The Malaysian Journal of ANALYTICAL SCIENCES ISSN 1394-2506

PHYTOCHEMICAL SCREENING, TOTAL PHENOL ESTIMATION, ANTIOXIDANT ACTIVITY OF *BLAINVILLEA ACMELLA* LEAF AND STEM SUCCESSIVE EXTRACTS

(Ujian Penyaringan Fitokimia, Penganggaran Fenol dan Aktiviti Antioksidan *Blainvillea Acmella* dan Ekstrak Berturutan Daun dan Stem)

Piush Sharma[1]*, Ganesh N. Sharma[2], B. Shrivastava[2], Hemant R. Jadhav[3]

[1]*Maharishi Arvind College of Pharmacy, Ambabari, Jaipur (Raj). India-302023*
[2]*School of Pharmaceutical Sciences, Jaipur National University, Jaipur (Raj) India-302025*
[3]*Department of Pharmacy, Birla Institute of Technology & Science, Pilani, Rajasthan, India-333031*

**Corresponding author: joshipiush@gmail.com*

Abstract

The aim of present work was to investigate antioxidant potential of different extracts of *Blainvillea acmella* leaf and stem. The successive extraction of individual plant part was carried out using solvents of different polarity viz. n-hexane, ethyl acetate, methanol and water. Preliminary phytochemical screening of all the extracts was done. The present total phenolic contents were estimated by Folin-Ciocalteu reagent method and expressed as µg/mg of gallic acid equivalent. The antioxidant potential and reducing power of all the prepared extracts were measured against DPPH, as compared to standard ascorbic acid, and BHA respectively. The result data indicate that the phenolic contents were higher in methanolic extracts of leaf (73.67±0.38mg/g) followed by ethyl acetate (29.08±0.38mg/g), aqueous (21.50±0.28 mg/g), and n-Hexane (9.29±0.38 mg/g); gallic acid equivalent. The similar pattern in stem part was also observed i.e. methanolic extracts (41.90±0.45mg/g), ethyl acetate (21.92±0.28 mg/g), aqueous (15.13±0.18mg/g), and n-Hexane (3.69±0.28 mg/g). The antioxidant capacity of methanolic extract of both the part i.e leaf and stem was found to be maximum, as IC50 values were 226.49±0.16, 402.05±1.10 respectively. The reducing power was also highest in methanol extract of both parts. The result data conclude that the higher antioxidant as well as reducing power may be due to present phenolic contents.

Keywords*: Blainvillea acmella*, Antioxidant, Reducing Power, DPPH, IC50

Abstrak

Kajian ini bertujuan meneliti potensi bahan antioksidan dari ekstrak daun dan batang Blainvillea acmella yang berbeza. Pengekstrakan berturutan pada bahagian tertentu tumbuhan telah dijalankan dengan menggunakan pelarut berkutub berbeza seperti n-heksana, etil asetat, metanol dan air. Saringan awal fitokimia terhadap semua sampel ekstrak telah dilakukan. Jumlah kandungan fenolik telah dianggar dengan mengunapakai kaedah reagen Folin-Ciocalteu dan dinyatakan sebagai kesetaraan µg/ mg asid Gallic. Potensi antioksidan dan kuasa penurun bahan sampel terekstrak diukur melawan DPPH, sebagai perbandingan masing – masing terhadap asid askorbik piawai dan BHA. Data kajian menunjukkan bahawa kandungan fenolik adalah lebih tinggi di dalam daun mengunakan pengekstrak metanol (73.67 ± 0.38mg /g) diikuti etil asetat (29.08 ± 0.38mg /g), air (21.50 ± 0.28 mg /g) dan n-heksana (9.29 ± 0.38 mg/g) kesetaraan asid Gallic. Corak yang sama di bahagian batang juga diperhatikan iaitu ekstrak metanol (41.90 ± 0.45mg /g), etil asetat (21.92 ± 0.28 mg/g), air (15.13 ± 0.18mg/ g), dan n-Heksana (3.69 ± 0.28 mg/g). Kapasiti antioksidan mengunakan pengekstrak metanol daripada kedua-dua bahagian iaitu daun dan batang didapati maksimum, dengan nilai IC50 masing – masing adalah 226.49 ± 0.16 dan 402.05 ± 1.10. Kuasa pengurnagn juga paling tinggi di dalam ekstrak metanol bagi kedua-dua bahagian. Kesimpulan bahawa kehadiran kandungan fenolik menyebabkan nilai antioksidan dan kuasa pengurangan menjadi lebih tinggi.

Kata kunci*: Blainvillea acmella*, Antioksidan, Kuasa penurun, DPPH, IC50

Introduction

The free radicals are involved in a number of biochemical processes and also represent an essential part of aerobic life and metabolism. These radicals are continuously produced in body's normal utilization of oxygen such as respiration and some cell-mediated immune functions, and may also be generated through environmental pollutants, automobile exhaust fumes, pesticides, cigarette smoke, radiation, air pollutants etc.[1]**,** which become part and parcel of our daily inhaling/ingesting life, and appears no escape from them [2].

The antioxidants are fighter against these free radicals and terminate the chain reaction before vital molecules are damaged. Antioxidants prevent cellular damages, and the pathway of a variety of diseases [3]**.** A wide range of antioxidants, both natural and synthetic are being utilized in treatment of various diseases, mediated by free radicals [4, 5]. The synthetic compounds have their own adverse effects, so an attempt is being made regularly with use of natural resources.

The *Blainvillea acmella (Family: Asteraceae)* is one of the important annual herb**,** which is native of America and found throughout India. Also found in Indonesia, Malaysia, Myanmar, Nepal, Philippines, Thailand, Vietnam; Africa and Australia [6]. The various parts of the plant have been reported for the presence of number of phytoconstituents [7], although we tried to confirm the presence of these phytoconstituents in extracts of solvents with different polarity. The *Blainvillea acmella* has also been used traditionally for various purposes [8]. The plant have been found effective as ovicidal, larvicidal and pupicidal [9], diuretic [10], anti fungal [11], antipyretic [12.], anti-inflammatory [13], and few others. Main objective of this study was to estimate phenolic content, free radical scavenging activity and reducing power of the leaf and stem of *Blainvillea acmella* extracted successively with n-Hexane, Ethyl Acetate, Methanol and Water.

Materials and Methods

Reagents and chemicals

All the chemicals used in the study were of analytical grade. The reagents used in the preliminary phytochemical study were freshly prepared. Folin Ciocalteu reagent (Qualigens), Gallic acid (Fluka), DPPH (Sigma), Ascorbic acid, Iron chloride, Butylated hydroxy anisole (BHA), Trichloroacetic acid (Merck), Potassium ferricyanide (Thomas Baker) were incorporated in study.

Collection and authentification of plant parts

The leaves and stem of *Blainvillea acmella* was collected from *tehsil* Aamer, Jaipur (Raj.) India, in the month of September to November (most suited period as the phytoconstituents and secondary metabolite are abundant in plant part) and was authenticated by Joint Director, Botanical survey of India, Jodhpur. Specimen samples are stored at herbarium of Maharishi Arvind college of Pharmacy, Ambabari, Jaipur.

Preparation of plant part extracts

The leaf and stem part was cleaned and shade dried. The samples were broken into small pieces with cutter mill, powdered and passed through sieve no. 44. The leaf and stem samples separately; were extracted successively using soxhlet apparatus with n-hexane, ethyl acetate and methanol. Finally remaining marc was extracted with water. The collected extracts were vacuum dried and were labeled as indicated in Table 1.

Table 1. Labels of successive extracts of leaf and stem of *Blainvillea acmella*

Plant Part	n-Hexane	Ethyl Acetate	Methanol	Water
Leaf	BALH	BALEA	BALM	BALW
Stem	BASH	BASEA	BASM	BASW

Preliminary phytochemical screening of extracts

The collected extracts were subjected to preliminary phytochemical screening for qualitative determination of phytoconstituents [14, 15].

Estimation of total phenolic content

The total polyphenolic content of all extracts were measured by Folin- Ciocalteu reagent method, and are expressed as µg/mg of gallic acid equivalent. The absorbance was measured at 760 nm using UV spectrophotometer (Jasco V530 – UV/VIS/NIR) [16].

Estimation of free radical scavenging activity by DPPH method

Scavenging free radical potential of collected extracts was evaluated against a methanolic solution of 1, 1- diphenyl-2- picryl hydrazyl (DPPH) as method describe by Cengiz 2008[17]. The 200µM methanolic solution of DPPH was prepared. 1ml of different concentration (10µg to 4 mg/ml) of extract solution and standard Ascorbic acid solution (10- 60 µg/ml) were taken in different vials. To this 1 ml of methanolic solution of DPPH was added, shaken well and mixture was allowed to stand at room temperature for 20 min. A blank was also prepared in the similar way and the absorbance was measured at 517nm. Scavenging activity was expressed as the percentage inhibition calculated using the Equation 1.

$$\% \text{ Anti-radical activity} = \frac{\text{Control Abs - Sample Abs}}{\text{Control Abs}} \times 100 \qquad \text{(Eq. 1)}$$

Reducing power assay

The reducing capability was measured by the transformation of Fe^{3+}- Fe^{2+} in presence of different extracts. Different concentrations of extracts (250-2500 µg) in 1ml of water were mixed with 2.5 ml of phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferrocyanide. The mixture was incubated at 50^0C for 20 min. 2.5 ml (10%) of trichloroacetic acid was added to the mixture, and was centrifuged at 3000 RPM for 10 min. 2.5 ml of upper layer solution was mixed with 2.5 ml distilled water and 0.5 ml of 0.1% Fecl3 solution and the absorbance was measured at 700 nm. Increased absorbance of reaction mixture indicated increased reducing power [18, 19]. BHA solution (1000 µg/ml) prepared in phosphate buffer was used as standard.

Results and Discussion

Percentage yield of extracts (%)

Blainvillea acmella extract yield was minimum for methanol and hexane extract, for leaf 1.36% and for stem 0.62% respectively, while extractive value was maximum for ethyl acetate (Leaf 4.93%) and aqueous (3.34%). The result data are given in Table 2.

Table 2. Percentage yield of extracts by successive extraction

Plant Part	Extract (% w/w)			
	n-Hexane	Ethyl Acetate	Methanol	Water
Leaf	2.02	4.93	1.36	3.83
Stem	0.62	2.20	1.23	3.34

Preliminary phytochemical screening results

The phytochemical screening result data are shown in Table 3, which implies that the carbohydrates, flavanoids and phenolic compounds are present in methanolic extracts of leaf and stem.

Table 3. Preliminary phytochemical screening of *Blainvillea acmella*

Constituents	Test	***Blainvillea acmella***							
		Leaf				**Stem**			
		n-Hexane	**Ethyl Acetate**	**Methanol**	**Water**	**n-Hexane**	**Ethyl Acetate**	**Methanol**	**Water**
Alkaloids	Dragendroff's test	-	-	+	-	-	-	-	-
	Mayer's test	-	-	+	-	-	-	-	-
	Hager's test	-	-	+	-	-	-	-	-
	Wagner's test	-	-	+	-	-	-	-	-
Carbohydrates	Molish test	-	-	-	+	-	-	+	-
	Fehling's test	-	-	-	+	-	-	+	-
	Barfoed's test	-	-	-	+	-	-	+	-
Glycosides	Kellar-Killani test	-	-	-	-	-	-	+	-
	Borntrager's test	-	-	-	-		-	-	-
	Legal's test	-	+	+	+	-	+	+	-
Flavonoids	Shinoda test	-	-	+	-	-	+	+	-
Saponin	Foam test	-	-	-	-	-	-	-	-
	Haemolytic test	-	-	-	-	-	-	-	-
Sterols/ Steroids	Salkowaski reaction	+	-	-	-	-	-	-	-
Tannins and Phenolic compounds	5% Ferric chloride solution	+	+	+	+	-	+	+	+
	Lead acetate solution	+	+	+	+	-	+	+	+
	Dil. Potassium permanganate solution	+	+	+	+	-	+	+	+
	Bromine water	+	+	+	+	-	+	+	+
Amino acids	Ninhydrin test	-	-	-	-	-	-	-	-
Proteins	Biuret test	-	-	-	-	-	-	-	-

Total polyphenolic content determination

Blainvillea acmella methanolic extract was found to contain maximum phenolic content (73.67mg/g from leaves, 41.90mg/g from stem), while its hexane extract shows 9.29mg/g from leaves, 3.69mg/g from stem. The present phenolic contents were found as given in Table 4.

Table 4. Total polyphenolic content in *Blainvillea acmella* extracts

Plant Part	Total Phenolic Content mg/g equivalent to Gallic Acid			
	n-Hexane	Ethyl Acetate	Methanol	Water
Leaf	9.29±0.38	29.08±0.38	73.67±0.38	21.50±0.28
Stem	3.69±0.28	21.92±0.28	41.90±0.45	15.13±0.18

Antioxidant assay of extracts

Results for DPPH free radical scavenging activity

Scavenging activity of various extracts and ascorbic acid was studied against DPPH radicals. All the samples were analysed in triplicate. The DPPH assay of BALH extract found to produce no response (% inhibition) even at concentration 4000µg/ml. The BALEA, BALM and BPLW extract at the level of 300µg/ml, 250µg/ml and 350µg/ml showed > 50% inhibition respectively, along with concentration dependent response. The methanolic extract of *Blainvillea acmella* leaves exhibited a maximum DPPH scavenging activity. IC50 value (Figure 1) was 226.49±0.16 µg/ml followed by ethyl acetate and water extract whose scavenging activities (IC50) were 283.35±0.56 and 337±0.44 µg/ml, respectively. The IC50 value of ascorbic acid was also 36.14±0.26, while n-hexane extract did not produced any response.

BASH extract also produced similar results up to concentration 4000µg/ml as of BPLH extract i.e. no response was generated. The BASEA, BASM, BASW extract, at the concentration level of 500µg/ml, 400µg/ml and 600µg/ml was found to exhibit > 50% inhibition, which was a concentration dependant response. The IC50 value (the concentration with scavenging activity of 50%) of methanolic extract of stem part was, 402.05±1.10, followed by ethyl acetate and water extract, whose IC50 values were 498.56±0.87, and 579.85±1-34 µg/ml respectively (Figure 2). Again no response was generated with hexane extract of stem part also.

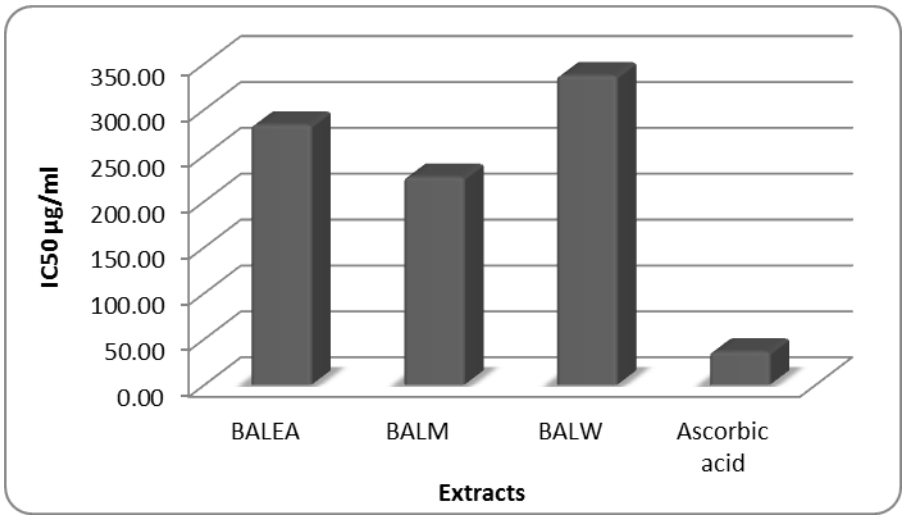

Figure 1. IC 50 of *Blainvillea acmella* leaves extracts

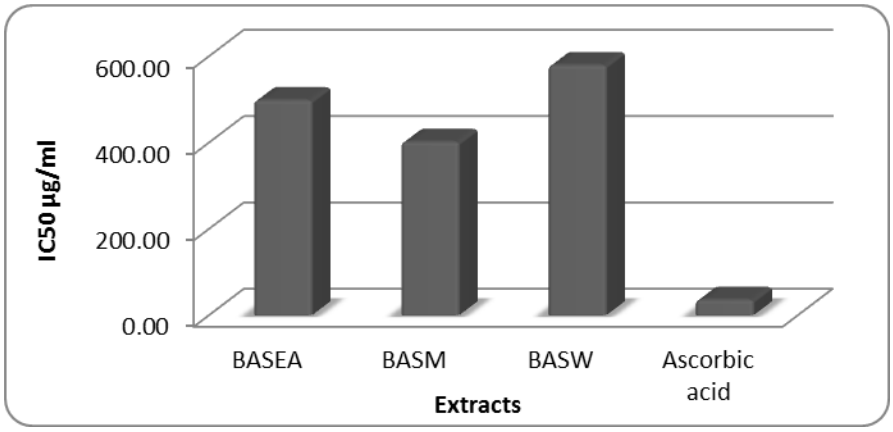

Figure 2. IC 50 of *Blainvillea acmella* stem extracts

Results for reducing power activity

The reducing power of different extracts of leaf and stem of *Blainvillea acmella* was assayed and compared with butylated hydroxy anisole (BHA) (Figure 3 and 4). Maximum reducing capacity was recorded with methanolic extract of leaf (BALM), although the response generated with BALEA and BALW were also significant as compared to standard, and was lowest with BALH. The similar finding was also observed with different extracts of *Blainvillea acmella* stem. The result data were found as in Table 5 and 6.

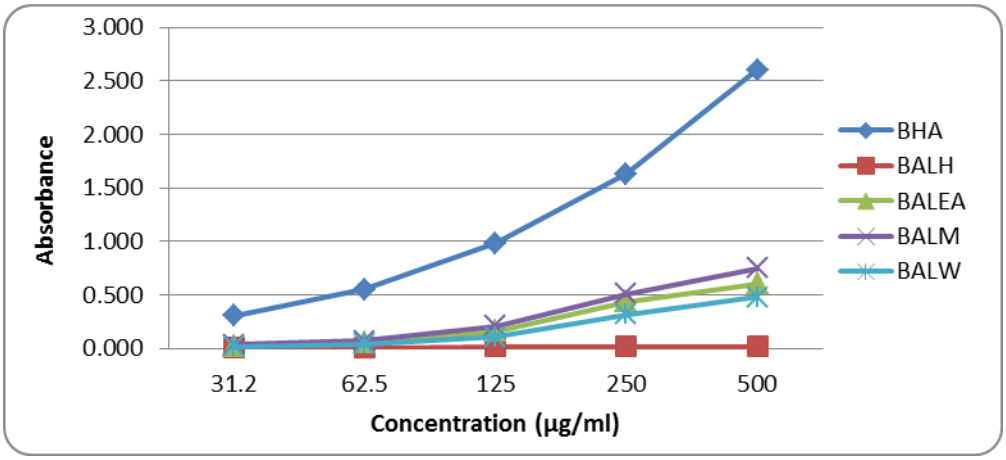

Figure 3. Reductive potential of different concentrations of *Blainvillea acmella* leaves extracts

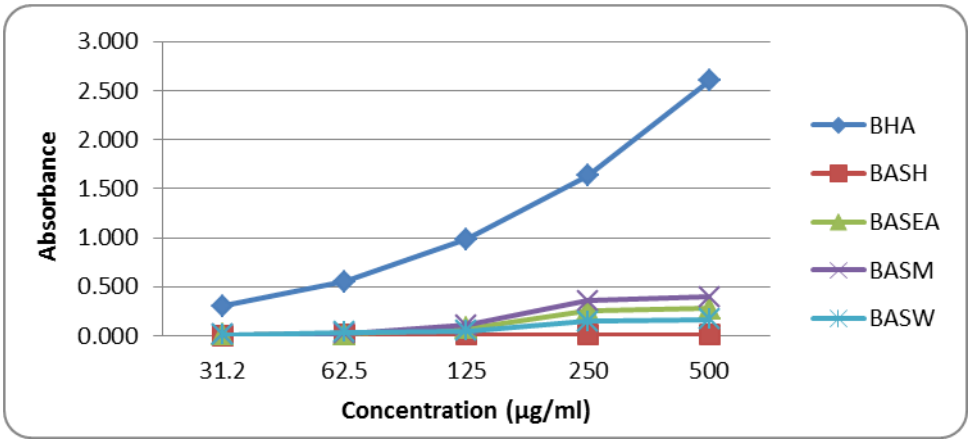

Figure 4. Reductive potential of different concentrations of *Blainvillea acmella* stem extract

Table 5. Reducing power of *Blainvillea acmella* leaves extracts

Sequence No.	Conc (µg/ml)	BHA	BALH	BALEA	BALM	BALW
1	31.2	0.306±0.001	0.006±0.001	0.024±0.001	0.034±0.001	0.017±0.001
2	62.5	0.551±0.002	0.009±0.002	0.053±0.002	0.071±0.002	0.041±0.002
3	125	0.985±0.001	0.018±0.001	0.164±0.002	0.208±0.001	0.107±0.002
4	250	1.631±0.001	0.021±0.001	0.438±0.002	0.513±0.002	0.319±0.002
5	500	2.603±0.002	0.021±0.001	0.605±0.002	0.749±0.003	0.483±0.002

n=3, Data are given as mean ± S.D

Table 6. Reducing power of *Blainvillea acmella stem* extracts

Sequence No.	Conc (µg/ml)	BHA	BASH	BASEA	BASM	BASW
1	31.2	0.306±0.001	0.004±0.001	0.010±0.001	0.012±0.001	0.010±0.001
2	62.5	0.551±0.002	0.006±0.002	0.015±0.001	0.022±0.002	0.036±0.002
3	125	0.985±0.001	0.008±0.001	0.076±0.002	0.105±0.002	0.050±0.002
4	250	1.631±0.001	0.009±0.001	0.260±0.002	0.357±0.002	0.149±0.002
5	500	2.603±0.002	0.009±0.001	0.280±0.002	0.392±0.002	0.167±0.002

n=3, Data are given as mean ± S.D

Conclusion

An attempt has been made here to establish the antioxidant measures of the *Blainvillea acmella*, which might be helpful in establishing its therapeutic values. The leaf and stem part of the plant were extracted successively using

solvents of different polarity. The extracts were subjected to phtyochemical screening to confirm presence of phytoconstituents and secondary metabolites. The results data regarding presence of total phenolic contents implies that the methanolic extract of both the part was most abundant, as compared to other extracts. From this we can conclude that the phenolic compounds can be better isolated with solvents of higher polarity range. The antioxidant potential as well as reducing power of the extracts was determined and was found better in methanolic extract. Still it was higher with leaf extract as compared to stem extract.

At the same time the results also demonstrate that the IC50 values of different extracts may be co-related with present phenolic content as it was in proportion to that of concentration of the extract and present phenolic moieties. The results confirm the superiority of the different leaf extracts than the stem part extracts. In this study, it seemed that, the higher total phenolic content of plants extracts resulted in higher antioxidant activity.

Acknowledgement

Authors are thankful to School of Pharmaceutical Sciences, Jaipur National University, Jaipur (Raj.) for providing necessary support and facility to carry out this research work.

References

1. Tiwari, A. K. (2001). Imbalance in antioxidant defence and human disease, multiple approach of natural antioxidants therapy. *Current Science,* 81(9):1179-1187.
2. Tiwari, A. K. (2004). Antioxidants, new-generation therapeutic base for treatment of polygenic disorders. *Current Science*, 86(8): 1092-1102.
3. Balz, F. (1994). Molecular biological mechanisms of antioxidant action. *The FASEB Journal*, 13(9): 963-964.
4. Byungpal, P. Y. (1994). Cellular defenses against damage from reactive oxygen species. *Physiological Reviews*, 74(1): 139-162.
5. Halliwell, B., Gutteridge, J. M. & Cross, C. E. (1992). Free radicals, antioxidants, and human disease: where are we now? *The Journal of Labarotary and Clinical Medicine,* 119(6): 598-620.
6. Wei, B. N. (2011). Blainvillea acmella (Linnaeus) Philipson, *Flora of China*, 20-21: 868.
7. Singh, P., Sharma, A. K., Joshi, K. C., Jakupovic, J. & Bohlmann, F. (1985). Acanthospermolides and other constituents from *Blainvillea acmella*. *Phytochemistry*, 24(9): 2023-2028.
8. Kshirsagar, R. D. & Singh, N. P. (2001). Some less known ethnomedicinal uses from Mysore and Coorg, districts, Karnataka, Southern India. *Ancient Science of Life*, 20(3):20-25.
9. Saraf, D. K. & Dixit, V. K. (2002). Spilanthes acmella Murr. : Study on Its Extract Spilanthol as Larvicidal Compound. *Asian Journal of Experimental Sciences,* 16(1-2): 9-19.
10. Ratnasooriya, W. D., Pieris, K. P., Samaratunga, U. & Jayakody, J. R. (2004). Diuretic activity of Spilanthes acmella flowers in rats. *Journal of Ethnopharmacology*, 91(2-3): 317-320.
11. Rani, S. A. & Murty, S. U. (2006). Antifungal potential of flower head extract of Spilanthes acmella Linn. *African Journal of Biomedical Research*, 9: 67-69.
12. Chakraborty, A., Devi, B. R., Sanjebam, R., Khumbong, S. & Thokchom, I. S. (2010). Preliminary studies on local anesthetic and antipyretic activities of Spilanthes acmella Murr. in experimental animal models. *Indian Journal of Pharmacology*, 42(5): 277-279
13. Chakraborty, A., Devi, R. K. B., Rita, S., Sharatchandra, K. H. & Singh, T. I. (2004). Preliminary studies on antiinflammatory and analgesic activities of Spilanthes acmella in experimental animal models. *Indian Journal of Pharmacology*, 36(3):148-150.
14. Khandelwal, K. R. (2010). Practical Pharmacognosy. *Nirali Prakashan*, Pune, India. 25.1-25.9.
15. Harborne, J. B. (1998) Phytochemical methods. *Chapman and Hall*, London. 90-203.
16. Maurya, S. & Singh D. (2010). Quantitative analysis of total phenolic content in *Adhatoda vasica* Nees extracts. *International Journal of Pharmtech Research*, 2(4):2403-2406.
17. Cengiz, S., Bektas, T., Dimitra, D., Moschos, P. & Mansur, H. (2008). Studies on the antioxidant activity of the essential oil and methanol extract of *Marrubium globosum* subsp. *Globosum lamiaceae* by three different chemical assays. *Bioresource Technology*, 99(10):4239-4246.
18. Ilkay, O., Murat, K., Mahmoud, A. A., Sezer, S. F., Gulderen, Y. & Bilge, S. (2009). Free radical scavenging properties and phenolic characterization of some edible plants. *Food Chemistry*, 114(1): 276–281.

19. Jeng, L. M., Hsiu, C. L., Chin, C. C. (2002). Antioxidant properties of several medicinal mushrooms. *Journal of Agriculture and Food Chemistry*, 50(21):6072-6077.