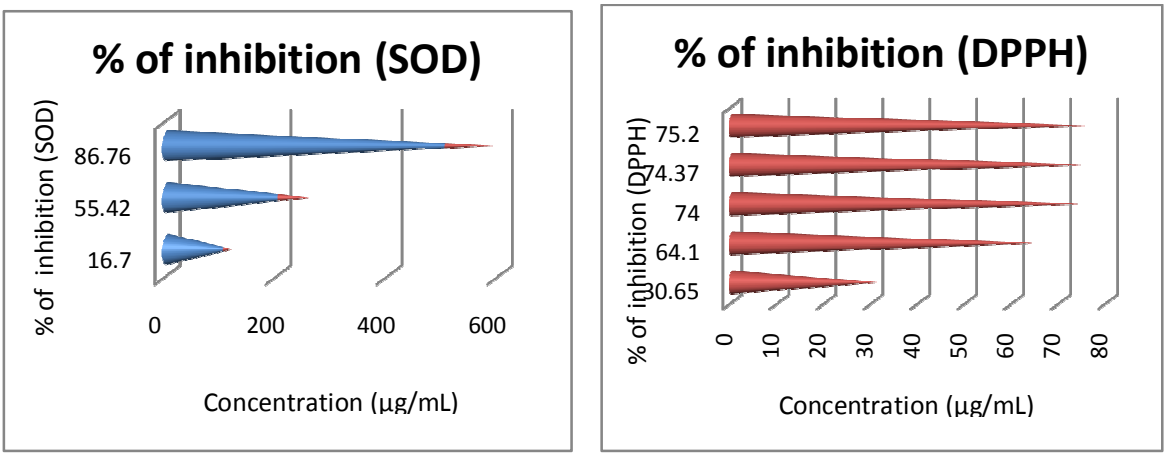


Figure 1 and 2: Antioxidant assay (SOD and DPPH) in *S. ghoshinis*

Conclusion

The findings of study indicate that *Spilanthes ghoshinis* extract contains detectable amounts of phenolic and flavonoids compounds and exhibits good antioxidant and free radical scavenging activities. Assays show that *Spilanthes ghoshinis* extract is a significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses. Further studies are in progress for the isolation of active constituents responsible for antioxidant activity.

Social relevance and expected out come

In the present scenario, demand for medicinal products or natural formulations are accepted by the common people. This study will give a good outlook for developing how drug formulation in a cheaper manner as *S.ghoshinis* is distributed all over India. Ayurvedic industry will be greatly benefited by this study area, as this medicinal plant has got enormous antioxidant property.

Applicable industry

Pharmaceutical industry

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