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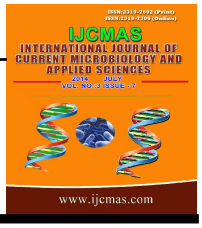

Original Research Article

Determination of Antioxidant Potential in *Spilanthes acmella* using DPPH assay

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Keywords

Antioxidants, free radicals, *Spilanthes acmella*, DPPH, scavenging ability.

A B S T R A C T

Medicinal plants are rich sources of natural antioxidants which are used in the prevention and treatment of diseases like artherosclerosis, heart stroke, diabetes, cancer and to delay the process of aging. *Spilanthes acmella* Murr. (Family:Asteraceae) commonly known as Akarkara or toothache plant is an edible herb traditionally used in the treatment of many diseases and well known for its culinary use as a spice. It is an important source of highly valuable bioactive compounds. The roots, flower heads and whole aerial parts yield a compound known as spilanthol amide which is a powerful insecticide and local anaesthetic. In the present study, antioxidant activity of root extracts of *Spilanthes acmella* was evaluated *in vitro* using DPPH free radical scavenging method. Different concentrations of methanolic extract of roots showed radical scavenging activity with an IC_{50} value 16.3 ug/ml. This study suggests that *Spilanthes acmella* is an effective plant in terms of antioxidant potential and can be exploited for development of plant based antioxidant formulations.

Introduction

Medicinal plants are rich sources of secondary metabolites like flavonoids, flavones, isoflavones, flavonones, anthocyanins, catechins, polyphenols and tannins etc. Many of these chemical compounds posses many biological activities like antimicrobial, antioxidant etc. Antioxidants are the chemicals which scavenge the free radicals and help in preventing and treatment of several diseases. Human body produces oxygen free radicals and other reactive oxygen species (ROS) as by products through numerous physiological and biochemical processes[2].

Overproduction of free radicals can cause oxidative damage to biomolecules such as lipids, proteins, DNA etc. thereby leading to many chronic diseases such as atherosclerosis, cancer, diabetics, rheumatoid arthritis, cardiovascular diseases, chronic inflammation, stroke and septic shock, aging and other degenerative diseases in humans(Bayani Uttara et al.).The most common synthetic antioxidants in use today are butylated hydroxy anisole (BHA) and butylated hydroxy toluene (BHT), but side effects and health risks such as carcinogenic effects has restricted their use

and prompted the investigation of natural antioxidants from plant sources.

Spilanthes acmella Murr. is widely distributed in tropical and subtropical regions of the world (Kishan Lal Tiwari et al.,2011). It is an annual herb with flowers arranged in head inflorescence. It has been well documented for its uses as a spice, antiseptic, anti-bacterial, antifungal, antimalarial and as a remedy for toothache, flu, cough and tuberculosis (Ang Boon Haw and Chan Lai Keng,2003) . Traditionally this plant is used in the treatment of dysentery, rheumatism, as a snake bite remedy, to treat stammering in children and many other diseases (Kishan Lal Tiwari et al.,2011).

The whole aerial parts, flower heads and roots (Plate 1) yield a compound known as spilanthol amide which has a saliva inducing effect (Shefali Arora et al.,2011) and is a powerful insecticide and local anaesthetic (Kishan Lal Tiwari et al.,2011). In addition to spilanthol, *S.acmella* is an important source of highly valuable bioactive compounds such as phenolics , coumarin (scopoletin) and triterpenoids (Supaluk Prachayasittikul et al.,2009).

Presently there is a huge demand for spilanthol isolated from this plant in toothpaste industry. There is also much demand of this plant in cosmetic industry in the production of antiwrinkle formulations like Gatuline, which is being used as Botox replacement (Kishan Lal Tiwari et al.,2011). The present work is aimed for evaluating the antioxidant potential of different concentrations of root extracts of *Spilanthes acmella*.

Materials and Methods

Plant material: The roots of *Spilanthes acmella* were collected from the plant established in Botanical garden, Osmania University College for Women, Koti, Hyderabad.

Extraction: The roots collected from field grown plants were washed thoroughly and shade dried. The dried material was ground to a course powder. The powdered material was (50g) was extracted with methanol for 3-4 hours at 65^0C in a Soxhlet apparatus. Solvent was recovered under reduced pressure to obtain crude extracts. Different concentrations of the extract were employed in the study.

DPPH free radical scavenging assay:

The radical scavenging activity of *Spilanthes acmella* root extracts was determined as described by Gayatri et al. 4.3mg of DPPH **(**2, 2-Diphenyl -1-picrylhydrazyl) was dissolved in methanol (6.6ml) to prepare 0.3mM DPPH solution and it was protected from light by covering the test tubes with aluminium foil. DPPH (150µl) was added to 3ml of methanol and absorbance was noticed immediately at 516nm for control reading. Different concentrations of test samples i.e 25 µl, 50 µl, 100 µl, 150 µl, 200 µl and 250 µl were taken and each of the samples was diluted with methanol up to 3ml, to it 150 µl DPPH was added. The samples were kept in dark for 15 min after which the optical density was observed at 516nm using methanol as blank. The synthetic antioxidant, Rutin hydrate was used as positive control.

The free radical scavenging activity (%Antiradical activity) was calculated using the formula:

$$\%\text{Antioxidant activity} = \frac{(\text{Control Absorbance} - \text{Sample Absorbance})}{\text{Control Absorbance}} \times 100$$

Results and Discussion

The DPPH free radical scavenging assay is one of the widely used techniques for screening the antioxidant potential of plant extracts. In the present work, we have evaluated the root extracts of *S.acmella* for its antiradical activity (Table1). The root extract exhibited antioxidant activity at all the concentrations of test solutions. With the increase in concentration of the plant root extract (25-250µg/ml.), the percentage of antioxidant activity also increased (63.2-83.3%). Among all maximum antioxidant activity (83.3%) was observed at 250 µl concentration with IC_{50} value of 16.3 µg/ml. The standard Rutin hydrate showed 98% antioxidant activity with IC^{50} value at 6.4µg/ml concentration.

The use of DPPH radical provides an easy, rapid and convenient method to evaluate the antioxidants and radical scavengers (Soler Rivas et al.,2000). A freshly prepared DPPH solution exhibits a purple colour with absorption maximum at 517 nm. The DPPH radical reacts with suitable reducing agents, the electrons become paired off and solution looses colour depending upon the number of electrons taken up (Blois MS,1958). The test conducted for evaluating antioxidant activity in *Spilanthes acmella* showed that the DPPH radical scavenging activity increased with the increase in concentration of the extract (Fig1) similarly, increase in antioxidant activity with increase in extract concentration was observed in *Eicchornia crassipes* (Thamaraiselvi et al.,2012) and *Maranta arundinacea* (Nishaa S.etal.,2012).

Plate.1 *Spilanthes acmella* Plant

a. Plant

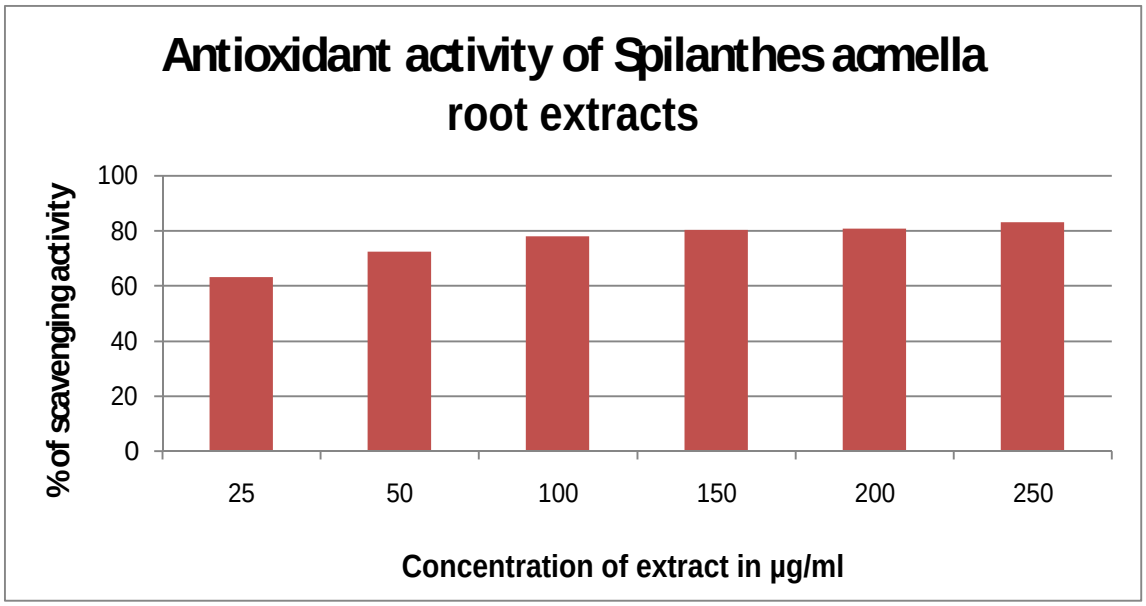
b. Flower heads

c. Roots

Table.1 Antioxidant activity of methanolic root extracts of *Spilanthes acmella*.

Concentration of extract in $\mu g/ml$	% of free radical scavenging activity
25	63.2
50	72.6
100	78.2
150	80.4
200	81.0
250	83.3

Fig.1 Percentage antioxidant activity of *Spilanthes acmella* root extracts.



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References

Ang Boon Haw and Chan Lai Keng. 2003.Micropropagation of *Spilanthes acmella* L., a bio-insecticide plant through proliferation of multiple shoots J. Appl. Hort.; 5(2):65-68.

Bayani Uttara, Ajay V. Singh, Paolo Zambani, R.T Mahajan. 2012. Oxidative Stress and Neurodegenerative Diseases: A Review of Upstream and Downstream Antioxidant Therapeutic Options. Der Pharmacia Sinica; 3 (2):271-277.

Blois MS. Antioxidant determination by the use of stable free radicals. 1958. *Nature* 26:1199-1203.

Gayatri S.Kamble, Rasika C.Torane, Kavita S.Mundhe, Nirmala R.Deshpande, Jyoti P.Salvekar. 2011. Evolution of Free Radical Potential of *Embelia basal*.J. Chem.Pharm.Res.3(2):465-471.

Kishan Lal Tiwari, Shailesh Kumar Jadhav, Veenu Joshi.2011. An updated review on medicinal herb genus *Spilanthes.* J Chin Integr Med.;9(11):1170-1178.

Nishaa.S, Vishnupriya.M, Sasikumar.J.M, Hephzibah P Christabel, Gopalakrishnan. V.K. 2012. Antioxidant activity of ethanolic extract of Maranta arundinacea .L tuberous Rhizomes. Asian J Pharm Clin Res;5(4),85-88

Shefali Arora, Saurabh Vijay and Deepak Kumar.2011. Phytochemical and antimicrobial studies on the leaves of Spilanthes acmella. J. Chem. Pharm. Res.; 3(5):145-150.

Soler-Rivas C, Espin JC and Wichers HJ 2000. An easy and fast test to compare total free radical scavenger capacity of foodstuffs. *Phytochem. Analysis* ,11:1-9.

Supaluk Prachayasittikul, Saowapa Suphapong, Apilak Worachartcheewan, Ratana Lawung, Somsak Ruchirawat and Virapong Prachayasittikul 2009 Bioactive metabolites from Spilanthes acmella Murr.Molecules;4(12);850-867.

Thamaraiselvi, P. Lalitha,P. Jayanthi Pelagia. 2012.Study of antioxidant activity of ethanolic extract of fresh Eichhornia crassipes (Mart.)Solms.Der Pharmacia Sinica;3 (2):271-277.

Vivek Kumar Gupta,Surendra Kumar Sharma.Plants as natural antioxidants.Natural Product Radiance.2006;5(4):326-334.