

***In-Vitro* Antioxidant Activity, Total Phenolic, Flavonoid and Tannin Contents in the *Ajuga Bracteosa* Wall. Ex Benth, Grown at Middle Hill Climatic Condition of Western Himalayas**

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ABSTRACT

The antioxidant activity of aqueous and alcoholic extracts of different plant parts viz, leaves, flower, stem and root of *Ajuga bracteosa* was investigated against various *in-vitro* antioxidant assays. The total phenolic, flavonoid and tannin contents also estimated. The results revealed the significant antioxidant potential and variation in the IC₅₀, EC₅₀ and phytochemical contents among all the plant parts. The aqueous extract of leaves exhibited significantly (P<0.05) highest antioxidant activity on 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid), 2,2-diphenyl-1-picryl hydrazyl, and potassium ferricyanide reducing power assay, with IC₅₀ values of 0.2707±0.0008, 0.4409±0.0020, and EC₅₀ value 0.3413±0.0030 mg/mL, respectively, followed by the other parts of the plant. The leaves extract also possess the highest total phenolics, flavonoid and tannin contents among all the parts. Similarly, the aqueous extract is better than the alcoholic extract of different parts as far as phytochemical contents and antioxidant activity concerned. The present study revealed that the aqueous extract of leaves had the highest antioxidant potential, which correlated with the high level of total phenolic flavonoid, and tannin contents. Therefore, higher the phytochemical contents, higher will be the antioxidant potential.

Keywords: Inhibition concentration; Phytochemical contents; Ascorbic acid

1. INTRODUCTION

An imbalance between the production of reactive oxygen species (ROS) and normal physiology of the body originates oxidative stress. The oxidation by free radicals give rise to the disintegration of the cell membrane, DNA mutation and damage of membranous protein, also a number of diseases can initiate or propagate, like diabetes, liver injury, rheumatism, cardiovascular disorders and cancer¹. An antioxidant biomolecule can detain or interrupt the oxidation of molecules by oxidising themselves, so these are often called as reducing agents such as thiol, ascorbic acid, polyphenols etc². Thus, antioxidants may improve the quality of life by preventing the origin of degenerative diseases. Due to their beneficial effects, the investigations are focused on naturally occurring antioxidant molecules, especially plant phytochemicals viz., phenolic compounds, flavonoids, carotenoids, benzoic acid derivatives, proanthocyanidins, coumarins, stilbenes and lignins to replace the synthetic antioxidants compounds, which have various side effects. The medicinal plants contain several phytochemical constituents, which attributed to the antioxidant potential and prevent chronic disease progression^{3,4}.

Ajuga bracteosa Wall. Ex Benth (Lamiaceae) commonly known as Neelkanthi and in Kumaon region of Uttarakhand, it is called as 'Ratapatha' or Ratpatiya. It is a perennial erect or ascending hairy herb, distributed in subtropical and temperate

regions at an altitude of 1500 m⁵. Many compounds like γ -sitosterol, β -sitosterol, tri-acontanyl docosanoate, and tetra-cosanoic acid have been isolate from aerial parts⁶. This herb used since pre-historic times, and it also recommended in Ayurveda for the treatment of gout, rheumatism, amenorrhea and palsy. In the Kumaon region of Uttarakhand, the leaves of the plant used for the cure of diabetes as folk medicine, which is free radical damage to the body^{5,7}. Rahman⁸, et al. investigated the antioxidant and free radical scavenging activity in methanolic extract of different plants part viz., stem bark, root bark, leaves, and flowers of *Tabebuia pallid*. The study revealed that the highest total antioxidant capacity showed by the leaves of the plant, followed by the other parts.

The objective of the present study was to determine the total phenolic, tannin, and flavonoid contents, and also evaluate the *in vitro* antioxidant potential in terms of inhibition concentration (IC₅₀ value) in the different plant parts of *Ajuga bracteosa*. Further, the correlation between the phytochemical contents and the antioxidant activity also established.

2. MATERIALS AND METHODS

2.1 Collection and Preparation of Plant Material

The different plant parts viz. leaves, flowers, stems and roots of *Ajuga bracteosa* (100 g) were collected in the flowering season from Defence Institute of Bio-Energy Research Fd. Stn., Pithoragarh, Uttarakhand (India), located at 5500 feet altitude in 29°35'N 80°13'E in the middle

Himalayan region of Uttarakhand, India (Fig. 1), dehydrated (in a chamber below 40 °C), powder with the help of grinder and stored for the current study. The plant material (30 gm dried powder) was extracted by cold maceration with 300 mL water by occasionally shaking, and kept for 24 h. The organic filtrate collected was then concentrated and same method was followed for the alcoholic extract using ethanol (300 mL). For the determination of antioxidant activity, Total phenolic, flavonoids and tannin contents, the solution was prepared by dissolving concentrated plant extracts with water (100 mg/10 mL).



Figure 1. *Ajuga bracteosa* plant.

2.2 Chemicals

2,6-dichlorophenolindophenol, sodium carbonate, potassium permanganate (KMnO_4), potassium persulfate ($\text{K}_2\text{S}_2\text{O}_8$), ferric chloride (FeCl_3), potassium ferricyanide ($\text{K}_3\text{Fe}(\text{CN})_6$), aluminium chloride (AlCl_3), potassium acetate (CH_3COOK), Ascorbic acid, 1,1-diphenyl-2-picryl-hydrazyl (DPPH), tannic acid, 2,2'-azino-bis(3-ethyl benzothiazoline-6-sulfonic acids) diammonium salt (ABTS), Folin-Ciocalteu reagent and oxalic acid. All the reagents were purchased from Sigma Chemicals USA and of analytical grade.

2.3 Determination of Antioxidant Activity

For the determination of antioxidant activity by different antioxidant assays, the solution of plant extracts (100 mg/10 mL) diluted according to the required concentrations. The Ascorbic acid (0.1 mg/mL) was used as the reference standard for all methods.

2.3.1 Free Radical Scavenging by ABTS Assay

The antioxidant capacity was evaluated by the reported method⁹ depends on the scavenging of ABTS free radical (2,2'-azino-bis(3-ethyl benzothiazoline-6-sulfonic acid)). In this method, 7 mM ABTS solution and 2.45 mM potassium persulphate solution mixed in equal volume and allowed to react them for 12-16 hr in the dark to produce a blue-green chromogen, ABTS radical cation ($\text{ABTS}^{+\cdot}$). Before performing the assay, the blue-green resultant solution diluted with methanol for

obtaining the initial absorbance of 0.702 ± 0.001 at 734 nm. The antioxidant effects observed by mixing 1 mL of ABTS solution with 00-50 μL of test samples (make up to 1 mL) and measured the absorbance after 5-7 min at 734 nm, the decrease in the absorbance observed due to hydrogen donating availability of ABTS radical cation, inducing a change in colour of $\text{ABTS}^{+\cdot}$ radical cation to colourless ABTS¹⁰. The percentage of free radical scavenging activity of $\text{ABTS}^{+\cdot}$ radicals were determined at different concentrations by using the formula:

$$\text{FRSA (\%)} = \frac{[\text{Absorbance of control} - \text{Absorbance of test}]}{\text{Absorbance of control}} \times 100,$$

The IC_{50} value calculated as:

$$\text{IC}_{50} \text{ value (mg/mL)} = \frac{\text{Concentration of test}}{50\% \text{ nearest FRSA}} \times 50$$

2.3.2 Free Radical Scavenging by DPPH Assay

The antioxidant capacity was estimated by the reported method^{10,11} relies on the scavenging of DPPH free radical (1,1-diphenyl-2-picryl-hydrazyl). Herein, DPPH methanolic solution (2 mL, 0.135 mM) mixed with different aliquots of extract, after 40 min, the absorbance was measured at 517 nm against methanol as blank. The percentage of free radical scavenging activity of DPPH radicals was determined at different concentrations, also IC_{50} value calculated by using the formula mentioned above.

2.3.3 The Potassium Ferricyanide Reducing Power (PFRAP) Assay

The reducing ability was estimated by the earlier reported method¹². The compounds having the antioxidant activity form a potassium ferrocyanide complex by reacting with potassium ferricyanide. Further, with ferric trichloride, it produces a blue coloured ferric ferrocyanide complex, at 700 nm¹⁰. In brief, different aliquots of extracts mixed with the phosphate buffer (6.6 pH) and 2.5 mL of $\text{K}_3\text{Fe}(\text{CN})_6$ and then incubated for 20 min at 50 °C. After the incubation, 2.5 mL of 10% trichloroacetic acid added to the above mixture and centrifuged for 10 min. 2.5 mL of supernatant mixed with 2.5 mL of distilled water and 0.5 mL of 0.1 % FeCl_3 solution, and then measure the absorbance at 700 nm. EC_{50} value (mg/mL) was calculated to measure the reducing ability of the sample.

2.4 Determination of Total Phenolic Contents

The phenolic contents (TPC) of the sample determined following the Folin-Ciocalteu method¹³. In the method, the different extracts (100 μL) mixed with water up to 3 mL and 0.5 mL of Folin-Ciocalteu reagent. After 3 min, 20 % sodium carbonate (2 mL) was mixed thoroughly, and then boil the mixture for 1 min in a water bath. A blue colour was developed due to the formation of the complex by the redox reaction of phosphomolybdic acid (folin ciocalteu reagent) with a maximum absorbance at 650 nm. The total phenolic contents were measured by catechol calibration curve and expressed as catechol equivalent (CE) in mg/g on a dry weight basis.

2.5 Determination of Total Flavonoid Contents

The flavonoid contents (TFC) of the sample were measured by the aluminium chloride method¹⁴ (colourimetric method). In

the assay, test sample (100 µl) was mixed with 80% ethanol and subsequently with 95% ethanol. After that aluminium chloride solution added followed by potassium acetate solution. The mixture was thoroughly mixed and incubated for 30 min. Absorbance was then measured at 415nm versus the blank (except of Aluminium chloride). The total flavonoid contents were measure by quercetin calibration curve and expressed as quercetin equivalent (QE) in mg/g on a dry weight basis.

2.6 Determination of Total Tannin Contents

Total tannin contents (TTC) of the sample were measure by the Folin Denis method¹⁵. Based on the method, the test sample (100 µl) was pour to the volumetric flask (50 ml) containing 30 ml distilled water and then added Folin Denis reagent, 35% sodium carbonate and finally make up the volume. After that, test solutions heated at 10-20 °C for 30 min. Absorbance was then measure at 700 nm. The total tannin contents measured by tannic acid calibration curve and expressed as tannic acid equivalent (TAE) in mg/g on a dry weight basis.

2.7 Statistical Analysis

The data was characterise by Mean ± Standard Deviation (SD) (n=3). The obtained data were interprete by One-way analysis of variance (ANOVA) and Duncan's test at 0.05 probability level using SPSS 16.0 Software. The difference between the means (P<0.05) was consider to be significant.

3. RESULTS

3.1 Total Phenolic Contents (TPC)

In aqueous extract, phenolic contents varied from 5.27 to 10.46 mg CE/g d.w. Maximum phenolic contents (10.46 mg/g) found in aqueous extract of leaves followed by aqueous extract of the root (9.44 mg/g). While, least concentration of phenolics recorded from aqueous extract of stems. The phenolics contents in the alcoholic extract were ranged from 2.51 to 6.46 mg CE/g d.w. In the alcoholic extract, highest phenolics contents found in leaves (6.46 mg/g) followed by the alcoholic extract of the roots (6.11 mg/g). Maximum phenolic contents were found in aqueous extracts of different parts as compared to the alcoholic extracts. Table 1 shows the total phenolic contents present in aqueous and alcoholic extracts of different parts of the plant, and the leaf extract showed a significantly higher amount followed by the other parts.

3.2 Total Flavonoid Contents

In the present study, flavonoid contents ranged from 1.92 to 7.50 mg QE/g d.w. in aqueous extracts of *Ajuga bracteosa*. The maximum flavonoids (7.50 mg/g) were display by the aqueous extract of leaves followed by roots extract (5.80 mg/g). In the alcoholic extract of plant, flavonoids varied from 0.88 to 4.66 mg QE/g d.w. The highest flavonoid contents (4.66 mg/g) found in the leaves followed by an alcoholic extract of roots (4.19 mg/g), while, least quantity of flavonoids exhibited in the stem. Aqueous extract displayed the highest flavonoid contents followed by the alcoholic extract. Table 1 shows the total flavonoid contents present in aqueous and alcoholic extracts of different parts of the plant, and the leaf extract showed a significantly higher amount followed by the other parts.

3.3 Total Tannin Contents

In aqueous extracts of all plant parts of *Ajuga bracteos*, the tannin contents varied from 7.29 to 21.06 mg TAE/g) d.w. The maximum quantity of tannin (21.06 mg/g) recorded in the leaves followed by the root of the plant (16.66 mg/g). While the stem shows, least tannin contents. In alcoholic extracts of the plant, tannins ranged from 3.12 to 12.72 mg TAE/g) d.w. The highest tannins found in leaves (12.72 mg/g) followed by the roots (10.64 mg/g). While minimum tannin contents were present in the stem of the plant. Aqueous extract displayed the highest tannin contents followed by the alcoholic extract. Table 1 shows the total tannin contents present in aqueous and alcoholic extracts of different parts of the plant, and the leaf extract showed a significantly higher amount followed by the other parts.

3.4 Determination of Antioxidant Activity

The in-vitro antioxidant activity was assessed by three different assays viz. ABTS, DPPH, and PFRAP assay. The capacity of the extracts to inhibit the ABTS, DPPH radicals and reducing power were expressing by estimating IC₅₀ value (mg/mL) for both aqueous and alcoholic extracts. Table 2 shows the antioxidant activity in aqueous and alcoholic extracts of different parts of the plant as shown in Table 2.

3.4.1 Free Radical Scavenging by ABTS Assay

The inhibition concentration (IC₅₀) was estimate in the study, minimum IC₅₀ value means maximum anti-oxidant activity. In aqueous extract; minimum IC₅₀ value i.e. maximum

Table 1. Total phenolics, flavonoid and tannin contents in the aqueous and alcoholic extracts of different plant parts of *Ajuga bracteosa*

Sample	Total phenolic contents (mgCE/g) d.w.		Flavonoid contents (mgQE/g) d.w.		Tannin contents (mgTAE/g) d.w.	
	Aq	Alc	Aq	Alc	Aq	Alc
Leaves	10.46±0.09a	6.466±0.0709a	7.503±0.075a	4.663±0.122a	21.06±0.3983a	12.723±0.4041a
Flowers	9.136±0.1159c	5.49±0.0519c	3.366±0.023c	1.66±0.00c	14.346±0.4041c	10.08±0.3459b
Stem	5.276±0.095d	2.516±0.1429d	1.923±0.0461d	0.886±0.023d	7.29±0.35d	3.125±0.345c
Root	9.44±0.09b	6.11±0.1558b	5.806±0.0461b	4.193±0.0461b	16.663±0.345b	10.646±0.5291b

(* AQ- Aqueous extract, Alc Alcoholic extract) The value represented as (Mean ± SD) (n = 3).

The values not sharing the same letter are significantly different (Duncan's test) at P < 0.05 probability level.

Table 2. Antioxidant activity in the aqueous and alcoholic extracts of different plant parts of *Ajuga bracteosa*

Sample	ABTS (IC ₅₀ = mg/mL) d.w.		DPPH (IC ₅₀ = mg/mL) d.w.		PFRAP (EC ₅₀ = mg/mL) d.w.	
	Aq	Alc	Aq	Alc	Aq	Alc
	Leaves	0.2707±0.0008b	0.3521±0.0097b	0.4409±0.0020b	0.5418±0.0122b	0.3413±0.0030b
Flowers	0.4931±0.0388d	0.6752±0.056d	0.6161±0.0015d	0.6883±0.0030d	0.4573±0.0011d	0.4983±0.00577d
Stem	0.7173±0.0017e	1.1773±0.0013e	1.4323±0.0513e	1.5563±0.0466e	0.8083±0.0097e	0.9003±0.0085e
Root	0.3118±0.0005c	0.563±0.0031c	0.4933±0.0008c	0.602±0.0018c	0.372±0.0017c	0.4667±0.0045c
Ascorbic acid	0.00449±0.00001a		0.0096±0.00002a		0.0066±0.00006a	

(* AQ- Aqueous extract, Alc Alcoholic extract) the value are represented as (Mean ± SD) (n = 3).

The values not sharing the same letter are significantly different (Duncan's test) at P < 0.05 probability level.

antioxidant activity was exhibited by leaves (0.2707 mg/mL) followed by the root (0.3118 mg/mL). While the stem extract shows least antioxidant activity. Similarly, the alcoholic extract of leaves showed the highest antioxidant activity (0.3521mg/mL) followed by roots of the plant (0.563 mg/mL) and minimum antioxidant activity was exhibit by the stem. The ascorbic acid used as a reference standard, and the IC₅₀ value was 0.00449 mg/mL.

3.4.2 Free Radical Scavenging by DPPH Assay

The DPPH assay commonly used and relatively brief method for the determination antioxidant potential¹⁶. The disappearance of the colour of DPPH radical at 517 nm wavelengths monitored by decreased optical density¹⁷⁻¹⁹. The aqueous extract of leaves displayed the highest antioxidant activity (0.4409 mg/mL) followed by the root extract (0.4933 mg/mL) and minimum antioxidant activity displayed by the stem of the plant. In the alcoholic extracts of the plant, IC₅₀ varied from 0.5418 to 1.5563 mg/mL. The highest antioxidant activity displayed by the leaves extract (0.5418 mg/mL) followed by root extract (0.602 mg/mL). The ascorbic acid used as a reference standard, and the IC₅₀ value was 0.0096 mg/mL.

3.4.3 The PFRAP Assay

The antioxidant potential by reducing power assay is associated with the reduction process, in which splitting of free radical chain occurs by contributing a hydrogen atom¹⁸. In the assay, the antioxidant reduces the Fe³⁺/Ferricyanide complex to ferrous and blue colour, thus formed, which measured at 700 nm¹⁹. The reducing power (EC₅₀) in aqueous extracts

of plants ranged from 0.3413 to 0.8083 mg/mL. The results revealed that the maximum reducing power observed in leaves extract (0.3413 mg/mL) followed by root extract (0.372 mg/mL) and minimum activity displayed by the stem. In alcoholic extract, leaves extract displayed highest reducing power (0.3743 mg/mL) followed by in roots extract (0.4667 mg/mL).

3.5 Correlation Analysis

The correlation analysis between the phytochemical contents (TPC, TFC and TTC) and antioxidant ability (IC₅₀ and EC₅₀ Values) of extracts represented in Table 3, the phytochemical contents exhibited highly significant correlation with the ABTS, DPPH and PFRAP assay. The results suggested that the phytochemical contents viz, total phenolic, flavonoid and tannin contents significantly contributed to the antioxidant activity of the different parts of the plant extracts as shown in Table 3.

4. DISCUSSION

Antioxidant compounds are known for their capabilities to prevent or delay the oxidation of other molecules. They inhibit the free radical chain reactions by donating an electron without becoming themselves free radicals, and nowadays, it becomes a vital part of our body. In the present study, antioxidant activity and phytochemical contents of the different plant parts of *Ajuga bracteosa* has been assessing. The results revealed the antioxidant potential and phytochemical contents of the aqueous and alcoholic extracts of the plant. The recent work by Kayani¹⁹, et al. on *Ajuga bracteosa* to evaluate the antioxidant activity and phytochemical contents in methanolic and chloroform extract of aerial and root portion. It suggested

Table 3. Correlation Analysis between antioxidant activities (IC₅₀ and EC₅₀ Values) and phytochemicals content of different plant parts of *Ajuga bracteosa*

	ABTS Assay	DPPH Assay	PFRAP Assay	TPC	TFC	TTC
ABTS Assay (IC ₅₀)	1	0.881**	0.919**	-0.887**	-0.887**	-0.916**
DPPH Assay (IC ₅₀)		1	0.994**	-0.788*	-0.77*	-0.85**
PFRAP Assay (IC ₅₀)			1	-0.81*	-0.809*	-0.877**
TPC				1	0.853**	0.967**
TFC					1	0.923**
TTC						1

* Indicates correlation significance at P < 0.05, ** Indicates correlation significance at P < 0.01 (2-tailed).

that the aerial part shows higher antioxidant activity followed by the root. The variation observed between the antioxidant activity, and phytochemical contents depends on the part of the plant and the extraction solvent used for the study. The present study compared the antioxidant potential of aqueous and alcoholic extracts of the different parts against different free radicals. The antioxidant activity estimated by inhibition concentration (IC_{50}); the leaves displayed better antioxidant activity followed by the other parts of the plant. Similarly, the leaves showed the higher total phenolics, flavonoids and tannin contents followed by other parts. The phenolic compounds present in vegetables and fruits have attracted a lot of attention due to their potential anti-oxidant activities²²⁻²⁴. The results revealed the variation of IC_{50} , EC_{50} values, total phenolic, flavonoids and tannin contents that may attributed to different part of the plant used for the study. Further, this difference could attribute to an uneven distribution of phytochemical contents in the different parts of the plant and therefore, varying its antioxidant activity³. The results are in a satisfactory agreement with the previous work¹⁹⁻²¹. In the present study, correlation analysis represents a negative correlation among the IC_{50} , EC_{50} values and phytochemical contents, which reflects that higher the phytochemical contents, lesser the IC_{50} value, therefore higher the antioxidant activity. This result was consistent with the previous research, reported the correlation between phytochemical contents and antioxidant potential⁴.

5. CONCLUSION

Hence, it can concluded from the study that among different plant parts of *Ajuga bractiosa*, leaves of the plant displayed highest antioxidant activity followed by roots and least antioxidant activity exhibited by the stem of the plant. As far as phytochemical content are concerned leaves possessed maximum phenolic, flavonoids and tannins contents followed by the root of the plant. It is further, observed that aqueous extract of the plant exhibited better antioxidant activity and phytochemical contents than alcoholic extract. Therefore, the antioxidant activity of different parts of the plants attributed to the total phenolic, tannin and flavonoid contents. Consequently, the study suggests that the leaves can employed as a direct source of antioxidants, and there is a need for further study of its isolated compounds and biological activities.

CONFLICT OF INTEREST

There is no conflict of interest among the authors.

REFERENCE

- Liao, K.L. & Yin, M.C. Individual and combined antioxidant effects of seven phenolic agents in human erythrocyte membrane ghosts and phosphatidylcholine liposome systems: Importance of the partition coefficient. *J. Agri. Food Chem.*, 2000, **48**, 2266-2270. doi: 10.1021/jf990946w
- Alam, M.N.; Bristi, N.J, & Rafiquzzaman, M. Review on *in-vivo* and *in-vitro* methods evaluation of antioxidant activity. *Saudi Pharm. J.*, 2013, **21**, 143–152. doi: 10.1016/j.jsps.2012.05.002
- Sylvie, D.D.; Anatole, P.C.; Cabral, B.P. & Veronique, P.B. Comparison of *in-vitro* antioxidant properties of extracts from three plants used for medical purpose in Cameroon: *Acalypha racemosa*, *Garcinia lucida* and *Hymenocardia lyrata*. *Asian Pac. J. Trop. Biomed.*, 2014, **4**(2), S625-S632. doi:10.12980/APJTB.4.201414B168
- Tamuly, C.; Hazarika, M.; Bora, J. & Gajurel, P.R. Antioxidant activities and phenolic content of *Piper wallichii* (miq.) Hand.-Mazz. *Int. J. Food Prop.*, 2014, **17**, 309–320, doi: 10.1080/10942912.2011.631250
- Singh, K.P.; Meena, H.; Pandey, H.K. & Mohd, N. *Ajuga bracteosa*: A central Himalayan plant shows potent hypoglycemic effect in rats. *Indian J. Pharm. Pharmacol.*, 2016, **3**(4), 210-213. doi: 10.18231/2393-9087.2016.0008
- Hussain, M.; Bibi, Y.; Iqbal, N.R.; Iqbal, M. & Aslam, S. A review of therapeutic potential of *Ajuga bracteosa*: A critically endangered plant from Himalaya. *J. Coast. Life Med.*, 2016, **4**(11), 918-924.
- Kumar, D. & Bisht, R. Antidiabetic potency of leaves of *Ajuga bracteosa* on hypoglycemic activity in alloxan induced diabetic chicks. *Int. J. Adv. Res.*, 2017, **5**(12), 836-841. doi:10.21474/IJAR01/6027
- Rahman, M.M.; Islam, M.B.; Biswas, M. & Alam, A.H.M.K. *In-vitro* antioxidant and free radical scavenging activity of different parts of *Tabebuia pallida* growing in Bangladesh. *BMC Res. Notes.*, 2015,**8**, 621. doi: 10.1186/s13104-015-1618-6
- Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M. & Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Rad. Biol. Med.*, 1999, **26**, 1231-123. doi: 10.1016/S0891-5849(98)00315-3.
- Meena, H.; Pandey, H.K.; Pandey, P.; Arya, M.C. & Ahmed, Z. Evaluation of antioxidant activity of two important memory enhancing medicinal plants *Baccopa monnieri* and *Centella asiatica*. *Indian J. Pharmacol.*, 2012, **44**, 114-7. doi: 10.4103/0253-7613.91880
- Kedare, S.B. & Singh, R.P. Genesis and development of DPPH method of antioxidant assay. *J. Food Sci. Technol.*, 2011, **48**(4), 412–422. doi: 10.1007/s13197-011-0251-1
- Maruthamuthu, V. & Kandasamy, R. Ferric reducing anti-oxidant power assay in plant extract. *Bangladesh J. Pharmacol.*, 2016, **11**, 570-572. doi: 10.3329/bjp.v11i3.27663
- Shoib, A.B. & Malik, S.A. Determination of total phenolic and flavonoids contents, antimicrobial and antioxidant activity of a root extract of *Arisaema jacquemontii* Blume. *J. Taibah Univ. Sci.*, 2015, **9**(4), 449-454. doi: 10.1016/j.jtusci.2014.11.
- Chang, C.C.; Yang, M.H.; Wen, H.M. & Chern, J.C. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J. Food Drug Anal.*, 2002, **10**, 178-182.

15. Sadasivam, S. & Manickam, A. Biochemical methods. *New age International*, Delhi, 2007, **3**, 195-197 p.
16. Kumar, A.; Thamilselvan, V.; Senthikumar, G.P.; Mazumder U.K. & Gupta, M. Antioxidant and free radical scavenging effects of *Lippia nodiflora*. *Pharm. Biol.*, 2008, **46**, 762–71.
doi: 10.1016/j.foodchem.2006.10.066
17. Blois, M.S. Antioxidant activity determination by the use of a stable free radical. *Nature.*, 1958, **181**, 1199-1200.
doi: 10.1038/1811199a0
18. Duan, X.; Wu, G. & Jiang, Y. Evaluation of antioxidant properties of phenolics from litchi fruit in relation to pericarp browning prevention. *Molecule.*, 2007, **12**, 759-771.
doi: 10.3390/12040759.
19. Kayani, W.K.; Dilshad, E.; Ahmed, T.; Ismail, H. & Mirza, B. Evaluation of *Ajuga bracteosa* for antioxidant, anti-inflammatory, analgesic, antidepressant and anticoagulant activities. *BMC Complem. Altern. M.*, 2016, **16**, 375.
doi: 10.1186/s12906-016-1363-y
20. Toiu, A.; Mocan, A.; Vlase, L.; Parvu, A.E. & Vodnar, D.C. Comparative phytochemical profile, antioxidant, antimicrobial and *in-vivo* antiinflammatory activity of different extracts of traditionally used Romanian *Ajuga genevensis* L. and *A. reptans* L. (Lamiaceae). *Molecules*, 2019, **24**, 1597.
doi:10.3390/molecules24081597
21. Kumar, S.; Mohammad, S.J.; Kumar, P. & Kumar, R. Chemical composition, *in-vitro* antibacterial, and antioxidant activity of essential oil from leaves of *Ajuga parviflora* Benth. *Asian J. Pharm. Clin. Res.*, 2018, **11**(2), 57-6.
doi: 10.22159/ajpcr.2018.v11s2.28529
22. Pan, Y.; Wang, K.; Huang, S.; Wang, H.; Mu, X. & He, C. Antioxidant activity of microwave assisted extract of longan (*Dimocarpus longan* Lour.) peel. *Food chem.*, 2008, **106**, 1264-1270.
doi: 10.1016/j.foodchem.2007.07.033
23. Amin, A. & Yazdanparast, R. Antioxidant and free radical scavenging potential of *Achillea santolina* extract. *Food Chem.*, 2007, **104**, 21-29.
doi: 10.1016/j.foodchem.2003.05.007
24. Miliauskas, G.; Venskutonis, P.R. & Van Beek, T.A. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. *Food Chem.*, 2004, **85**, 231-237.
doi: 10.1016/j.foodchem.2003.05.007

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CONTRIBUTIONS

Ms Anchala Guglani, MPharm, is currently working as Junior Research Fellow in Defence Institute of Bio-Energy Research, DRDO, Pithoragarh and pursuing her PhD from Kumaun University. Her area of interest includes pharmacological and toxicological evaluation of herbal products, phytochemical and biochemical evaluation of herbal plants and their products. She has contributed in the designing and performed experiments. She was also involved in analysis, compilation of data and writing the manuscript.

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Dr Madhu Bala, presently working as Director, DRDO-Defence Institute of Bio-Energy Research, Haldwani. Her area of interest includes Radiation Biology. In the current study, she has provided active and continuous guidance for this work.