Phytochemical and radical scavenging activity of *Bauhinia. Variegate* (Kachnar) stem

Para Dholakia[1], Jyoti Yadav[1], Shalini Singh[1], Shalini Yadav[1], Shruti Pal[1], PrabhjotKaur Sabharwal[1*]

[1] Department of Food Technology, ShaheedRajguru College of Applied Sciences, University of Delhi

Abstract

Medicinal plants have been used as chief antidote against numerous diseases since time immemorial. *Bauhinia variegate*, commonly known as kachnar has been indigenously used in Indian household for its therapeutic properties. It is a potent antioxidant and is rich in phenolic and flavonoid compounds. The present paper aims to analyze the antioxidant property of plant stem on the basis of free radical scavenging activity, phenolic content and flavonoid content. Tender and soft plant stems were collected from Vasundhra Enclave, New Delhi. A standardized protocol was followed for extraction from the plant stem and the extract thus obtained was analyzed for antioxidant activity. Total phenolic, flavonoid content was found to be 15.24% and 0.01% respectively. Radical scavenging activity was found to be 48.3% at 0.82 mg mL^{-1}. The presence of these secondary metabolites suggests that the plant might be of medicinal and industrial importance. The overall antioxidant activity can be attributed to flavonoids, phenolic and other phytochemical constituents indicating stem can be used as therapeutic agent and as a potential antioxidant.

KEYWORDS: Antioxidant, phenolic, flavonoid, phytochemical, DPPH assay,

free radical scavenging activity

1. Introduction

Bauhinia variegata(variegated orchid tree) a member of family Fabaceae and sub family Caesalpinioideae is commonly known as commonly known as RaktaKanchan in Sanskrit[1] and mountain ebony (English) or kachnar (Hindi). It is a well-known ornamental tree of tropical and sub-tropical climate with hot and dry summers and mild winters. It is native of Asia and is widely found in the Indian subcontinent and in regions of China. *Bauhinia variegata*is distributed in sub-Himalayan and outer Himalaya of the Punjab and Sikkim state, India. It is also found in Burma and China, most tropical countries, including Africa and South America[3,4].*Bauhiniavariegate* (Kachnar) is well practiced in Indian indigenous health science; it has been frequently used in folk medicine for varied purposes; to treat different kinds of pathologies, particularly diabetes, infections, as well as pain and inflammation owing to the presence of numerous phytochemicals[3]. The chemical constituents isolated so far from the plant are β-sitosterol, kaempferol-3-glucoside, tannins, carbohydrates, amides, reducing sugars, vitamin C, crude protein, fibres, calcium, phosphorus, quercetin, rutin, quercetin, apigenin, apigenin- 7-Oglucoside, heptatriacontan-12, 13-diol and dotetracontan-15-en-9-ol[4]. Plant polyphenols act as strong antioxidants and they protect cell constituents against oxidative damage, thus averting the deleterious effects on nucleic acids, proteins and lipids in cells[5]. High antioxidant capacities are observed in plants with high level of phenolic compounds[6]. The redox properties of phenolic compounds enable them to act as reducing agents, hydrogen donors and singlet oxygen scavengers, whichaccount for the antioxidant

© International Journal of Science, Technology and Management

potential of medicinal plants[7]. The phenolic content is considered as animportant plant constituent as its hydroxyl groups have considerable scavenging ability[8].Flavonoids prevent hydroxyl radical induced damage by donating an electron to neutralize the species[10,11]. Plants synthesize compounds with biological activity, namely antioxidant, as secondary products that are mainly phenolic metabolites ranging from simple to highly polymerized compounds[12]. Many epidemiological studies have shown that the consumption of phenolic rich foods is associated with the prevention of chronic diseases[13]. Biological properties of different *Bauhinia* species shows therapeutic properties are mainly due to the presence of flavonoids. Plant phenolics, in particular phenolic acids, tannins and flavonoids are known to be potent antioxidants and occur in vegetables, fruits, nuts, seeds, roots and barks. In the case of phenolic compounds, the ability of the phenolics to act as antioxidants depends on the redox potential of their phenolic hydroxyl groups that allow them to act as reducing agents, hydrogen-donating antioxidants and oxygen quenchers[12]. These findings indicate that free radical scavenging is of immense value in the prevention and treatment of deadly disease. If the plant contains phenolics in considerable quantity, the plant can be commercially exploited and used as therapeutic agent. High yield of total phenolics from the stem bark of *Bauhinia variegate* has been obtained pointing that it can be utilised as a remarkable source for the preparation of not only nutraceuticals but also for the treatment of other major health problems. Different parts of *Bauhinia variegate* like leaves, bark and flowers have reported decent free radical scavenging activity[9]. Overall *Bauhinia*

variegate can be considered as a model herbal drug for experimental studies including free radical induced disorders like cancer, diabetes, atherosclerosis etc.

The bark of Kachnar plant is useful in the treatment of skin diseases, scrofula and ulcers. Bark decoction have been used for diarrhea control, as an astringent alternative and for treating scrofula, skin diseases and ulcers. In a study, it was found that the body weight and feed intake of hyper caloric diet fed female rats was reduced significantly when treated with methanolic extract of stem and root barks of *B.variegata*, which could be ascertained to the presence of â-sitosterol in the stems[11]. The stem bark extract of *B. variegata*has shown to exhibit significant hepato protective activity[12].The ethanolic extract of the stem bark of *B.variegata*is an immune modulatory agent and most likely acts by stimulating both the specific and non- specific arms of immunity. Aqueous extract and isolated compound of *B. variegate* stem bark showed primarily significant activity on in vitro human neutrophils in all parameters, which are comparable to standard and control at different concentration signifying the potential immuno-stimulating effect. One cup of this decoction is taken thrice a day against cancer[13].

The current study is aimed at extraction of antioxidantcompounds using water as solvent from *B.variegate stem*. The extract is screened for antioxidant potential using three parameters;DPPH assay, total phenoliccontent and total flavonoid content.

2. Materials and Methods

2.1 Collection of plant material

The stem of *Bauhinia variegate* was collected from Herbal garden of ShaheedRajguru College of Applied Sciences for Women, Vasundhra

Enclave (Figure 1). The stems were washed thrice with tap water to remove all the dust and debris & then finally washed with distilled water and blotted smoothly between folds of filterpaper to remove excess amount of water. The final weight of stem was observed as 140.05g.

Figure 1: Fresh stem sample of *B.variegata*

2.2 Processing of plant material

The washed and dried stem was crushed in mortar pestle using 100mL of distilled water. Water was used as solvent for extraction. Extraction was carried out in pan using 300 mL distilled water (Figure 2). The solution was boiled to finally obtain a viscous concentrate. The concentrated solution obtainedwas filtered through muslin cloth and finally double filtered usingWhatman filter no. 42.

Figure 2: Crushed sample in mortar pestle, boiling sample in water after crushing, stem residue after filtration (L to R)

2.3 Preparation of extract

The filtered solution was concentrated in an aluminium dish by continuous boiling till a dark coloured concentrated sample (weight: 1.653 g) was obtained. The extract was taken in a volumetric to make the volume upto 100 times the weight of extracted sample (weight of extracted sample is 1.653 g then total volume used will be 165.3 mL). These extract was stored in refrigerator till further usage.

Weight of concentrated sample= 1.653 g Volume of water used=165.3 mL

Thus, the extract concentration is 100 mg mL^{-1}.

2.4 Chemicals and reagents used

Gallic acid, α,α diphenylβpicrylhydrazyl (DPPH), Quercitin solution were procured from SRL chemicals (Mumbai, India), Sodium carbonate, sodium nitrite, aluminum chloride, sodium hydroxide, methanol were procured from Qualigens chemicals (Mumbai, India). Folins reagent was procured from SD fine chemicals (Mumbai, India). All the chemicals and solvent used in the study were of analytical grade. Double distilled water was used in the preparation of stock solvents and extraction.

2.5 Preparation of chemicals

- 0.02% Gallic acid : 0.01 g Gallic acid in 50 mL distilled water
- 20% sodium carbonate: 10 g Na_2CO_3 in 50 mL distilled water
- α,αdiphenyl-β-picrylhydrazyl (DPPH): 0.02g DPPH in 50mL distilled water
- Quercitin solution: 50 mg quercitin solution in 100mL distilled water
- 5% sodium nitrite: 2.5 g

 ISSN: 2229 - 6646

$NaNO_2$ in 50 mL distilled water

- 10% aluminium chloride: 2g $AlCl_3$ in 20 mL distilled water
- 1 M sodium hydroxide: 4 g NaOH in 100mL distilled water
- Folins reagent: Mix folins reagent with distilled water in the equal parts (1:1)

2.6 DPPH Assay

This method was developed by Blois (1958) with the viewpoint to determine the antioxidant activity in a like manner by using a stable free radical α, α-diphenyl-β-picrylhydrazyl (DPPH; $C_{18}H_{12}N_5O_6$, M = 394.33). The assay is based on the measurement of the scavenging capacity of antioxidants towards it.

Figure 4: Color change in DPPH assay

Procedure:

Six standard solutions of strength (100,200,400,600,800 & 1000 ppm) by taking 0.1, 0.2, 0.4, 0.6, 0.8 & 1 mL of sample extract in different test tubes were prepared. Volume was made upto 3 mL in each test tube by addition of methanol. Then 1mL DPPH solution was added (Table 1).After 30 minutes absorbance was taken at 518nm using spectrophotometer (Figure 4). The scavenging effect was calculated using formula:

Scavenging effect%= $[(Abs_c - Abs_s)/ (Abs_c)] \times 100$

© International Journal of Science, Technology and Management

Table 1: Procedure table of DPPH assay

Concentration (mL)	Sample (mL)	Methanol (mL)	DPPH (mL)
100	0.1	2.9	1
200	0.2	2.8	1
400	0.4	2.6	1
600	0.6	2.4	1
800	0.8	2.2	1
1000	1.0	2.0	1

2.7 Total Phenolic Content (TPC) assay

Six standard Gallic acid solution of concentration 200 µg/mL (20,40,60,80,100&120 ppm) was prepared. 0.1, 0.2, 0.3, 0.4, 0.5& 0.6) mL of this was pipetted out in six different test tubes. Each test tube's volume was made up to 5mL by adding distilled water. Then 0.5 mL of Folin-Ciocalteu phenol reagent was added. After 5 minutes, 1.5 mL of 20% Na_2CO_3 was added in each test tube. The volume of test tube was made up with 10 mLdistilled water (Table 2). This was incubated for 2 hrs at 30-32°C[16]. Intense blue colorwas observed and optical density was measured at 750nm using spectrophotometer.

© International Journal of Science, Technology and Management

Table 2:Procedure table of TPC assay

Gallic Acid	**Distilled water (mL)**	**Folins reagent (mL)**	**20% NaCO3**	**Distilled water (mL)**	**OD at 750 nm**
(mL)			**(mL)**		
0(blank)	5	0.5	1.5	3	
0.1	4.9	0.5	1.5	3	0.081
0.2	4.8	0.5	1.5	3	0.086
0.3	4.7	0.5	1.5	3	0.155
0.4	4.6	0.5	1.5	3	0.128
0.5	4.5	0.5	1.5	3	0.217
0.6	4.4	0.5	1.5	3	0.259
Sample					
KS(10x)	4	0.5	1.5	3	0.866

2.8 Total Flavonoids Content (TFC) assay

Six standard quercetin solution of concentration 500µg/mL (100, 200, 300, 400, 600, 800 & 1000 ppm) were prepared in methanol. Then pipetted out (0.2, 0.4, 0.6, 0.8, 1.2, 1.6 & 2) mL of the respective solution in six different test tubes. Each test tube's volume was made up to 5mL by adding distilled water. 0.3 mLof 5% $NaNO_2$was added in each test tube. After 5 minutes 0.3 mL of 10% $AlCl_3$was added in each test tube. Post 1 minute 2 mL of 1M NaOH was added to every test tube. The volume was made upto10 mL with distilled water. It was incubated for 1 hour at 30-32° C. Optical density was measured at 510nm using spectrophotometer.

Table 3: Procedure table for TFC assay

Querceti nbromela in (mL)	Distille d Water (mL)	NaNo 2 (mL)	AlCl 3 (mL)	NaOH (mL)	Make up vol. to 10mL	OD at 510 nm
0	5	0.3	0.3	2	2.4	0
0.2	4.8	0.3	0.3	2	2.4	0.087
0.4	4.6	0.3	0.3	2	2.4	0.095
0.6	4.4	0.3	0.3	2	2.4	0.135
0.8	4.2	0.3	0.3	2	2.4	0.148
1.2	3.8	0.3	0.3	2	2.4	0.163
1.6	3.4	0.3	0.3	2	2.4	0.239
2	3	0.3	0.3	2	2.4	0.346
Sample						
KS(10x)	4	0.3	0.3	2	2.4	0.093

3. Results& Discussion

3.1 DPPH Assay

DPPH is a stable organic free radical, which loses its absorption spectrum band at 515–528 nm when it accepts an electron or a free radical species. The DPPH assay is a simple, acceptable and most widely used technique to evaluate the radical scavenging potency[17]. The radical inhibiting activity of the extract obtained was measured by the ability to scavenge DPPH free radicals. Figure 5 shows the standard curve of extract. Free radical scavenging capacity was

© International Journal of Science, Technology and Management

highest at 800µg/mL or 80mg/g with 56.140% inhibition. Our extract showed 48.39% inhibition at 0.82 mg mL^{-1}(Figure 6). Pandeyet al.[18] have reported IC_{50} value at 7.68 mg mL^{-1} concentration of acidic bark extract.

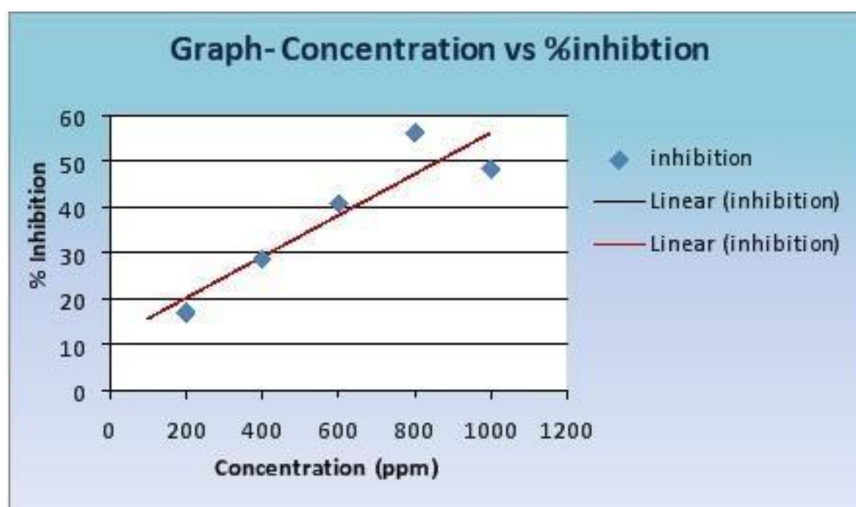

Figure 5: Standard curve of of *B. variegate* stem

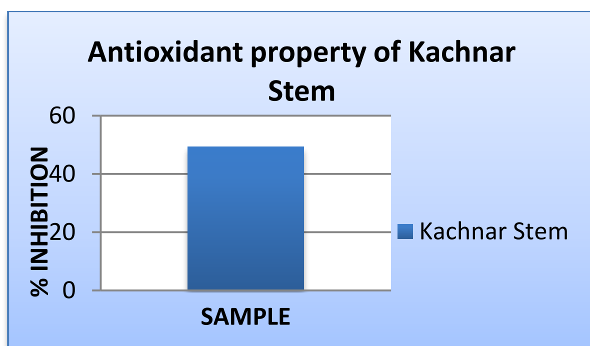

Figure 6: Free radical scavenging activity *B. variegata*stem at 0.82 mg mL^{-1}

© International Journal of Science, Technology and Management

DPPH is a stable, nitrogen-centered free radical which produces violet/purple colour in ethanol solution and fades to shades of yellow colour in the presence of antioxidants. One peculiarity of this method is that it allows testing of both lipophilic and hydrophilic compounds in comparison to other methods that are restricted in the nature of antioxidants that they can be used to quantify[19].

3.2 Total phenolic content (TPC)

Phenolic content was measured using folin's reagent in each extract. The results were derived from standard curve of gallic acid (20-120mg/mL) and expressed in gallic acid equivalents (GAE) per mL dry extract weight (figure 7). Equation of the curve obtained was $y=0.0019x+0.0323$ (r^2 = 0.972). For diluted extract (10X) the value was found 251.9 + 0.047 $mgGAEmL^{-1}$ or 152.41 mgGAE g^{-1} or 15.24% total phenolic percent (Table 4). Similar value has been reported in literature for Kachnar bark [18].

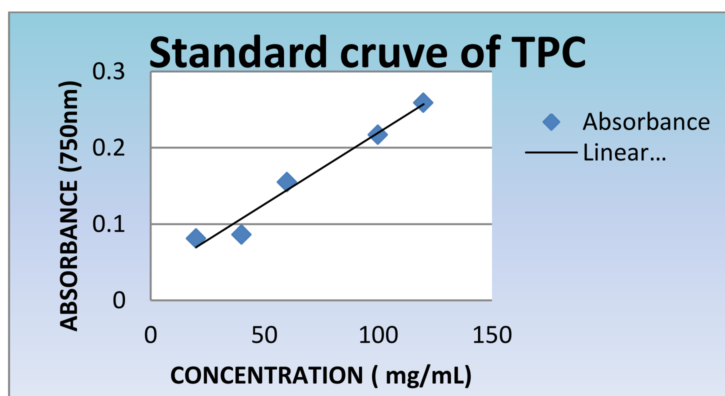

Figure 7: Standard calibration curve of TPC

Recent studies have shown that many dietary polyphenolic constituents derived from plants are more effective antioxidants in vitro than vitamins E or

© International Journal of Science, Technology and Management

C, and thus might contribute significantly to the protective effects in vivo. It is now possible to establish the antioxidant activities of plant-derived flavonoids in the aqueous and lipophilic phases, and to assess the extent to which the total antioxidant potentials of wine and tea can be accounted for by the activities of individual polyphenols[20] .

Table 4: Total phenolic content (TPC) of *B.variegata*stem

Sample	Absorbance	Concentration(mg/mL)
KS(10x)	0.511	251.947

The recovery of phenolic compounds from plant materials is also influenced by the extraction time and temperature, which reflects the conflicting actions of solubilisation and analyte degradation by oxidation. An increase in the extraction temperature can promote higher analyte solubility by increasing both solubility and mass transfer rate[21]. The extraction procedures and solvents are responsible for dissolving the endogenous compounds of the plants.Phenolic compounds are ubiquitous secondary metabolites in plants. They are known to have antioxidant activity and it is likely that the activity of these extracts is due to this compounds[22].

3.3 Total flavonoid content (TFC)

Flavonoids are secondary metabolites with antioxidant activity, the potency of which depends on the number and position of free OH groups. Flavonoid content was measured using quercetin solution in each extract. The results were derived from stnadrad calibration curve ofquercetin solution (100-1000mg mL^{-1}) and expressed as quercetin solution equivalents per gram dry extract weight (Figure 8). The equation of the standard curve obtained

is y=0.0003x+ 0.0436 (r^2= 0.9266). For diluted extract ofKachnar stem (10X), the value obtained was 199.5 + 0.0506 µg of quercetinmL^{-1} extract or 0.120 mgQE g^{-1} or can also be represented as percent concentration 0.012% (Table 5). Pandeyet al.[18] have reported TFC value of 0.01%, which is in range with our result.

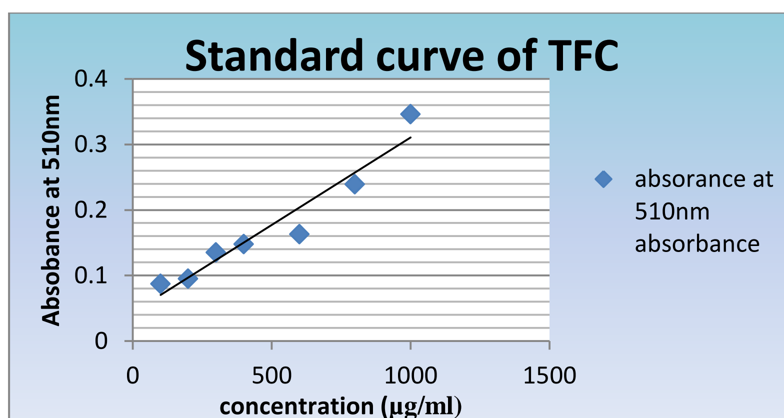

Figure 8: Standard calibration curve of TFC

Table 5: Total flavonoids content (TFC) result table of *B.variegata*stem

Sample	Absorbance at 510nm	Concentration (µg/mL)
KS(10x)	0.093	199.5506

Flavonoids show anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activity. Flavonoids are generally distributed throughout the plant kingdom and are 15 carbon compounds.Tannin and phenol are necessary for the animal body for repair and maintenance[23]. Flavonoids are one class of secondary plant metabolites that are also known as Vitamin P. These metabolites are mostly used in plants to produce yellow and other

pigments, which play an important role in the coloursof plants[24].

4. Conclusion

In the present study, aqueous extract of *B. variegate* bark and its fractions were evaluated for antioxidant, TPC and TFC activity. The potent antioxidative activity of *B. variegata*bark extract/fractions is attributed due toits rich phenolic/flavonoid compounds.A significant correlation was observed between antioxidant activity and the total phenolic/flavonoid content. Although the parameters used in this study were not disease specific, the quantification of antioxidant properties can serve as a guide for the use of these plants for related diseases. The plant (*B. variegata*) with high antioxidant activity and might be proposed as nutraceuticals and preservative. It is concluded that the antioxidant capacities, total phenolic content of the stem are considered as good sources of antioxidants as observed in DPPH scavenging assay.

5. Scope of future work

5.1. *B. variegata*stem shows promising antioxidant activity. Further work is desired to find out antioxidant activity of different parts of the plant.

5.2. In the current study aqueous extraction has been employed. Different solvent can be utilized for extraction of antioxidant compounds to zero in a solvent that gives highest antioxidant activity.

5.3. The results for DPPH, TPC and TFC are encouraging, thus the concentrate from stem can be used as a potential functional food/ nutraceutical.

5.4. Studies can also be conducted to analyze the efficacy of concentrate as a natural preservative in food.

6. References

1. G, Gupta, P K, Sahu. Kanchnar bauhinia variegata as a medicinal herb a systematic review. A review on Bauhinia variegata Linn. Int Res J Pharm, 2012; 3(1):48-51.
2. Gamble J S, Flora of the Presidency of Madras, Vol. 2. Botanical Survey of India, Calcutta
3. ArvindNegi, Nimisha Sharma, Reetika Pant, Mamta F. Singh. Determination of total phenolic content of the stem bark of Bauhinia variegata Linn. an approach to standardization. The Pharma Research, 2012; Volume 7, Issue 2. Page16-22.
4. Vijay Kumar M M J, Eswarappa B, Yadav D. Bodke, Jayadevaiah K V, Basavaraja H S.Isolation of Phytoconstituents from the Stem Bark of Bauhinia Variegata Linn. Pharma Tutor Magazine, 2014; Vol. 2, Issue9
5. Rice-Evans C. Current Medicinal Chemistry, June 2001; Volume 8, Number 7, 1, pp. 797-807(11)
6. Moyo M, Ndhlala A R, Finnie J F, Staden J V. Phenolic composition, antioxidant andacetylcholinesterase inhibitory activities of Sclerocaryabirrea and Harpephyllumcaffrum (Anacardiaceae). Food Chem, 2010; 123:69-76.
7. F LukmanulHakkim, C Gowri Shankar, and S Girija. Chemical Composition and Antioxidant Property of Holy Basil (Ocimum sanctum L.) Leaves, Stems, and InflorescenceandtheirinVitroCallus Cultures.J.Agric.FoodChem, 2007;55,9109– 9117.
8. A T Diplock. Will the ‘good fairies’ please prove to us that vitamin E lessens human degenerative of disease? Free Rad. Res, 27 1997; pp.511–53
9. SouzaSouza R S O, Albuquerque U P, Monteiro J M, Amorin E L C: Jurema-preta (Mimosa tenuiflora

[wild.] poir) a review of its traditional use, phytochemistry and pharmacology. Braz Arch BiolTechn, 2008; 51(5):937-947.

10. HusainSR, Cillard J and Cillard P. Hydroxyl radical scavenging activity of flavonoids. Phytochemistry, 26:1987;2489-2497.
11. Balamurugan, G. and Muralidharan, P. 2010. Antiobesity effect of Bauhinia variegata bark extract on female rats fed on hypercaloric diet. Bangladesh Journal of Pharmacology, 5(1):8-12
12. Bodakhe, S.H. and Ram, A. 2007. Hepatoprotective properties of Bauhinia variegata bark extract. YakugakuZasshi, 127(9):1503-1507.
13. Ghaisas, M.M.; Shaikh, S.A. and Deshpande, A.D. 2009. Evaluation of immunomodulatory activity of ethanolic extract of the stem bark of Bauhiniavariegata Linn. International Journal of Green Pharmacy, 3(1): 70-74
14. Kedare , S.B. & Singh, R.P.J Food SciTechnol(201148:412) https://doi.org/10.1007/s13197-011-0251
15. Plank, D.W.;Szpylka, J.;Sapirstein, H.;Woollard,D.;Zapf, C.M.;Lee,V.;Chen,C.-Y.O.;Liu, R.H.;Tsao, R.; Düsterloh, A.; et al.Determination of Antioxidant Activity in Foods and Beverages by Reaction with 2,2′Diphenyl1Picrylhydrazyl (DPPH): Collaborative Study First Action 2012.04. J. AOAC Int. 2012, 95,1562–1569, doi:10.5740/jaoacint.CS2012_04.
16. AOAC SMPR 2015.009: Estimation of Total Phenolic Content Using the Folin-C Assay, Journal of AOAC INTERNATIONAL, Volume 98, Issue 4, 1 July 2015, Pages 1109–

© International Journal of Science, Technology and Management

1110, https://doi.org/10.5740/jaoac.int.SMPR2015.009

17. African Journal of Pharmacy and Pharmacology Vol. 5(12), pp. 1494-1500, 29 September, 2011,http://www.academicjournals.org/AJPP/ISSN1996-11 Academic Journals
18. A.K. Pandey, VijayalakshmiOjha, Swati Yadav and Sonu Kumar Sahu, 2011. PhytochemicalEvaluation and Radical Scavenging Activity of Bauhinia variegata, Saracaasoka and Terminaliaarjuna Barks. Research Journal of Phytochemistry, 5: 89-97.
19. J.Olorunjuwon,Olugbami, MichaelA. Gbadegesin, and Oyeronke. A. Odunola, Pharmacognosy Res. 2015 Jan-Mar;7(1): 49–56. doi:10.4103/09748490.147200/PMCID:PMC4285649/PMID: 25598635
20. Atherinee Rice-Evans, Nicholas Miller, George Paganga/Trends in plant science 2 (4),152-159,1997
21. Di J, Mumper RJ. Plant Phenolics: Extraction, Analysis and Their Antioxidant and Anticancer Properties. Molecules.2010; 15(10):7313-7352.
22. Advances in Pharmacological Sciences Volume 2019, Article ID 7428593, 4 page https://doi.org/10.1155/2019/7428593
23. Olasupo AD, Aborisade AB, Olagoke OV (2018) Phytochemical Analysis and Antibacterial Activities of Spinach Leaf. Am J PhytomedClinTher Vol.6No.2:8
24. Journal of Applied Pharmaceutical Science Vol.5 (01), pp.052-057,January,2014,DOI: 10.7324/JAPS.2015.50110 ,ISSN 2231-3354