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RESEARCH ARTICLE

SECONDARY METABOLITE PROFILING OF *INDIGOFERA VISCOSA* LAM. STEM BARK

Mani, J., *Sudheer Mohammed, M.M. and Narayanasamy, A.

Department of Botany, Government Arts College (Autonomous), Coimbatore - 18, Tamil Nadu, India

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ABSTRACT

Objective: The present investigation aimed to study the secondary metabolite profiling of stem of *Indigofera viscosa* Lam. **Methods:** The stem was extracted by successive method using different solvents of varying polarity. The extracts were tested for screening of secondary metabolites, characterization of the compounds were carried out using FTIR. **Results & Discussion:** Alkaloids, flavonoids, tannins, steroids, triterpenoids, glycosides, saponins, gum and mucilages and fixed oils were found in different extracts. Ethanol and water extracts showed significant results with respect of secondary metabolites in screening. Significant level of phenolic content and flavonoid content was observed in quantification assays. Various functional groups such as amine, alcohols, phenols, carboxylic acids, alkynes, etc. were identified through FTIR analysis. **conclusion:** The results of the present study will be helpful to chemically standardize the plant and it will be useful in tapping the medicinal uses of *Indigofera viscosa*.

INTRODUCTION

Humans have always relied on natural products and have continually explored their surroundings to improve various aspects of life (Colegate and Molyneux, 2007). Medicinal plants, since times immemorial, have been used in virtually all cultures as a source of medicine (Hoareau and DaSilva, 1999). Medicinal potential of plant shave stored in the form of phytochemicals. These chemicals are classified as primary and secondary metabolites with divergent functions (Croteau *et al*., 2000). Unlike primary metabolites responsible for growth and development of plants, secondary metabolites have a key role in survival of the plants (Agostini-Costa *et al*., 2012) and they are unique sources for pharmaceuticals, food additives, flavors, and other industrial materials (Zhao *et al*., 2005).Keeping the above mentioned importance of medicinal plants in view, *Indigofera viscosa* Lam. [=*Indigofera colutea* (Brum.f.) Merr.] is selected for the present study to find out the secondary metabolite profiling in the stem. *Indigofera viscosa* (family Fabaceae) is a medicinally important herb, growingup to 3 feet in height.

***Corresponding author:* Sudheer Mohammed, M.M.,**
Department of Botany, Government Arts College (Autonomous), Coimbatore - 18, Tamil Nadu, India.

The plant is commonly known as 'Rusty Indigo' and 'Sticky Indigo', which is found in barren lands and corners of cultivated lands throughout in Tamil Nadu. The plant is used as a medicine for jaundice, stomach pain, diarrhea, dysentery, scabies, cuts and wounds and bruises by local peoples in different parts of the world (Chifundera, 2001; Neuwinger, 2000; Copeland, 2007). The plant was studied for polyphenol content and antioxidant activity and it compared with its allied species (Bakasso *et al*., 2008). But the plant is not thoroughly investigated for phytochemical analysis. In the present work we aimed to discover the bioactive compounds in stem of *Indigofera viscosa*.

MATERIALS AND METHODS

Collection, Preparation and Extraction of Plant materials: The plant was collected from Thondamuthur village, Coimbatore district, Tamil Nadu, the identity of the plant is confirmed by Botanical Survey of India, Southern Regional Centre, Coimbatore, Tamil Nadu. Fresh stems were collected from the same place and washed in running water until the specimen was clean, and air dried at room temperature. The dried samples were milled into fine powder manually with clean mortar and pestle.

The powder stored in clean glass beaker at room temperature for further analysis. Successive extraction was carried out as described by Das *et al*. (2010). 50g of each powders were successively extracted in soxhlet apparatus with petroleum ether, dichloromethane and ethanol solvents, the same specimen extracted with water. Each extraction process was successively ran for 72 hours. The extracts we restored in labelled sterile bottles at room temperature for subsequent analysis.

Qualitative tests for secondary metabolites: The stem extracts were subjected to qualitative secondary metabolite analysis. Presence of alkaloids, flavonoids, tannins, steroids, triterpenoids, saponins, glycosides, gum and mucilages and fixed oils were detected by various tests using standard procedures (Waldi, 1965; Evans, 1997; Trease and Evans, 2002; Segelman *et al*., 1969; Finar, 1986; Roopashree *et al.*, 2008; Kokate, 1999; Camporese *et al.*, 2003; Whistler and BeMiller, 1993).

Quantification of secondary metabolites

Total phenolics: Total phenolic contents was measured by using Folin-Ciocalteu reagent method described by Makkar (2003). 50 µl of ethanolic extract is taken into a test tube and made up to 1 ml with distilled water. A test tube with 1 ml of distilled water served as the blank. 500 µl of Folin – Ciocalteau Phenol reagent (1N) is added to both the test tubes including the blank. After 5 minutes, 2.5 ml of $NaCo_3$ solution (5%) was added to all the test tubes and the tubes were vortexed thoroughly. Absorbance was recorded after the formation of blue colour in the incubated test tubes at wavelength of 725 nm using UV- visible spectrophotometer after 40 minutes. Gallic acid standard was also prepared and the results were expressed as Gallic acid equivalents (GAE). The analyses were performed in triplicates.

Total tannins: Total phenolics contain both tannins and non-tannins. The amount of tannins is calculated by subtracting the non-tannins from total phenolics. For the determination of non-tannins (Makkar, 2003), 500 µl of plant sample is incubated with 100 mg of polyvinyl polypyrrolidone (PVPP) and 500 µl of distilled water taken in a 2 ml eppendorf tube for 4 hours at 4° C. After incubation, the eppendorf tubes are centrifuged at 4000 rpm for 10 minutes at 4° C. The supernatant contains only the non-tannin phenolics since the tannins would have been precipitated along with PVPP. The supernatant is collected and the non-tannin phenolics are determined by the same method described for the quantification of total phenolics. The analyses are also performed in triplicates and the results are expressed in Tannic Acid Equivalents (TAE). From these two results, the tannin content of the plant samples are calculated as follows,

Tannins = Total phenolics - Non tannin phenolics

Total flavonoids: Total flavonoids content was quantified according to the method described by Zhishen *et al*., (1999). About 500 µl of plant extract is taken in a test tube and 2 ml of distilled water was added to test tube. A test tube containing 2.5 ml of distilled water served as blank. Then, 150 µl of 5% $NaNO_2$ is added to both the test tubes followed by incubation at room temperature for 6 minutes.

After incubation, 150 µl of 10% $AlCl_3$ is added to both the test tubes including the blank, the test tubes are incubated for 6 minutes at room temperature. Then 2ml of4% NaOH is added to all the test tubes, then which are made up to 5 ml using distilled water. The test tubes are vortexed well and are allowed to stand for 15 minutes at room temperature. The pink colour developed due to the presence of flavonoids and is read spectrophotometrically at 510 nm and is compared with Rutin (standard). All the experiments done in triplicates and the results are expressed in Rutin equivalents (RE).

FTIR Spectroscopic analysis: The FTIR analysis was followed by KBr pellet method as described by Stuart (2004). The stem extract was examined for FTIR analysis. About 2-3mg of powdered form of stem extract was mixed with 200mg of perfectly dried Potassium Bromide (KBr) pellets, which is a standard ratio for good results. FTIR analysis was carried out with Shimadzhu FTIR spectrometer, the spectra was recorded in the absorption range between 4000 to 400 cm^{-1} at room temperature. Which is repeated for the three times for spectrum confirmation.

RESULTS AND DISCUSSION

Qualitative secondary metabolite screening: Secondary metabolite screening of *Indigofera viscosa* stem extracts revealed the presence of all the tested compounds (table 1) namely alkaloids, flavonoids, tannins, steroids, triterpenoids, saponins, glycosides, gum & mucilages and fixed oils. Unlike dichloromethane (DM) the petroleum ether (PE), ethanol (E) and water (W) extracts exhibits presence of more than half number of tested compounds. Saponins detected from water extract only. Glycosides and fixed oils present in petroleum ether only. Alkaloids and tannins screened from ethanol and water extracts. The phenolic compound flavonoids were observed in petroleum ether, ethanol and water extracts, the another compound tannin detected from ethanol and water extracts. The presence of various phytochemicals in the plant extracts received attention because of their biological activities. For example, presence oftannins and flavonoids in the plants exhibited various biological activities like antibacterial, antifungal, antioxidant and anthelmintic (Pulipati *et al*., 2017); Terpenoids reported for various important pharmacological activities i.e., anti-inflammatory, anti-cancer, anti-malarial, anti-viral and anti-bacterial activities (Mahato and Sen, 1997).The colour formation of flavonoids and tannins was high in ethanol extract when compare to other extracts, it may indicates the presence high concentration of phenolic compounds in ethanol extract. Therefore, the ethanol extract was examined for quantification assays. The same sample subjected to FTIR analysis.

Quantification of secondary metabolites: Though phenolic compounds found everywhere in plants, possess rich antioxidant activity (Okudu *et al*., 1994; Tepe *et al*., 2006). The results obtained in this study showed a significant level of total phenolic content. While, tannin content was little bit ofless amount in the extract. The total phenolic content and tannin content were compared with the standard gallic acid. The total phenolic content is 90.10±2.58 and the total tannin content is 39.19±5.46 mg of(gallic acid equivalent) GAE/gm of extract (Table 2). 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 colours of plants. Flavonoids are probably the most important natural phenols and are one of the widespread group of natural compounds. These compounds possess a broad spectrum of chemical and biological activities including free radical scavenging properties and they seem to display important anti-inflammatory, anti-allergic and anti-cancer activities (Pulipati *et al*., 2017; Crozier *et al.,* 2006). The flavonoid content of the present study is 82.93±5.25 mg of rutin/gm of extract (Table 2), which is moderate level. The phenolic and flavonoid compounds play a vital role in reproduction and growth of the plants. These also act as defensive agents against disease causing microbes and predators (Rice-Evans et al., 1996; Gautam *et al*., 2011). According to the literatures, flavonoids, phenolic and tannin are known to exhibit an array of biological activities. Therefore, quantitative analysis of such vital compounds is extremely significant to determine the quality ofdrugs.

Figure 1. Flowering twig of *Indigofera viscosa*

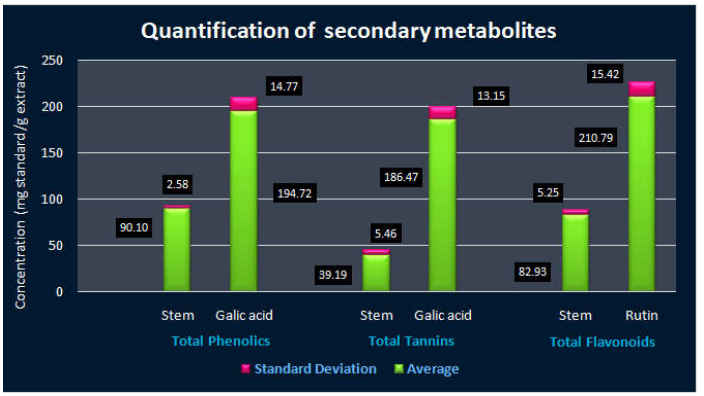

Figure 2. Quantification of secondary metabolites of *I. viscose* stem

Table 1. Secondary Metabolite Screening

S. No.	Chemical Constituent	Tests	Organic solvents			W
			PE	DM	E	
1	Alkaloids	a) Dragendorff's test	–	–	+	–
		b) Mayer's test	–	–	+	+
2	Flavonoids	10% HCl& 5% NaOH test	+	–	+	+
3	Tannins	5% $FeCl_3$ test	–	–	+	+
4	Steroids	Liebermann-Burchard's test	+	+	–	+
5	Triterpenoids	a) Liebermann-Burchard's test	+	–	+	+
		b) Salkowski's test	+	–	–	+
6	Saponins	Foam test	–	–	–	+
7	Glycosides	Keller - Kiliani test	+	–	–	–
8	Gum & Mucilages	Whistler & BeMiller test	+	–	+	+
9	Fixed oils	Spot test	+	–	–	–

* PE – Petroleum Ether; DM – DichloroMethane; E – Ethanol; W – Water

Table 2. Quantification of secondary metabolites of *I. viscosa* stem

Total phenolics (mg GAE / g extract)	Total tannins (mg TAE / g extract)	Total flavonoids (mg RE / g extract)
90.10 ± 2.58	39.19 ± 5.46	82.93 ± 5.25

GAE- gallic acid equivalents; TAE- tannic acid equivalents; RE- rutin equivalents. * Values are mean of triplicate determinations (n=3) ± standard deviation

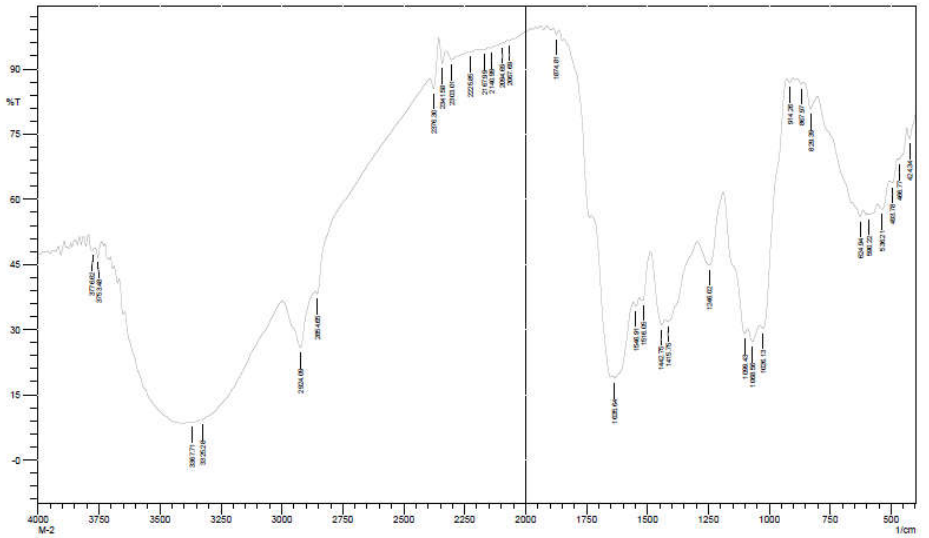

Figure 3. FTIR Spectrum of stem powder of *I. viscosa*

Table 3. FTIR peak values and functional groups

Peak value (Wave number cm-1)	Type of Bond	Functional group
3776.62	N-H rock	Amine
3753.48	O-H stretch	Alcohols
3367.71	O–H stretch, H–bonded	Alcohols, phenols
3325.28	O–H stretch, H–bonded	Alcohols, phenols
2924.09	O–H stretch	Carboxylic acids
2854.65	C-H stretch	Alkanes
2376.30	N-H stretch	Carboxylic acids
2341.58	N-H stretch	Carboxylic acids
2303.01	C=C stretch	Alkynes
2225.85	N-H stretch	Aminoacids
2167.99	C=C stretch	Alkynes
2140.99	–C≡C– stretch	Alkynes
2094.69	C≡N stretch	Nitriles
2067.69	C≡N stretch	Nitriles
1874.81	C=O stretch	Ester
1635.64	N–H bend	1° amines
1546.91	N–O asymmetric stretch	Nitro compounds
1516.05	N–O asymmetric stretch	Nitro compounds
1442.75	C–C stretch	Aromatics
1415.75	C–C stretch	Aromatics
1246.02	C–N stretch	Aliphatic amines
1099.43	C–O stretch	Alcohols, carboxylic acids, esters, ethers
1068.56	C-O stretch	Ethers
1026.13	C–N stretch	Aliphatic amines
914.26	O–H bend	Carboxylic acids
867.97	N–H wag	1°, 2° amines
829.39	C–H “oop”	Aromatics
624.94	–C≡C–H: C–H bend	Alkynes
590.22	C–Br stretch	Alkyl halides
536.21	C–Br stretch	Alkyl halides

FTIR analysis: Infrared technique does not require any reagent, so this is eco-friendly method. It has been proved to be a powerful analytical tool used in many fields (Lin *et al*., 2011). There are several reports available for FTIR studies on medicinal plants (Maruthamuthu and Ramanathan, 2016). FTIR analysis of the present study showed the presence various functional groups in ethanolic stem extract such as amines, alcohols, phenols, carboxylic acids, alkanes, alkynes, aminoacids, nitriles, ester, nitro compounds, aromatics, ethers, 1°, 2° amines and alkyl halides. Which is confirmed by the type of bond of the respective peak values (Figure 3; Table 3). These functional groups conform the presence major bioactive compounds such as alkaloids, tannins, flavonoids, terpenoids and saponins. FTIR spectroscopy of the current research proved that is a reliable and sensitive method for detection of bioactive compounds. The FTIR spectral range is not only used to determine the functional groups of a molecule, but it also provides a characteristic fingerprint data that can be used to uniquely identify the compounds. So, this method is more valuable to evaluate not only the quality and authenticity of powder but also the presence of adulterants if any.

Conclusion

Medicinal plants are highly admired for their diverse ethno pharmacological actions which could be the presence of secondary plant metabolites such as alkaloids, flavonoids, tannins, steroids, terpenoids, saponins, etc. Some of these plants are major source to reduce the high risk of certain acute and chronic diseases such as cancer, inflammation, jaundice, heart diseases, stroke, etc. The present study indicated that the petroleum ether, ethanol and water extracts yielded more number of constituents. Total amount of flavonoids, phenolics and tannins were considerable level in the tested sample. Different functional groups (amines, alcohols, phenols, carboxylic acids, nitro compounds, aromatics, etc.) were identified by FTIR analysis, which provide the fingerprint of the bioactive compounds such as alkaloids, flavonoids, tannins, terpenoids, etc. In addition a direction for further research is necessary to identify the chemical structure of new compounds and evaluate their therapeutic values.

REFERENCES

Agostini-Costa, T.S., Vieira, R.F., Bizzo, H.R., Silveira, D. and Gimenes, M.A. 2012. Secondary Metabolites, Chromatography and Its Applications, Dr. Sasikumar Dhanarasu (Ed.), ISBN: 978-953-51-0357-8, InTech, Available from: http://www.intechopen.com/books/chromatography-and-itsapplications/secondary-metabolites .

Bakasso, S., Lamien-Meda, A., Lamien, C.E., Keindrebeogo, M., Millogo, J.F., Ouedraogo, A.G. and Nacoulma, O.G. 2008. Polyphenol contents and antioxidant activities of five *Indigofera* species (Fabaceae) from Burkina Faso. *Pak. J. Biol. Sci.* Vol. 11; p. 1435-1442.

Camporese, A., Balick, M.J., Arvigo, R., Esposito, R.G., Morsellino, N., De Simone, F. and Tubaro, A. 2003. Screening of anti-bacterial activity of medicinal plants from Belize (Central America). *Journal of Ethnopharmacology*. Vol. 87; p. 103–107.

Chifundera, K. 2001. Contribution to the inventory of medicinal plants from the Bushi area, South Kivu Province, Democratic Republic of Congo. *Fitoterapia*. Vol. 72; p. 351-368.

Colegate, S.M. and Molyneux, R.J. 2007. Bioactive Natural Products: Detection, Isolation, and Structural Determination, 2nd Edition. CRC Press, Boca Raton, Florida, USA. p. 12.

Copeland, S.R. 2007. Vegetation and plant food reconstruction of lowermost Bed II, Olduvai Gorge, using modern analogs. *Journal of Human Evolution*. Vol. 53; p. 146-175.

Croteau, R., Kutchan, T.M. and Lewis, N.G. 2000). Natural products (secondary metabolites). In Buchanan B, Gruissem W, Jones R (Eds.), Biochemistry and molecular biology of plants. Rockville, MD: American Society of Plant Physiologists. p. 1250-1318.

Crozier, A., Clifford, M.N. and Ashihara, H. 2006. Plant Secondary Metabolites and the Human Diet. Blackwell Publishing. Oxford. England.

Das, K., Tiwari, R.K.S. and Shrivastava, D.K. 2010. Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends. *Journal of Medicinal Plants Research*. Vol. 4(2); p. 104-111.

Evans, W. C. 1997. Trease and Evans Pharmacognosy. 14th edition. Harcourt Brace and company, Asia pvt. Ltd., Singapore.

Finar, I.L. 1986. Stereo chemistry and the chemistry of natural products. Vol. 2. Longman, Singapore.

Gautam, B., Vadivel, V., Stuetz, W. and Biesalski, H.K. 2011. Bioactive compounds extracted from Indian wild legume seeds: antioxidant and type II diabetes related enzyme inhibition properties. *Int J Food SciNutr*. Vol. 15; p. 1-4.

Hoareau, L. and Da Silva, E.J. 1999. Medicinal plants: a re-emerging health aid. *Electronic Journal of Biotechnology*. Vol. 2(2); p. 56-70.

Kokate, C.K. 1999. Practical pharmacognosy. 4th Edition. Vallabhprakashan Publication, New Delhi, India.

Lin, H., Zhao, J.W., Sun, L., Chen, Q.S. and Zhou, F. 2011. Freshness measurement of eggs using near infrared (NIR) spectroscopy and multivariate data analysis. *Innovative Food Science & Emerging Technologies*. Vol. 12(2): pp. 182–186.

Mahato, S.B. and Sen, S. 1997. Advances in triterpenoid research, 1990-1994. *Phytochemistry*. Vol. 44(7): p. 1185-1236.

Makkar, H.P.S. 2003. Quantification of tannins in tree and shrub foliage: A laboratory Mannual. Dondrecht. The Netherlands: Kluwer academic publishers. p. 143-154.

Maruthamuthu, R. and Ramanathan, K. 2016. Phytochemical analysis of bark extract of *Cinnamomum verum:* A medicinal herb used for the treatment of coronary heart disease in Malayali tribes, Pachamalai hills, Tamil Nadu, India. *International Journal of Pharmacognosy and Phytochemical Research*. Vol. 8(7): p. 1218-1222.

Neuwinger, H.D. 2000. African traditional medicine: a dictionary of plant use and applications. Medpharm Scientific, Stuttgart, Germany.

Okudu T, Yoshida T, Hatano T. 1994. Food phytochemicals for cancer prevention II. In: Ho, C.T., Osawa, T., Huang, M.T., Rosen, R.T. Chemistry and antioxidative effects of phenolic compounds from licorice, tea and Compositae and Labiateae herbs: Washington, DC: American Chemical Society. p. 132-143.

Pulipati, S., Babu, P.S., Naveena, U., Parveen, S.K.R, Nausheen, S.K.S. and Sai, M.T.N. 2017. Determination of Total Phenolic, Tannin, Flavonoid Contents and Evaluation of Antioxidant Property of *Amaranthus tricolor* (L). *International Journal of Pharmacognosy and Phytochemical Research*. Vol. 9(6); p. 814-819.

Rice-Evans, C.A., Miller, N.J. and Paganga, G. 1996. Structure–antioxidant activity relationships of flavonoids and phenolic acids. Free Radic Biol Med. Vol. 20; p. 933-956.

Roopashree, T.S, Dang, R., Rani, S.R.H. and Narendra, C. 2008. Antibacterial activity of anti-psoriatic herbs: *Cassia tora, Momordica charantia* and *Calendula officinalis*. *International Journal of Applied Research in Natural Products*. Vol. 1(3); p. 20-28.

Segelman, A.B., Farnsworth, N.R. and Quimby, M.D. 1969. False negative saponins test results induced by the presence of tannins. *Lloydia*. Vol. 32; p. 52-58.

Stuart, B.H. 2004. Infrared Spectroscopy: Fundamentals and Applications. Volume 14 of Analytical Techniques in the Sciences (AnTs). John Wiley and Sons Ltd., Chichester, West Sussex, UK. p. 28-29.

Tepe, B., Sokmen, M., Akpulat, H.A. and Sokmen, A. 2006. Screening of the antioxidant potentials of six *Salvia* species from Turkey. *Food Chem.* Vol. 95; p. 200-204.

Trease, G.E. and Evans, W.C. 2002. Pharmacognosy. 15th Edition. W. B. Sanders Company Limited, London. p. 001-585.

Waldi, D. 1965. Spray Reagents for Thin Layer Chromatography. In: Egonstahl (Ed). *Thin layer chromatography –A- Laboratory Hand book.* Acadameic press Inc., Publishers, New York, U S A.

Whistler R.L. and BeMiller J.N. 1993. Industrial Gums; Polysacchraida and their Derivatives. Acadamic Press, London, U.K.

Zhao, J., Davis, L.C. and Verpoorte, R. 2005. Elicitor signal transduction leading to production of plant secondary metabolites. *Biotechnology Advances*. Vol. 23(4); p. 283–333.

Zhishen, J., Mengcheng, T. and Jianming, W., 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry*. Vol. 64; p. 555–559.

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