Mallikarjuna Rao T. *et al.* / *International Journal of Phytopharmacology.* 3(2), 2012, 216-220.

e- ISSN 0975 – 9328
Print ISSN 2229 – 7472

International Journal of Phytopharmacology

Journal homepage: www.onlineijp.com

IJP

ANTIOXIDANT ACTIVITY OF *SPILANTHES ACMELLA* EXTRACTS

T. Mallikarjuna Rao[1*], B. Ganga Rao[2], Y. Venkateswara Rao[1]

[1]Department of Botany, College of Science and Technology, Andhra University, Visakhapatnam, Andhra Pradesh-530003, India.
[2]A.U College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, Andhra Pradesh-530003, India.

ABSTRACT

Many medicinal plants were with a long history of use in folk medicine against a variety of diseases. Recently, many researchers have taken a great interest on medicinal plants for their phytochemical constituents and biological activities including antioxidant activity. The present study was aimed to evaluate phytochemical analysis, quantification of total phenolic, alkaloid contents and antioxidant activity of *Spilanthes acmella* extracts (70% Ethanol, Methanol, Ethyl Acetate and Hexane). The total phenolic and alkaloid contents were quantified by using gallic acid and atropine are as standards and antioxidant activity was evaluated by using three free radicals (Superoxide, Hydroxyl and DPPH). *Spilanthes acmella* extracts revealed the presence of different phytochemical constituents like steroids, terpenoids, flavanoids, alkaloids, glycosides, tannins, carbohydrates, oils and amino acids. The methanol extract have more phenolic and alkaloid contents i.e. 38.83±0.68 (mg/gm) and 26.37±0.16 (mg/gm) than other extracts. The extracts were produced concentration dependent percentage inhibition on free radicals and produced maximum activity at concentrations of 320 and 640µg. Among all extracts, methanolic extract showed better activity compared to other extracts with mean IC_{50} values on superoxide, hydroxyl and DPPH radicals were 189µg, 153µg and 313µg. The above results suggest that *Spilanthes acmella* extracts have antioxidant activity. Further research is in progress i.e. to isolation and characterization of active molecules (compounds) responsible for the antioxidant activity which can be used to treat various diseases.

Key words: *Spilanthes acmella*, Aerial parts, Total Phenolic content, Total alkaloid content, Free radicals, Antioxidant Activity.

INTRODUCTION

Oxidation—one of the body's natural chemical processes—can produce "free radicals," which are highly unstable molecules that can damage cells. Free radicals can cause damage, known as "oxidative stress," which is thought to play a role in the development of many diseases, including Alzheimer's disease, cancer, eye disease, heart disease, Parkinson's disease, and rheumatoid arthritis. In laboratory experiments, antioxidant molecules counter oxidative stress and its associated damage.

Many medicinal plants were with a long history of use in folk medicine in different countries against a variety of diseases. Recently, many researchers have taken a great interest in medicinal plants for their phytochemical constituents and related total potential biological activities including antioxidant activity (Amarowicz *et al.*, 2004; Miliauskas *et al.*, 2004; Rajesh Manian *et al.*, 2008).

Spilanthes acmella L. is the tooth- ache plant, an annual herb belonging to the family Compositae. The genus is widely distributed throughout the tropics, subtropics and can be found in damp pastures, at swamp margins, on rocks near the sea and as a weed of roadsides and cultivations. The flower heads are chewed to relieve the toothache and other mouth related troubles. Leaves are used externally in treatment of skin diseases. Root decoction is used as purgative. Leaf decoction is used as diuretic and lithotriptic. Whole plant is used in treatment of dysentery (URL).

Corresponding Author

Mallikarjuna Rao T
Email: tmrao1987@gmail.com

The present study was aimed to evaluate the antioxidant activity, phytochemical analysis and to quantify the total phenolic and alkaloid contents for different extracts of *Spilanthes acmella* aerial parts. The findings from this work may add to the overall value of the medicinal potential of this plant.

MATERIALS AND METHODS

Source of Chemicals and Drugs

All chemicals and solvents were of the analytical grade obtained from S.D. Fine Chemical Pvt. Ltd., Mumbai, Sigma Chemical Company, U.S.A., Loba Chemic, Mumbai.

Preparation of plant extracts

Freshly collected aerial parts of *Spillanthes acmella* plant was dried under shade and powdered. The coarse powder was extracted with 70% v/v ethanol, methanol, ethyl acetate and hexane separately in a Soxhlet apparatus. The liquid extracts were filtered and evaporated under reduced pressure by using rotary evaporator (Buchi R-210) until a soft mass obtained and then four extracts were used for further investigation.

Phytochemical Analysis

Phytochemical studies were carried out for hexane, ethyl acetate, hydro alcoholic and methanol extracts of *S. acmella* to detect the presence of different phytochemical constituents like steroids, terpinoides, tannins, flavanoids, saponins, glycosides, amino acids etc by using standard procedures (Faraz *et al.,* 2003; Harborne 1998; Edeoga *et al.,* 2005).

Quantification of Total Phenolic content (Singleton and Rossi 1965)

Total phenolic content was determined using the Folin-Ciocalteau reagent Folin-Ciocalteau colorimetry is based on a chemical reduction of the reagent, a mixture of tungsten and molybdenum oxides. The products of the metal oxide reduction have a blue absorption with a maximum at 765nm.The intensity of the light absorption at that wave length is proportional to the concentration of phenols. By using standard Gallic acid calibration curve, measure the concentration of phenolic content in Gallic acid total equivalents using unit's mg/gm. (GAE).

Quantification of Total Alkaloid Content (Fazel Shamsa *et al.,* 2008)

Total alkaloid content was determined by the Fazel *et al.,* method. The plant extract (1mg/ml) was dissolved in 2 N HCl and then filtered. The pH of phosphate buffer solution was adjusted to neutral with 0.1 N NaOH. One ml of this solution was transferred to a separating funnel and then 5 ml of BCG solution along with 5 ml of phosphate buffer were added. The mixture was shaken and the complex formed was extracted with chloroform by vigorous shaking. The extracts were collected in a 10 ml volumetric flask and diluted to volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm. By using standard atropine calibration curve, measured the concentration of alkaloid content in atropine equivalents using unit's mg/gm. (GAE).

In vitro anti oxidant activity

For the assessment of free radicals scavenging activity of hexane, ethyl acetate, Ethanol (70%v/v) and methanol extracts were dissolved in dimethyl sulphoxide (DMSO) respectively.

DPPH radical Scavenging activity

The scavenging activity for DPPH free radicals was measured according to the procedure described by Braca (Braca *et al.,* 2003). In DPPH assay method is based on the reduction of alcoholic DPPH solution (dark blue in colour) in the presence of a hydrogen donating antioxidant converted to the non radical form of yellow colored diphenyl-picrylhydrazine. Lower the absorbance higher the free radical scavenging activity (Anita Murali *et al.,* 2011).

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity is commonly used to evaluate the free radical scavenging effectiveness of various antioxidant substances (Kalidas *et al.,* 2008). Hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and the extracts for hydroxyl radicals generated from the Fe^{2+}/EDTA/H_2O_2 system (Fenton reaction). The hydroxyl radical attacks deoxyribose, which eventually results in the formation of thiobarbituric acid reacting substances (TBARS).

Superoxide radical Scavenging activity

Superoxide scavenging activity of the plant extract was determined by McCord & Fridovich method (Mc Cord and Fridovich 1968), which depends on light induced superoxide generation by riboflavin and the corresponding reduction of nitroblue tetrazolium.

RESULTS AND DISCUSSION

Phytochemical analysis and Quantification of Total Phenolic and Alkaloid contents

Qualitative phytochemical screening of *Spilanthes acmella* extracts revealed the presence of different phytochemical constituents like steroids, terpenoids, flavanoids, alkaloids, glycosides, tannins, carbohydrates, oils and amino acids. The extracts gave negative results for the quinines and saponins. The Quantified phenolic contents of *Spilanthes acmella* extracts were ranging from 13.52±0.19 to 38.83±0.68

(mg/gm). The methanol extract have more phenolic content i.e. 38.83±0.68 (mg/gm) than other extracts and the alkaloid content was ranging from 17.73±0.38 to 32.64±0.86 (mg/gm). The methanolic extract has more alkaloid content i.e. 26.37±0.16 (mg/gm) than other extracts. The results were showed in table 1.

In vitro anti oxidant activity

The reactive oxygen species or oxidants, which are formed in the human body due to exogenous and endogenous factors, are found to be responsible for many diseases. Antioxidants are reducing agents and limit oxidative damage to biological structures by passivating free radicals. Antioxidant compounds may function as free radical scavengers, completers of prooxidant metals, reducing agents and quenchers of singlet oxygen formation (Gurusamy *et al.*, 2010). The medicinal properties of plants have been investigated in the recent scientific developments throughout the world, due to their potent antioxidant activities, no side effects and economic viability. Day by day, a lot of researches have shown the potential of phytochemical antioxidants as health benefactors because of their ability to neutralize free radicals, reactive oxygen species, or oxidants responsible for the onset of cell damage.

Inhibition of DPPH radical

The DPPH radical is considered to be a model for a lipophilic radical. A chain in lipophilic radicals was initiated by the lipid autoxidation. DPPH is a stable free radical at room temperature and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Soares *et al.*, 1997). The reduction capability of DPPH was determined by the decrease in its absorbance at 517 nm, which is induced by anti-oxidants. Positive DPPH test suggests that the samples were free radical scavengers. The scavenging effect of *S.acmella* extracts and ascorbic acid on DPPH radical was compared. On the DPPH radical, *S.acmella* extracts had scavenging effects with increasing concentration in the range of 20–1280µg/ml. when compared with ascorbic acid, the scavenging effect of *S.acmella* extracts was lower. The IC_{50} values of Hydro-alcoholic, methanol, ethyl acetate and hexane extracts and ascorbic acid were found to be 226µg, 313µg, 377µg, 546µg and 16 µg respectively. The results were showed in Table 2 and Fig 1.

Table 1. Total phenolic and alkaloid contents (mg/gm) of *Spilanthes acmella* extracts

S.No	Name of the extract	Total Phenolic content (mg/gm)	Total alkaloid content (mg/gm)
1	Hexane	13.52±0.19	17.73±0.38
2	Ethyl acetate	27.46±0.32	21.47±0.25
3	Methanol	38.83±0.68	32.64±0.86
4	Hydro alcoholic (Ethanol 70%)	31.58±0.47	26.49±0.18

Fig 1. Concentration dependent percent inhibition of DPPH radical by different extracts of *Spilanthes acmella* and Ascorbic acid in *In-vitro* studies

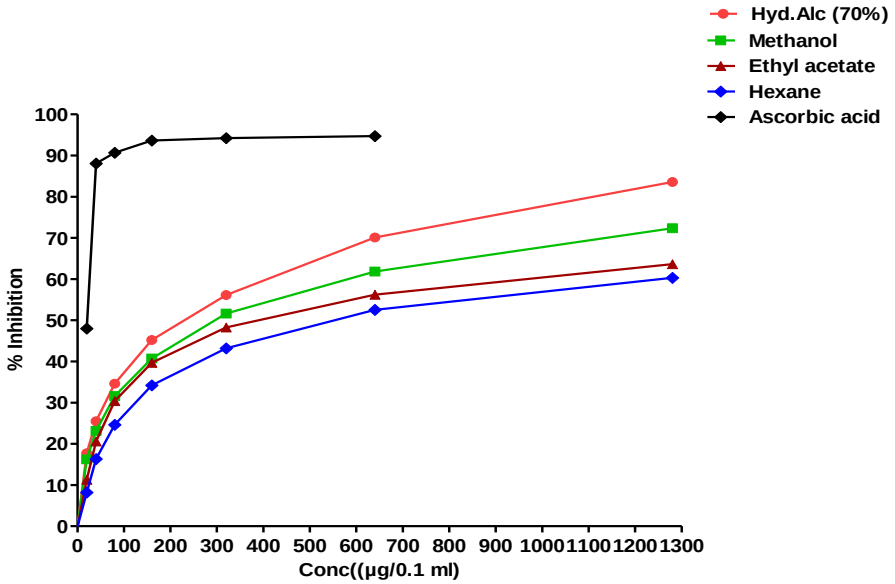

Fig 2. Concentration dependent percent inhibition of Hydroxyl radical by different extracts of *Spilanthes acmella* and Ascorbic acid in *In-vitro* studies

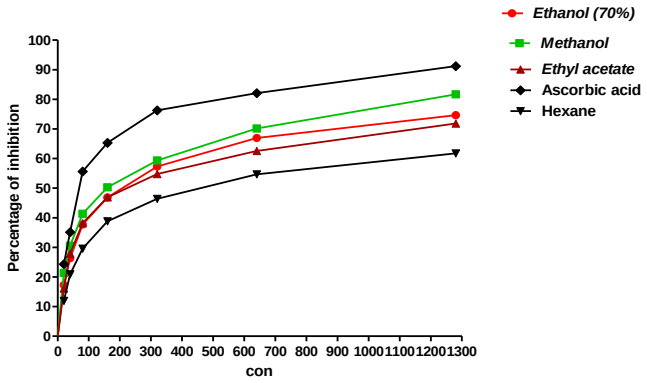

Fig 3. Concentration dependent percent inhibition of Superoxide radical by different extracts of *Spilanthes acmella* and Ascorbic acid in *In-vitro* studies

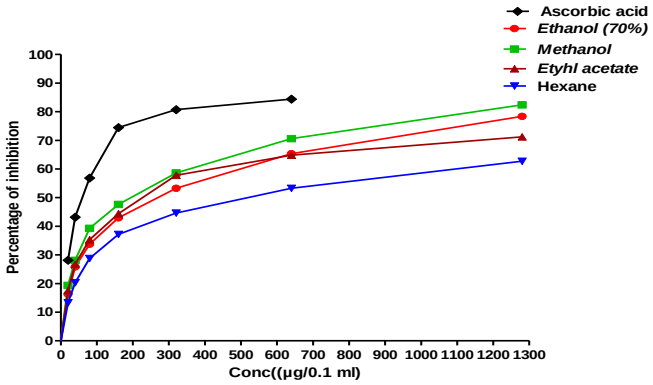

Table 2. *In-vitro* 50% inhibition concentration (IC_{50}) of different extracts of *Spilanthes acmella* on Superoxide, Hydroxyl and DPPH free radicals

Name of the extract of *Spilanthes acmella*	IC_{50} value (µg)		
	Superoxide radical	Hydroxyl radical	DPPH radical
Hyd. Alc.ext.	271	204	226
MeOH.ext.	189	154	313
EA.ext.	226	225	377
Hex.ext.	503	485	546
Ascorbic acid	59.3	66.0	16

Hydroxyl radical scavenging

The hydroxyl radical is an extremely reactive free radical formed in biological systems and has been implicated as a highly damaging species in free radical pathology, capable of damaging almost every molecule found in living cells. This radical has the capacity to join nucleotides in DNA and cause strand breakage which contributes to carcinogenesis, mutagenesis and cytotoxicity (Srinivasa Rao *et al.*, 2010). Hydroxyl radical scavenging capacity of an extract is directly related to its anti-oxidant activity. As shown in Table 2 and fig 2, the *S. acmella* extracts have potential inhibitory effect on hydroxyl radical. All results showed hydroxyl radical scavenging activity in dose dependent manner. The mean IC_{50} values for hydroxyl radical of Hydro-alcoholic, methanol, ethyl acetate and hexane extracts of *S. acmella* and ascorbic acid were found to be 204µg, 154µg, 225µg and 485µg and 66 µg respectively.

Superoxide radical scavenging

This free radical was formed when oxygen takes up one electron and as leaks in the mitochondrial electron transport but its formation is easily increased when exogenous components are present. Its first production site is the internal mitochondrial membrane. The superoxide free radical was very unstable, it plays a central role as other reactive intermediates are formed from it. Its main significance lies in its being a main source for the generation of hydrogen peroxide and as a reductant of transition metals, which are precursors to the formation of the lethal hydroxyl radical. So, in the present study, different extracts of *S. acmella* were tested for superoxide radical scavenging activity. The results were found that extracts of *S. acmella* possess concentration dependent scavenging activity on superoxide free radical. The mean IC_{50} values for superoxide radical of Hydro-alcoholic, methanol, ethyl acetate and hexane extracts of *S. acmella* and ascorbic acid were found to be 271µg, 189µg, 226µg, 503µg and 59.3 µg respectively. The results were showed in Table 2 and fig 3.

CONCLUSION

The scavenging activity of plants was may be due to the presence of some important chemical compounds like polyphenolic, alkaloids, glycosides, flavonoids, and steroids (Catherine Rice-Evans *et al.*, 1997; Lucia Račková *et al.*, 2004; Jadwiga Robak *et al.*, 1998; Ufuk Kolak *et al.*, 2006; Amar Djeridane *et al.*, 2010). These phytochemical compounds were commonly found in plants have been reported to have multiple biological effects (Eleni *et al.*, 2009), including antioxidant activity. The *S.acmella* extracts contain different phytochemical constituents like alkaloids, phenols, flavanoids, glycosides etc. The methanolic

extracts showed better scavenging activity compared to other extracts and contain more phenolic and alkaloid contents. By the above results the scavenging activity of *S.acmella* was may be due to the presence of some important chemical compounds like phenols, alkaloids, glycosides, flavonoids, and steroids. The *S. acmella* extracts have scavenging activity against superoxide radical, hydroxyl radical and DPPH radicals. The isolation and characterization of active molecules (compounds) responsible for antioxidant activity work is in progress.

ACKNOWLEDGMENTS

Deep thanks to A.U. College of Pharmaceutical Sciences, Department of Botany, Andhra University for providing all chemicals and equipment.

REFERENCES

Amar Djeridane, Mohamed Yousfi, Jean Michel Brunel, Pierre Stocker. Isolation and characterization of a new steroid derivative as a powerful antioxidant from *Cleome arabica* in screening the *In vitro* antioxidant capacity of 18 Algerian medicinal plants. *Food and Chemical Toxicology*. 48(10), 2010, 2599-2606.

Anita Murali, Purnima Ashok, Madhavan V. *In vitro* antioxidant activity and HPTLC Studies on the roots and rhizomes of *Smilax zeylanica l.* (smilacaceae). *Int J Pharm Pharm Sci*. 3(1), 2011, 192-195.

Harborne B. Phytochemical Methods: A Guide to Modern Techniques of Plants Analysis, 3 rd Edition,Chapman & Hall, 1998, London, England.

Braca A, Fico G, Morelli I, De Simone F, Tome F, De Tommasi N. Antioxidant and free radical scavenging activity of flavonol glycosides from different *Aconitum* species. *J Ethnopharmacol*. 86, 2003, 63-67.

Catherine Rice-Evans, Nicholas Miller, George Paganga. Antioxidant properties of phenolic compounds, *Trends in Plant Science*. 4(2), 1997, 152-159.

Eleni M Gioti, Yiannis C Fiamegos, Dimitris C Skalkos, Constantine D Stalikas. Antioxidant activity and bioactive components of the aerial parts of *Hypericum perforatum* L. from Epirus, Greece. *Food Chemistry*. 117(3), 2009, 398-404.

Fazel Shamsa, Hamidreza Monsef, Rouhollah Ghamooshi, Mohammadreza Verdian-rizi. Spectrophotometric determination of total alkaloids in some Iranian medicinal plants. *Thai J. Pharm. Sci*. 32, 2008, 17-20.

Miliauskas G, PR Venskutonis, TA van Beek. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. *Food and Chemical Toxicology*. 85(2), 2004, 231-237.

Edeoga HO, DE Okwu, BO Mbaebre. Phytochemical constituent of some Nigerian Medicinal Plants. *Afr.J. Biotechnol*. 4, 2005, 685-688.

Jadwiga Robak, Ryszard J Gryglewski. Flavonoids are scavengers of superoxide anions. *Biochemical Pharmacology*. 37(5), 1998, 837-841.

Gurusamy K, P Saranya. *In vitro* Antioxidant Potential of Ethanolic Contents of *Eclipta alba* and *Wedelia chinensis*. *Journal of Pharmacy Research*. 3(12), 2010, 2825-2827.

Srinivasa Rao K, Pradeep Kumar Chaudhury, Anshuman Pradhan. Evaluation of anti-oxidant activities and total phenolic content of *Chromolaena odorata*. *Food and Chemical Toxicology*. 48, 2010, 729-732.

Lucia Račková, Magdaléna Májeková, Daniela Košťálová, Milan Štefek., Antiradical and antioxidant activities of alkaloids from *Mahonia aquifolium*. Structural aspects. *Bioorganic & Medicinal Chemistry*. 12(17), 2004, 4709-4715.

Faraz M, K Mohammed, G Narysanna and RV Hamid. Phytochemical Screening of Some Species of Iranian plants. *Iranian J Pharm Res*. 3, 2003, 77-82.

Mc Cord JM, Fridovich I. Superoxide dismutase: an enzymic function for erythrocuprein (hemocuprien). *J Biol Chem*. 244, 1969, 6049-6055.

Amarowicz R, RB Pegg, P Rahimi-Moghaddam, B Barl, JA Weil. Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. *Food Chem*. 84(4), 2004, 551-562.

Rajesh Manian, Nagarajan Anusuya, Perumal Siddhuraju, Sellamuthu Manian. The antioxidant activity and free radical scavenging potential of two different solvent extracts of *Camellia sinensis* (L.) O. Kuntz, *Ficus bengalensis* L. and *Ficus racemosa* L. *Food Chem*. 107(3), 2008, 1000-1007.

Kalidas S, B Kameswari, P Devi, B Madhumitha, R Meera, NJ Merlin. Phyto-Physico chemical evaluation, Antioxidant activities and Diuretic activity of Leaves of *Lagerstroemia reginae*. *Asian J. Research Chem*. 1(2), 2008, 83-87.

Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic acid-phosphotungstic acid reagents. *Amer J Enology Viticulture*. 16, 1965, 144-58.

Soares JR, Dinis TCP, Cunha AP, Almeida LM. Antioxidant activities of some extracts of Thymus zygi. *Free Radical Research*. 26, 1997, 469-478.

Ufuk Kolak, Mehmet Öztürk, Fevzi Özgökçe, Ayhan Ulubelen., Norditerpene alkaloids from *Delphinium linearilobum* and antioxidant activity. *Phytochemistry*. 67(19), 2006, 2170-2175.