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Determination of antioxidant activity, total phenolic and flavonoid contents in leaves, stem and roots of *Uraria picta* Desv

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Abstract

***Uraria picta* Desv. belonging to family Leguminosae: Papilionoidae is one of the important constituent of "Dashmoolarista", a well-established Ayurvedic drug of Indian system of medicine. The objectives of the present study were to determine the free radical scavenging activity, total phenolic and flavonoid contents in leaves, stem and roots of *U. picta*. Powdered samples of leaves, stem and roots were subjected to successive extraction with solvents of increasing polarities i.e. Ethanol, Water: Ethanol (Aqua- alcoholic) (20: 80) and Water (aqueous) using soxhlet apparatus. Total phenol, flavonoid and antioxidant activity were determined by using Folin-Ciocalteau method, aluminum chloride colorimetric technique and DPPH free radical scavenging methods respectively. The results exhibited the maximum phenolic (1.991±0.299%) and flavonoid (2.865±0.11%) contents in ethanolic extract of leaves. For stem, the highest phenolic content (1.208±0.115%) and highest flavonoid content (22.189±2.7%) were detected in aqueous and ethanolic extracts respectively. For roots, both the maximum phenolic (3.554±0.004%) and flavonoid (0.497±0.507%) contents were found in Aqua- alcoholic extract of roots. The ethanolic extracts of leaves and stem and aqueous extract of roots were found to contain the lowest IC_{50} and hence, the maximum antioxidant activity. Based on the findings, it can be concluded that among the different extracts of leaves, stem and roots of *U. picta*, the ethanolic extracts of leaves and stem and aqueous extract of roots exhibited the more promising antioxidant activity due to the presence of phenolic and flavonoid compounds.**

Key Words: ***Uraria picta, leaves, stem, roots, phenolic content, flavonoid content, antioxidant activity.***

Introduction

Since ancient time plants played an important role in the form of herbal drugs. Basically the medicinal value of plants lies in the bioactive phytochemical constituents that produce specific physiological action on the human body (Akinmoladun *et al.,* 2007). Phytochemicals can be classified into two groups viz. primary and secondary according to their function in the plant body. Primary metabolites (sugars, amino acids, proteins, lipids, chlorophyll etc) are required for growth while secondary metabolites (alkaloids, essential oils, flavonoids, tannins, terpenoids, saponins, phenolic compounds, cardiac glycosides etc.) play an important role in plant defense against herbivory (Stamp, 2003; Hill, 1952; Edeoga et al., 2005) and other interspecies defenses. Secondary metabolites form the backbone of the modern medicine (Goh, 1995). Antioxidants are the compounds which prevent free radicals generated through cell metabolism. These free radicals are very reactive and damage the healthy cells, tissues and other parts of our body. These radicals oxidize macromolecules in the body, such as lipids, proteins, nucleic acids andare responsible for various degenerative diseases such as atherosclerosis, ischemic heart disease, ageing, diabetes mellitus and cancer (Khatoon *et al.,* 2013; Agbo *et al.,* 2015). In present time, there is a lot of concern on plant based natural antioxidants which have been scientifically proved safer due to their ability to scavenge free radicals and chelate metals than their synthetic counterparts which are carcinogenic and cause liver damage (Zhu *et al.,* 2011). Plant phenolics are the widely distributed group of phytochemicals (Figure 1) (Saxena *et al.,* 2013) which have been reported to bestow with a number of biological activities including antioxidant due to their strong ability to capture the free radicals (Sahu and Saxena, 2013). *Uraria picta*

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Desv. (Syn. *Doodia picta* Roxb., *Hedysarum pictum* Jacq., Family- Fabaceae) is commonly known as Prishnaparni or Pithvan (Figure 2) and widely distributed throughout India, Bangladesh, Sri Lanka, Tropical Africa, Malay Islands, Philippines, Australia, Africa and almost all parts of Asia (McNeill *et al.,* 2006; Ohashi and Iokawa, 2007). It is one of the important constituent of "Dashmoolarista", a well-established Ayurvedic drug of Indian system of medicine, prepared from the roots of 10 medicinal plants and used for treating general fatigue, oral sores and several gynecological disorders (Yadav *et al.,* 2009). Dashmool is also used as basic ingredient in manufacture of over 109 drug formulations (Pathak *et al.,* 2005). Traditionally, the plant is used as an antidote to the venom of a dangerous Indian snake, *Echis carinata* (Kirtikar and Basu, 1993). Its leaves are a good antiseptic and are used against gonorrhea. Leaves of *U.picta* also showed antianxiety activity (Garg *et al.,* 2012). The fruits, pods are effective against oral sores in children, roots are being used against cough, chills and fever (Kirtikar and Basu, 1993; Yusuf *et al.,* 1994).

The search for novel natural antioxidants of plant origin is the major focus area of researchers these days. The scientific studies showed that antioxidant activity of plants appear to play a major role in the protective effect of plant medicine (Saeed *et al.,* 2012). The present study focused to determine the TPC, TFC and the antioxidant activity of alcoholic, aqua-alcoholic and aqueous extracts of leaves, stem and roots of *U. picta*.

Materials and Methods

Chemicals and reagents: All chemicals, reagents and solvents used in the study were of AR grade and distilled water was utilized wherever required.

Collection of plant materials: The plant materials (leaves, stem and roots) of *U. picta* were collected by following the guidelines of good agricultural and collection practices (GACP) for medicinal plants (Anon, 2003) from the Khandwa region of Madhya Pradesh.

Processing of plant materials: Plant materials were washed thoroughly in running water to remove soil and other foreign particles. These were cut into small pieces and dried in shade. Shade dried plant samples were powdered using grinder mill and powdered plant materials were stored in air –tight polythene bags for further chemical analysis.

Preparation of plant extracts: Powdered plant samples of leaves, stem and roots of *U. picta* were subjected to successive extraction with solvents of increasing polarities i.e. Ethanol, Ethanol: Water (Aqua- alcoholic) (80: 20) and Water (aqueous) using soxhlet apparatus (Varghese *et al.,* 2013). A total of 20g of dried plant powder of concerned part was extracted in 250 ml of each solvent in successive manner for 12 hrs. Solvents were evaporated to dryness to yield the respective extracts which were used for estimation of total phenols , flavonoids and antioxidant activity.

Estimation of total phenols: Total phenols were determined by Folin-Ciocalteau method (McDonald *et al.,* 2001). 10 mg of the extract was dissolved in a known volume of distilled water. 0.2 ml of samples was then taken in test tube and volume made up to 3 ml with distilled water. 0.5 ml of Folin-Ciocalteau reagent was then added. After 3 minutes, 2 ml of 20% Na_2CO_3 solution was added to each tube, mixed thoroughly, placed in boiling water for exactly 1 min, cooled and absorbance was taken at 650 nm against blank. The phenolic content was calculated from the standard graph of catechol (Figure 3).

Estimation of total flavonoids: Total flavonoids were determined by aluminum chloride colorimetric technique (Chang *et al.,* 2002). 10 mg of the extract was dissolved to 25 ml with 80% ethanol. 0.5 ml of filtrate was then mixed with 1.5 ml of 95 % ethanol, 0.1 ml of 10% $AlCl_3$, 0.1 ml of potassium acetate and 2.8 ml distilled water. The tubes were than incubated at room temperature for 30 minutes. Observations were measured at 415 nm. The flavonoid content was calculated from the standard graph of quercetin (Figure 4).

DPPH free radical scavenging activity assay: The free radical scavenging activity of the fractions was measured in-vitro by 1, 1 - diphenyl-2-picrylhydrazyl (DPPH) assay according to the method described earlier (Pandeya *et al.,* 2016) with some modification. The stock solution was prepared by dissolving 0.006 g DPPH with 100 ml methanol and stored at 20°C until required. 3 ml aliquot of this solution was mixed with 100 µl of the sample at various concentrations (10 – 50 µg/ml). The reaction mixture was shaken well and

incubated in the dark for 15 min at room temperature and the absorbance was taken at 517 nm. The control was prepared as above without any sample. The percentage inhibition was estimated based on the percentage of DPPH radical scavenged as the following equation:

% Radical Scavenging Activity (RSA) = {(Abs control − Abs sample)}/ (Abs control) × 100

Where Abs control is the absorbance of the DPPH radical + methanol; Abs sample is the absorbance of DPPH radical + sample extract.

Calculation of IC_{50}: IC_{50} value is the concentration of the sample required to inhibit 50% of radicals. IC_{50} values of the sample extracts were calculated.

Statistical analysis: Each experiment was carried out in triplicate and results are expressed as Mean ± SD (n=3) wherever needed.

Results and Discussion

Total phenolic and flavonoid contents:

The decoction of *U. picta* root is used since ancient time in formulation of Dashmoola. The decoctions of different plant parts of *U. picta* have also been reported with diverse type of pharmaceutical activities (Bhattacharya and Datta, 2010). Flavonoid and phenolic compounds are the majorly dispersed secondary metabolites in plant kingdom which are recognized as the potent antioxidant as well as many other important bioactive agents benefitting the human health (Tungmunnithum *et al.,* 2018). The plant extracts are biologically active due to presence of numerous compounds and safer to intake because they have synergic as well as antagonistic effects (Lindsay and Nadja, 2019). Since a few decades ago, the research focusing on flavonoids and phenolics of plant origin due to their beneficial effects on human health has increased significantly.

Under the present study, the total phenolic and flavonoid contents in leaf, stem and root extracts of *U. picta* were detected which are given in Table 1.

It can be seen from Table 1 that ethanolic extract of leaves contained maximum phenolic (3.554±0.004%) and flavonoid (2.865±0.11%) contents. Stem contained maximum phenolic content (1.208±0.115%) in aqueous extract and maximum flavonoid content (22.189±2.7%) in ethanolic extract. Similarly, for roots, both the maximum phenolic (3.554±0.004%) and flavonoid (0.497±0.507%) contents were found in Aqua-alcoholic extract. On overall comparison, aqua-alcoholic extract of roots contained maximum phenolic content (3.554±0.004%) while ethanolic extract of stem contained maximum flavonoid content (22.189±2.7%).

Antioxidant activity:

The antioxidant activity of the plant extracts were measured by using DPPH free radical. The DPPH free radical is considered hydrophobic free radical and most common assay system to study antioxidant activity (Pandeya *et al.,* 2016). In brief, the DPPH powder was dissolved in methanol in order to generate DPPH free radical and in the DPPH solution the plant extract was added and kept for 30 min in dark. The molecules or antioxidants present in plant extract with weak A-H bonding will react with a stable free radical $DPPH^{\bullet}$ causing decolorization of DPPH solution. The decolorization of DPPH was measured at 517 nm (Shimada *et al.,* 1992).

Antioxidant activities (IC_{50}values, µg/ ml) of ascorbic acid standard as well as leaves, stem and root extracts of *U. picta* are presented in Table 2. The graphical representations of antioxidant activities of ascorbic acid, leaves, stem and root extracts are given as Figure 5, 6, 7, 8, 9, 10, 11, 12, 13 and 14 respectively. Figures 5-14 shows that inhibition pattern of DPPH free radical is dose dependent. The IC_{50} values vary from part to part and extract to extract because of the presence of different amount of antioxidants.

Table 2 showed that ethanolic extract of leaves, ethanolic extract of stem and aqueous extract of roots have the lowest IC_{50} values which intended that these extracts have the highest radical scavenging activities. On overall comparison, the aqueous extract of roots was found to exhibit maximum antioxidant activity.

Various studies reported a number of biological activities including antioxidant activity of the phenolic compounds (flavonoids, phenolic acids) of plant origin (Uritani *et al.,* 1994) and present investigation showed the presence of phenolic compounds in different extracts of *U. picta*. The study revealed that all extracts except aqua-alcoholic extract of roots possess better antioxidant activity than ascorbic acid. Aqua-alcoholic extract of stem and ethanolic extract of roots did not exhibit antioxidant activity. The earlier studies were

Table1. Total phenolic and flavonoid contents in leaf, stem and root extracts of *U. picta*

Plant name	Parts used	Extracts	Phenols (%) (Mean ± SD)	Flavonoids (%) (Mean ± SD)
U. picta	**Leaves**	Ethanol	1.991±0.299	2.865±0.11
		Aqueous	1.202±0.203	0.43±0.002
		Aqua- alcoholic (80:20)	0.777±0.102	0.236±0.255
	Stem	Ethanol	0.982±0.02	22.189±2.7
		Aqueous	1.208±0.115	0.611±2.7
		Aqua- alcoholic (80:20)	0.882±0.34	0.347±0.43
	Roots	Ethanol	1.662±0.009	0.494±0.04
		Aqueous	0.552±0.010	0.312±0.007
		Aqua- alcoholic (80:20)	3.554±0.004	0.497±0.507

Table.2. Antioxidant activity of leaves, roots and stem of *U. picta*

Plant name	Parts used	Extracts	IC_{50} (µg mL^{-1})
U. picta	Leaves	Ethanol	24.93±0.003
		Aqueous	36.89±0.029
		Aqua- alcoholic (80:20)	32.11±0.005
	Stem	Ethanol	32.11±0.011
		Aqueous	39.14±0.007
		Aqua- alcoholic (80:20)	ND*
	Roots	Ethanol	ND*
		Aqueous	21.18±0.009
		Aqua- alcoholic (80:20)	44.21±0.009
Ascorbic acid (Vitamin C)			42.27±0.399

***Not Detected**

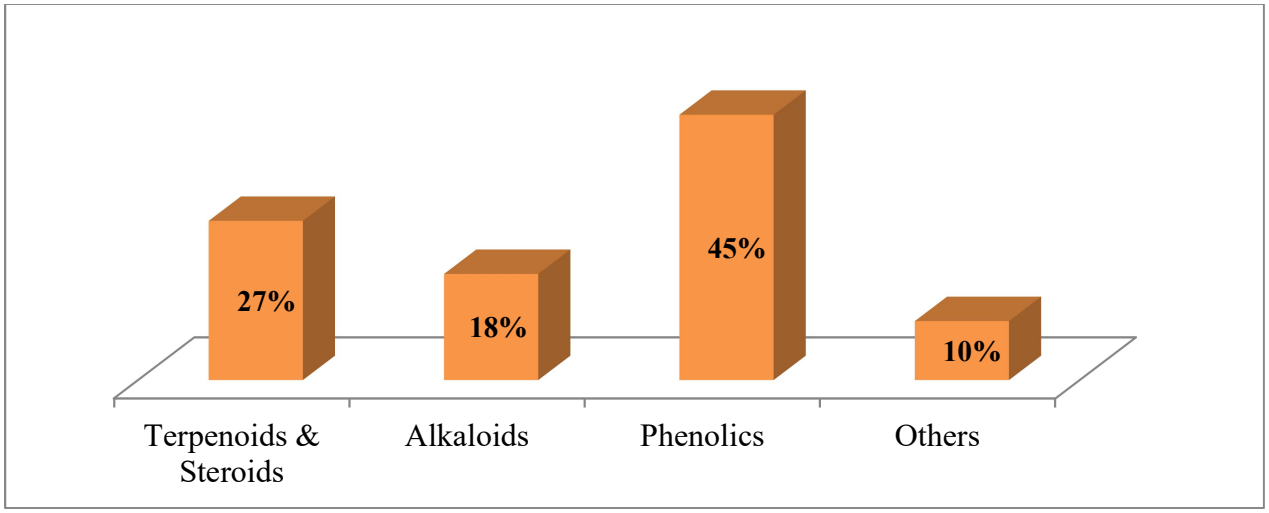

Figure 1. Major groups of plant phytochemicals

Figure 2. *Uraria picta* plant.

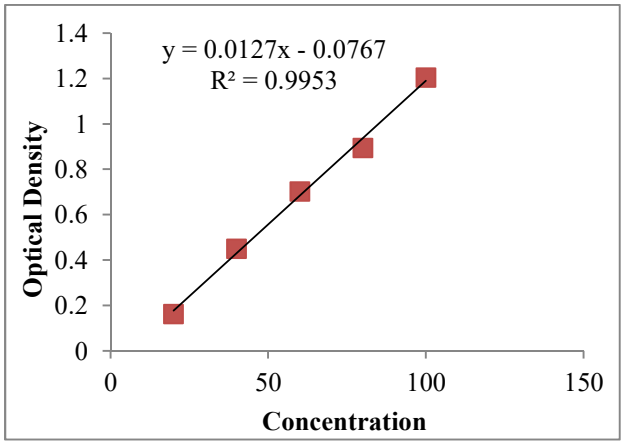

Figure 3. Standard graph of Catechol.

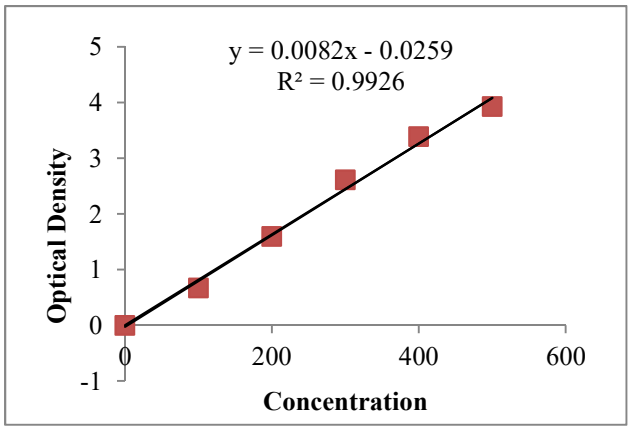

Figure 4. Standard graph of Quercetin.

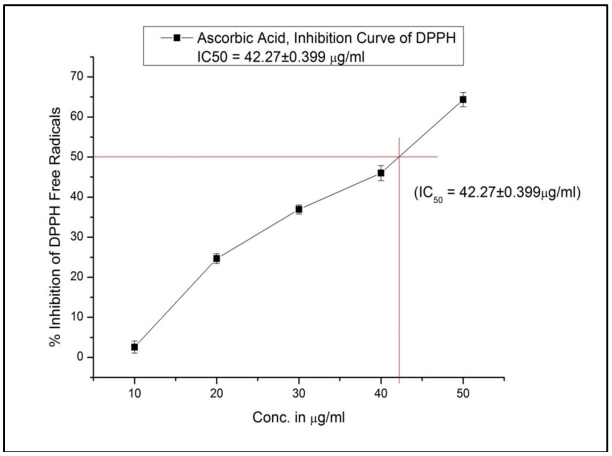

Figure 5. DPPH radical scavenging activity of Ascorbic acid.

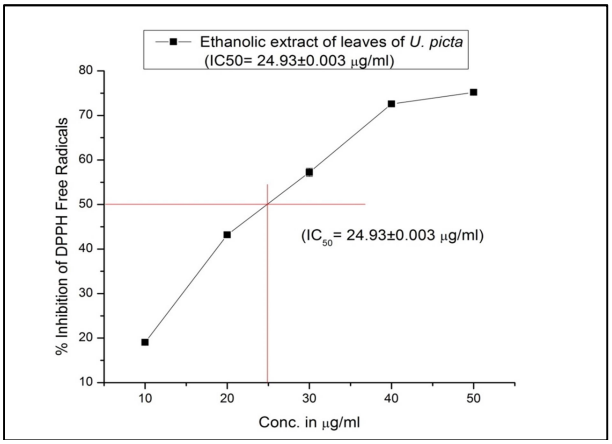

Figure 6. DPPH radical scavenging activity of ethanolic extract of *U. picta* leaves.

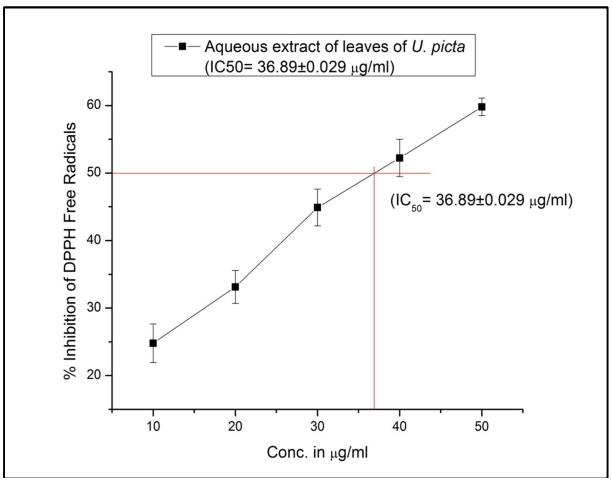

Figure 7. DPPH radical scavenging activity of aqueous extract of *U. picta* leaves.

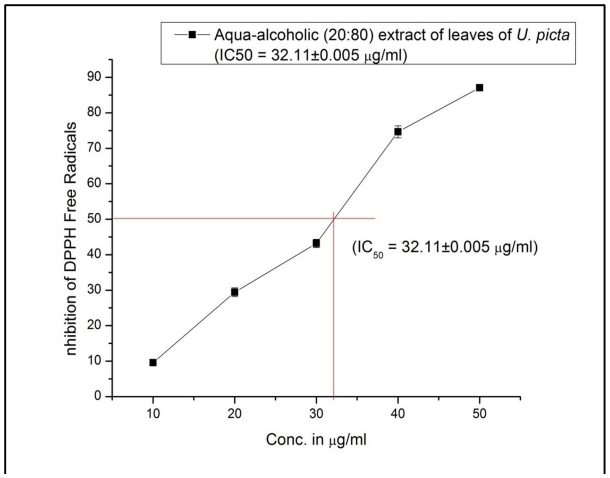

Figure 8. DPPH radical scavenging activity of Aqua-alcoholic extract (20:80) of *U. picta* leaves.

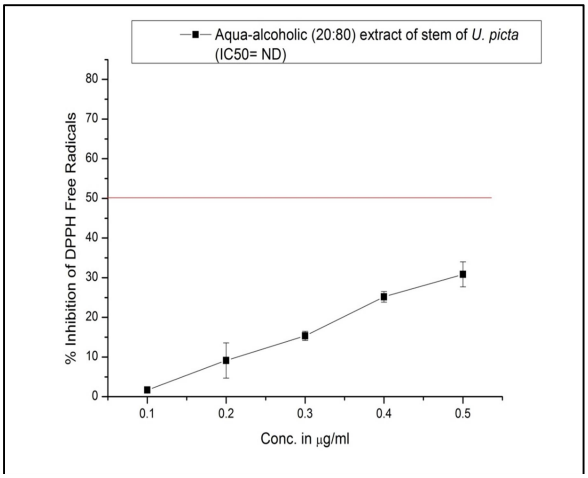

Figure 11. DPPH radical scavenging activity of Aqua-alcoholic extract (20:80) of *U. picta* stem.

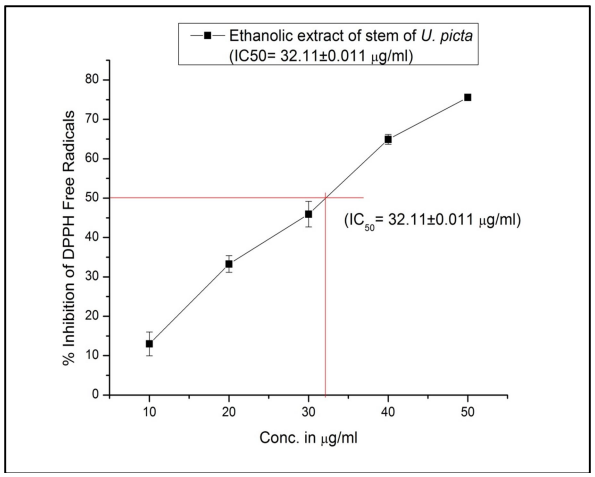

Figure 9. DPPH radical scavenging activity of ethanolic extract of *U. picta* stem.

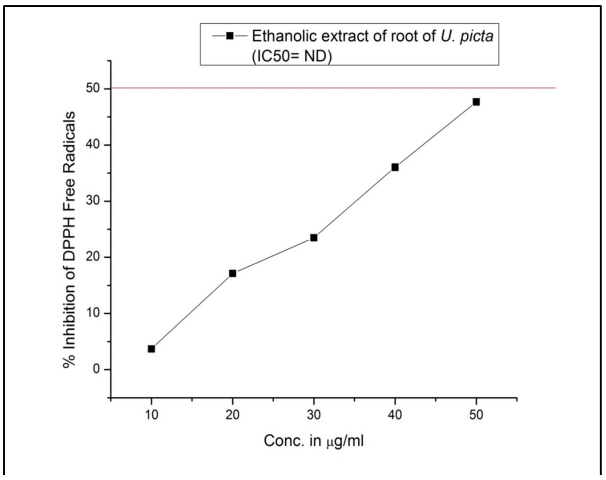

Figure 12. DPPH radical scavenging activity of ethanolic extract of *U. picta* root.

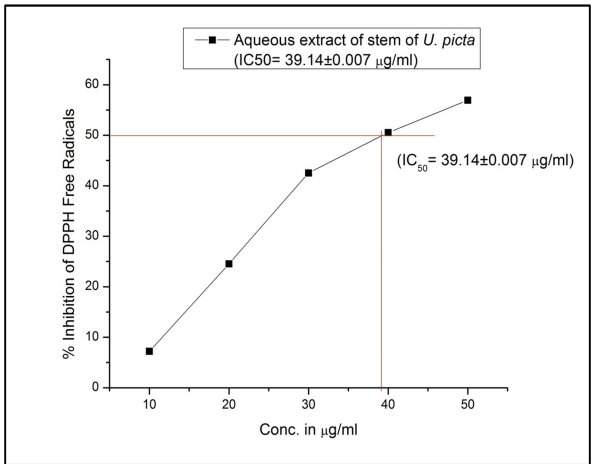

Figure 10. DPPH radical scavenging activity of aqueous extract of *U. picta* stem.

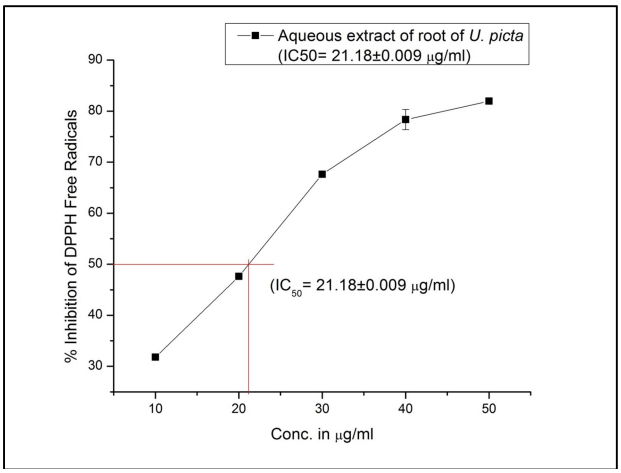

Figure 13. DPPH radical scavenging activity of aqueous extract of *U. picta* root.

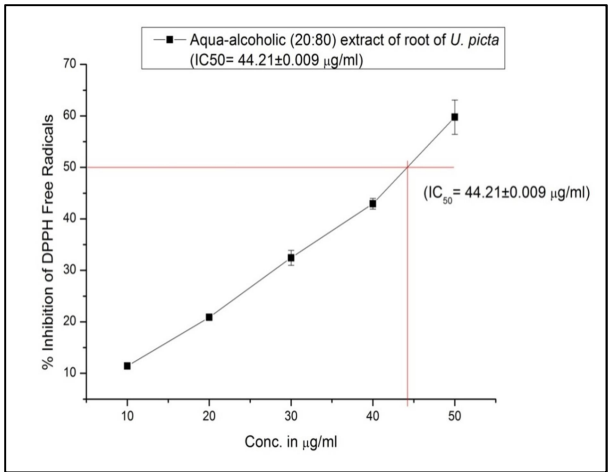

Figure 14. DPPH radical scavenging activity of Aqua-alcoholic extract (20:80) of *U. picta* root.

also carried out to investigate the antioxidant activity of ethanolic extract of entire plant (Patel *et al.,* 2011), aqueous extracts of whole plant (Patel *et al.,* 2011) and aqueous extract of leaves of *U. picta* (Odubanjo *et al.,* 2013). The findings revealed the DPPH radical scavenging activity of ethanolic and aqueous extracts of whole plant as 198.1µg/ml and 152 µg/ml respectively. Moreover, the aqueous extract of leaves of *U. picta* inhibited acetylcholine esterase (AChE) and butyryl choline sterase (BChE) in a dose dependent manner and exhibited radical scavenging ability due to the phytochemicals present in the extract. In the present work, plant parts viz. leaves, roots and stem were investigated separately for antioxidant potential using polar solvents which showed better results.

Conclusion

From the present study, it can be concluded that leaves, stem and roots of *U. picta* are a good source of antioxidant agents which is due to the presence of phenolic, flavonoid and their derivatives. Thus, this work is suggesting the utilization of *U. picta* as natural antioxidant. However, the investigation will further be needed for isolation and characterization of responsible antioxidant components of this plant.

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References

Agbo, M.O., Uzor, P.F., Akazie-Nneji, U.N., Eze-Odurukwe, C.U., Ogbatue, U. B. and Mbaoji, E.C. 2015. Antioxidant, total phenolic and flavonoid content of selected Nigerian medicinal plants. ***Dhaka University Journal of Pharmaceutical Sciences,*** 14(1): 35-41.

Akinmoladun, A.C., Ibukun, E.O., Afor, E., Obuotor, E.M. and Farombi, E.O. 2007. Phytochemical constituent and antioxidant activity of extract from the leaves of *Ocimum gratissimum*. ***Scientific Research and Essays,*** 2(5): 163-166.

Anonymous. 2003. WHO guidelines on good agricultural and collection practices (GACP) for medicinal plants. World Health Organization, Geneva.

Bhattacharya, A. and Datta, A.K. 2010. *Uraria picta*: An overview. ***Medicinal and Aromatic Plant Science and Biotechnology,*** 4(1):1 - 4.

Chang, C., Yang, M., Wen, H. and Chern, J. 2002. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. ***Journal of Food and Drug Analysis,*** 10: 178–182.

Edeoga, H.O., Okwu, D.E. and Mbaebie, B.O. 2005. Phytochemical constituents of some Nigerian medicinal plants. ***African Journal of Biotechnology,*** 4(7): 685-688.

Garg, N., Garg, M., Maan, A.S., Sandhu, B.S., Mittal, S. and Goyal, S. 2012. Phytochemical studies and anti-anxiety activity of *Uraria picta* leaves. ***Journal of Pharmaceutical Research and Opinion,*** 2(5): 39-40.

Goh, S.H., Chuah, C.H., Mok, J.S. L., and Soepadmo, E. 1995. Malaysian medicinal plants for the treatment of cardiovascular diseases. Selangor Darul Ehsan: Pelanduk Publication. Kaula Lumpur, Malaysia.

Hill, A.F. 1952. Economic Botany. A textbook of useful plants and plant products. 2nd edn. McGraw-Hill Book Company Inc, New York.

Khatoon, M., Islam, E., Islam, R., Rahman, A.A., Khurshid Alam, A.H.M., Khondkar, P., Rashid, M. and Parvin, S. 2013. Estimation of total phenol and in vitro antioxidant activity of *Albizia procera* leaves. ***BMC Research Notes,*** 6: 121.

Kirtikar, K.R. and Basu, B.D. 1993. Indian Medicinal Plants, Vol 2. International Book Publishers Dehradun, India.

Lindsay, K.C. and Nadja B.C. 2019. Synergy and antagonism in natural product extracts: when 1 + 1 does not equal 2. ***Natural Product Reports,*** 36: 869-888.

McDonald, S., Prenzler, P.D., Autolovich, M. and Robards, K. 2001. Phenolic content and antioxidant activity of olive extracts. ***Food Chemistry,*** 73: 73-84.

McNeill, J., Barrie, F.R., Burdet, H.M., Demoulin, V., Hawksworth, D.L., Marhold, D., Nicolson, D. H., Prado, J., Silva, P.C., Skog, J.E., Wiersema, J.H. and Turland, N.J. 2006. International Code of Botanical Nomenclature (Vienna Code). Koeltz Scientific Books, Konigstein – Germany.

Odubanjo, V.O., Oboh, G. and Ibukun, E.O. 2013. Antioxidant and anticholinesterase activities of aqueous extract of *Uraria picta* (Jacq.) DC. ***African journal of pharmacy and pharmacology,*** 7(41): 2768-2773.

Ohashi, H. and Iokawa, Y. 2007. A Revision of *Uraria* (Leguminosae) in Taiwan. ***Taiwania,*** 52: 177–183.

Pandeya, K.B., Tripathi, I.P., Mishra, M.K. and Jaiswal N. 2016. Antiradical and antioxidant properties of some oxovanadium (IV) complexes of l - amino acids. ***Journal of biological Sciences and Medicine,*** 2(4): 38-47.

Patel, B.D., Kamariya, Y.H. and Patel, M.B. 2011. Antioxidant potential of aqueous extract of entire plant of *Uraria picta* Desv. ***International Journal of Pharmaceutical Research,*** 3(4), 92-96.

Patel, B.D., Kamariya, Y.H. and Patel, M.B. 2011. Free radical scavenging potential of ethanolic extract of *Uraria picta* Linn. ***Pharmacology online,*** 2: 134-145.

Pathak, J.M., Krishnamurthy, R., Chandorkar, M.S., Gulkari, V.D. and Gupta, R. 2005. Identification of high yielding genotypes of Dashmool Shalparni (*Desmodium gangeticum*) drug plant and its cultivation under high density planting. ***Indian Journal of Horticulture,*** 62: 378 – 384.

Saeed, N., Khan, M. R. and Shabbir, M. 2012. Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts *Torilis leptophylla* L. ***BMC Complementary and Alternative Medicine,*** 12: 221.

Sahu, N. and Saxena, J. 2013. Total Phenolic & total flavonoid content of *Bougainvillea glabra* choisy and Calforina gold flower Extracts. ***International Journal of Pharmacy & Technology,*** 5: 5581-5585.

Saxena, M., Jyoti, S., Nema, R., Dharmendra, S. and Abhishek, G. 2013. Phytochemistry of Medicinal Plants. ***Journal of Pharmacognosy and Phytochemistry,*** 1(6): 168-182.

Shimada, K., Fujikawa, K., Yahara, K. and Nakamura, T. 1992. Antioxidative properties of xanthone on the auto oxidation of soybean in cylcodextrin emulsion, ***Journal of Agricultural and Food Chemistry,*** 40: 945-948.

Stamp, N. 2003. Out of the quagmire of plant defense hypotheses. ***The Quarterly Review of Biology,*** 78: 23-55.

Tungmunnithum, D., Thongboonyou, A., Pholboon, A. and Yangsabai, A. 2018. Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: An overview. ***Medicines (Basel),*** 5(3): 93.

Uritani, I., Garcia, V.V. and Mendoza, E.M.T. 1994. Postharvest biochemistry of plant food- materials in the tropics. 1st ed. Japan Scientific Societies Press, Tokyo, Japan, pp. 241-251.

Varghese, S., Narmadha, R., Gomathi, D., Kalaiselvi, M. and Devaki, K. 2013. Phytochemical screening and HPTLC finger printing analysis of *Citrullus lanatus* (Thunb.) seed. ***Journal of Acute Disease***, 2(2): 122-126002E.

Yadav, A.K., Yadav, D., Shanker, K., Verma, R K., Saxena, A. K. and Gupta, M.M. 2009. Flavone glycoside based validated RP-LC method for quality evaluation of Prishniparni (*Uraria picta*). ***Chromatographia,*** 69: 653 – 658.

Yusuf, M., Chowdhury, J.U., Wahab, M.A. and Begum, J. 1994. Medicinal plants of Bangladesh. BSCIR Laboratories, Chittagong, Bangladesh.

Zhu, K.X., Lian, C.X., Guo, X.N., Peng, W. and Zhou, H.M. 2011. Antioxidant activities and total phenolic contents of various extracts from defatted wheat germ. ***Food Chemistry,*** 126: 1122 - 1126.