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Variation of Phenolics, Antioxidant Activity and Carotenoids Contents in Some Medicinal Plants

H.M. Munjur Murshed, Md. Nesar Uddin, M. Ashrafuzzaman✉

Department of Crop Botany, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh

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Correspondence
M. Ashrafuzzaman
✉: drashraf2007@yahoo.com

ABSTRACT

Plants being important source of medicine play significant role in human health. Total phenolics, free radical scavenging capacity and carotenoids contents in six medicinal plants [Peltata (*Cyclea peltata*), Pudina (*Mentha piperita*), Bon tulsi (*Ocimum americanum*), Kalo tulsi (*Ocimum sanctum*), Akanadi (*Stephania japonica*) and Gulancha (*Tinospora cordifolia*)] from two families (Lamiaceae and Menispermaceae) available at the Bangladesh Agricultural University botanical garden were studied in the present experiment. Total phenolics content in the six medicinal plants ranged from 340.03 (*M. piperita*) to 890.58 (*O. americanum*) mg GAE 100 g^{-1} leaf fresh weight. The IC_{50} value for scavenging 2, 2- diphenyl-l- picrylhydrazyl (DPPH) free radicals ranged from 3.27 (*O. americanum*) to 57.85 (*T. cordifolia*) mg mL^{-1} leaf extract and carotenoid content was maximum in *M. piperita* leaf (0.380 mg g^{-1} fresh weight) among the six test species. The high content of phenolics in *O. americanum* represents the plant species as an important natural source of antioxidants with high potential value for drug preparation.

Introduction

Medicinal plants contain a complex set of chemical substances that influence the human and other organisms in different and versatile ways. Many secondary metaboites (Islam *et al*., 2018a; Islam *et al*., 2018b), anti-inflammatory, antimicrobic, spasmolytic, and neuroprotective actions (Rice-Evans *et al*., 1997). The medicinal plants selected for the present investigation such as Peltata (*C. peltata*), Pudina (*M. piperita*), Bon tulsi (*O. americanum*), Kalo tulsi (*O. sanctum*), Akanadi (*S. japonica*) and Gulancha (*T. cordifolia*) have long been used in the folk medicine due to their potential health promoting and pharmacological attributes. The juice of C. *peltata* leaves is applied to abscesses and decoction is given in dysentery (Kirubha *et al*., 2012). The leaves of *C. peltata* are being used traditionally as coolant, antidandruff, antipyretic and diuretic drug (Kingston *et al*., 2007; Bhandary *et al*., 1995). The paste prepared from leaves with water is taken orally along with cow's milk two times a day for a period of two days to get relief from lumbago (Lalitha *et al.,* 2011).

M. piperita L., commonly known as pudina in Bangla, a medicinally important plant belongs to the Family Lamiaceae. This family is a rich source of polyphenolic compounds and hence could possess strong antioxidant properties (Bimakr *et al.*, 2011). Multiple biological activities of *Peppermint* essential oil and extract might be ascribed to the presence of some components, such as flavonoids, phenols, carotenes, betaine, choline, tannins and volatile oil composed primarily of menthol, menthone, menthofuran and menthyl acetate (Leung, 1980).

O. sanctum L. has also been suggested to possess anti-fertility, anti-cancer, anti-diabetic, anti-fungal, anti-microbial, hepatoprotective, cardioprotective, anti-emetic, anti-spasmodic, analgesic, adaptogenic and diaphoretic action (Prakash and Gupta, 2005). With regard to folk medicine, *O. americanum* is used to treat insomnia, constipation, cough, and microbial infections (Yucharoen *et al.*, 2011).

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S. japonica L. belongs to the family Menispermaceae, a slender wiry climber or twining shrub is widely used in the traditional medicine of Bangladesh in treatment of a wide range of diseases and disorders including inflammation, cancer, asthma, fever, sleep disturbance, edema, and bone fracture (Jahan *et al*., 2010). Especially its leaves, which are extensively used to treat different kinds of painful conditions, more specifically, the crushed leaves for body pain (Seraj *et al*., 2013; Jahan *et al.*, 2010) and warmed leaves for rheumatism (Rahman *et al*., 2007).

T. cordifolia or Gulancha finds a special mention for its use in folk and tribal medicine in different parts of the country since time immemorial. It has been shown to possess anti-allergic (Nayam palli *et al*., 1986), anti-diabetic (Wadood *et al*., 1992), anti-hepatotoxic and anti-pyretic (Rege *et al.,* 1984) properties.

Analyzing these plants for their total soluble phenolics, carotenoids and anti-oxidative power to scavenge free radical will provide a better understanding of these medicinal plants. Keeping this view in mind the present study, therefore, was conducted to assess and compare total phenolics and carotenoids contents in above mentioned medicinal plants; and to determine free radical scavenging ability of the plant extracts.

Materials and Methods

Plant material

The medicinal plants [*C. peltata* (Menispemaceae), *M. piperita* (Lamiaceae), *O. americanum* (Lamiaceae), *O. sanctum* (Lamiaceae), *S. japonica* (Menispemaceae) and *T. cordifolia* (Menispemaceae)] available at the Bangladesh Agricultural University Botanical Garden were choosen for the study.

Sample collection

For each sample three different healthy plants from three different colonies were selected and considered as three replicates. Tender leaves from the selected plants and colonies were collected in the morning and immediately placed in zip lock bags with proper tagging to avoid moisture loss. Leaf samples were immediately brought to the laboratory for chemical analyses. The collected leaves were chopped into small pieces separately to produce working samples for analysis.

Total phenolics content assay

Total phenolics were assayed with the method modified after Albano and Miguel (2011). Exactly 5 g of the working samples for leaf were taken to a 250 mL beaker and 100 mL ice cooled methanol were added to it and then each sample was homogenized for 2 minutes using a Homogenizer (model- OV-5 VELP, Italy). The mixture was kept in dark condition for 30 minutes and centrifuged for 5 minutes at 1500 rpm and then its supernatant was treated as working sample extract. An aliquot amount of the extract was used for determining total phenolics content or DPPH scavenging activity. Gallic acid was used here as standard. Exactly 330 µL from different concentrations of gallic acid solutions or suitable amount of plant extracts were taken into a 50 mL test tube. Then 0.16 mL of Folin-Ciocalteu reagent and 3 mL of Na_2CO_3 (10%) solution was added to 1 mL of gallic acid solution. The mixture was kept in dark condition for half an hour at room temperature (25°C). Then absorbance was measured at 760 nm. The absorbance value is the reflection of the total phenolics content in the sample. After plotting the absorbance in ordinate against the concentration a linear relationship was obtained which was used as a standard curve for the determination of the total phenolics content of the test samples.

DPPH radical scavenging assay

Free radical scavenging activity of the plant extracts was determined by using a stable 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) (Brand-Williams et al., 1995). DPPH is a free radical of violet colour. The antioxidants in the sample scavenge the free radicals and turn it into yellow colour. The change of colour from violet to yellow is proportional to the radical scavenging activity. Briefly, the assay contained 2.7 mL of 0.1 mM DPPH in methanol and made up to 3 mL with 300 µL plant extracts (working sample). The contents were mixed well immediately and then incubated for 30 min at room temperature (25°C). The degree of reduction of absorbance was recorded at 517 nm using DR 6000 UV Spectrophotometer.

The percentage of scavenging activity was calculated as:
(Ac – As)/ Ac × 100

where 'Ac' is the absorbance of control (without extract) and 'As' is the absorbance of sample with plant extract. Percentage of radical scavenging activity was plotted against the corresponding concentration of the extract to obtain IC_{50} value. IC_{50} is defined as the amount of antioxidant material required to scavenge 50% of free radical in the assay system. The IC_{50} values are inversely proportional to the antioxidant activity (Nisha *et al*., 2009).

Total carotenoids

Total carotenoids can be determined in a whole pigment extract of green plant tissue by spectrophotometer (Lichtenthaler, 1987). From the fresh composite leaf sample, 50 mg were taken in a glass bottle and 200 µL distilled water were added to it. Then 16 mL ethanol

were added and shaken properly and finally, the content was kept in dark condition for 24h. Absorbance reading was taken in the following day in a spectrophotometer (DR 6000, Hach, USA) at 470, 649, 664 and 750 nm wave lengths. Afterward, amount of total carotenoids (sum of carotene and xanthophyll) were calculated using the following formulae:

Carotenoids

(Cx+c) = (4.785 A470+3.657 A664−12.76A649) ×16.2/FW where, A649 = Absorbance at 649 nm; A664 = Absorbance at 664 nm; A470 = Absorbance at 470 nm; and FW = Fresh weight of plant tissue (mg).

Statistical analyses

Mean values of each parameter studied for the six different medicinal plants were subjected to one-way ANOVA analysis using Minitab 17.3 to determine whether significant differences among the means existed or not. In case of having significant *F-ratio,* means were subjected to Tukey's post-hoc test to observe the significant differences among the mean values.

Results and Discussion

Total soluble phenolics

Methanolic extracts of leaves from six medicinal plants viz. *C. peltata*, *M. piperita*, *O. americanum*, *O. santum*, *S. japonica* and *T. cordifolia* were tested to determine total phenolics as gallic acid equivalent (GAE) per 100 g fresh weight (Fig. 1A). Phenolics content in leaves varied significantly among the tested plants and it ranged from 340.03 to 890.58 mg GAE 100 g^{-1} FW fresh weight (Fig. 1A). Leaf extracts of *O. americanum* contained the highest amount of soluble phenolics (890.58 mg GAE 100 g^{-1} FW) followed by in *C. peltata* (680.07 mg GAE 100 g^{-1} FW) in the same statistical rank and it represents that the leaves of those plants have maximum amount of soluble phenolic compounds. The lowest amount of phenol was detected in *M. piperita* (340.03 mg GAE 100 g^{-1} FW) leaves. Considering the phenolic content of *M. piperita* (340.03 mg GAE 100 g^{-1} FW) as 100%, the relative phenolic contents in *T. cordifolia, S. japonica, C. peltata, O. americanum and O. sanctum* were 245.8, 206.9, 200, 261.9 and 170%, respectively. In this study total phenolics content in the methanolic extracts of leaf was found in the order as: Pudina (*M. piperita*) < Kalo tulsi (*O. sanctum*) < Peltata (*C. peltata*) < Akanadi (*S. japonica*) < Gulancha (*T. cordifolia*) < Bon tulsi (*O. americanum*) (Fig. 1A).

Carotenoid content

Total carotenoids content in leaves varied widely among the tested plants and ranged from 0.160 to 0.380 mg g^{-1} FW (Fig. 1B). *M. piperita* leaf extract contained the highest amount of carotenoids (0.380 mg g^{-1} FW) followed by the second highest in *T. cordifolia* (0.296 mg g^{-1} FW). The carotenoids content was minimum in *O. americanum* leaf (0.160 mg g^{-1} FW) which was statistically in similar rank to *S. japonica* (0.189 mg g^{-1} FW). The leaves of other species showed the carotenoids content in the leaf extract of *Cyclea peltata* (0.257 mg g^{-1} FW) and *Ocimum americanum* (0.233 mg g^{-1} FW) were intermediate. Based on the carotenoids content in *M. piperita* leaf extract as 100%, the relative carotenoids in *T. cordifolia, S. japonica, C. peltata*, *O. americanum* and *O. sanctum* were 77.8, 49.7, 67.63, 42.10 and 61.31%, respectively.

DPPH radical scavenging capacity

The IC_{50} value (the amount of antioxidant material required to scavenge 50% of free radical in the assay system) of leaf extract to scavenge DPPH radical varied significantly among the tested plants and ranged from 3.27 (Bon tulsi) to 57.85 mg mL^{-1} (Gulancha) leaf methanolic extract (Fig. 1C). Bon tulsi, pudina and kalo tulsi had the lowest and shared statistically identical IC_{50} values for leaf methanolic extracts to scavenge DPPH. Considering IC_{50} values of leaf extract from *M. piperita* (3.70 mg mL^{-1}) to scavenge DPPH as 100%, the relative IC_{50} of *T. cordifolia, S. japonica, C. peltata*, *O. americanum* and *O. sanctum* were 1563.5, 477.0, 845.4, 88.4 and 107.3 %, respectively. The DPPH antioxidant assay is based on the ability of DPPH, a stable free radical, to decolorize in the presence of antioxidant DPPH stable free radical method and it is a sensitive way to determine the antioxidant activity of plant extracts. The IC_{50} values are inversely proportional to the antioxidant activity of plant extract.

This study was performed to determine antioxidant activity, total phenol and carotenoids contents of some selected medicinal plants that are traditionally being used as folk medicine. Among the six medicinal plants studied Gulancha (*Tinospora cordifolia*), Akanadi (*Stephania japonica*) and] and Peltata (*Cyclea peltata*) were from Menispermaceae family while Pudina (*Mentha piperita*), Bon tulsi (*Ocimum americanum*) and Kalo tulsi (*Ocimum sanctum*) were from Lamiaceae. Both families are famous for their therapeutic effects. In our study, all three Lamianceae species exhibited statistically very high potency to scavenge the DPPH free radicals as compared to the other three species from Menispermaceae. This is a strong indication that plants from Lamiaceae possess relatively higher amounts of antioxidants that scavenge the free radicals. The strong antioxidant activities of *O. americanum* and *M. piperita* leaf's methanolic extract could be attributed to their richness with phenolics and/or carotenoids (Fig. 1).

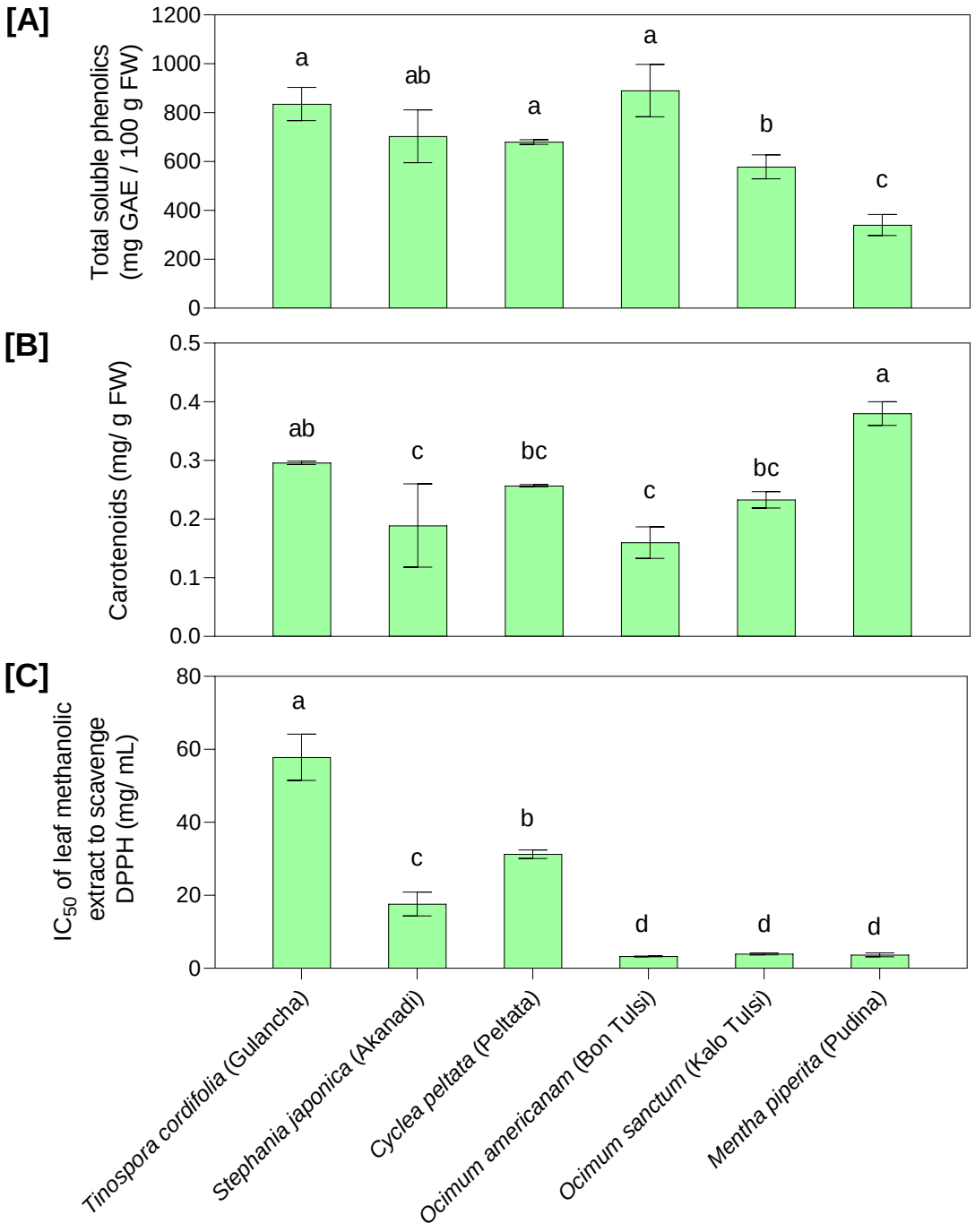

Figure 1. Variation in [A] phenolics content [B] carotenoids content and [C] DPPH radical scavenging capacity of the leaf extracts of six different medicinal plants. Each data point is the average of three replicates ± SEM. Bars sharing different letters are significantly different from each other at $P \leq 0.05$.

This Lamiaceae family in general is a rich source of polyphenolic compounds and hence could possess strong antioxidant properties (Bimakr *et al.*, 2011; Li et al., 2008). *Cyclea peltata* which has repeatedly been termed as a medicinal plant in the folk medicine is used in this study. A 70% methanolic leaf extract of *Cyclea peltata* has been shown to protect against cisplatin-induced renal toxicity and oxidative damages (Vijayan *et al*., 2007). Carotenoids act as photoprotective agents and may reduce the risk of sunburns, photoallergy and even some types of skin cancer. In this study, pudina leaf extract had highest quantity of carotenoids content indicating that *M. piperita* leaf is a good source of natural carotenoids and it can be a promising source for use in pharmacological products designed for antioxidant activity.

Conclusion

Results suggest that the species *O. americanum* (Bon tulsi) contained the highest amount of phenolics in its leaves. The highest radical scavenging ability was detected in *O. sanctum* (Kalo tulsi) and the lowest in *T. cordifolia* (Gulancha). *M. piperita* (Pudina) leaf contained the highest amount of carotenoids. Among the six medicinal plant species Gulancha, Peltata *and* Akanadi possessed low antioxidative activities while Pudina, Kalo tulsi, Bon tulsi were ranked as the highest i.e. the latter plants have great value in pharmacy and phytotherapy.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

Albano, S.M., Miguel, M.G. 2011. Biological activities of extracts of plants grown in Portugal. *Industrial Crops and Products*, 33: 338-343. https://doi.org/10.1016/j.indcrop.2010.11.012

Bhandary, M.J., Chandrashekar, K.R., Kaveriappa, K.M. 1995. Medical ethnobotany of the Siddis of Uttara Kannada district, Karnataka, India. *Journal of Ethnopharmacology*, 47: 149–58. https://doi.org/10.1016/0378-8741(95)01274-H

Bimakr, M., Rahman, R.A., Taip, F.S., Ganjloo, A., Salleh, L.M., Selamat, J., Hamid, A., Zaidul, I.S.M. 2011. Comparison of different extraction methods for the extraction of major bioactive flavonoid compounds from spearmint (*Mentha spicata* L.) leaves. *Food and bioproducts processing,* 89: 67–72. https://doi.org/10.1016/j.fbp.2010.03.002

Brand-Williams, W. Cuvelier, M.E., Berset, C.L.W.T. 1995. Use of a free radical method to evaluate antioxidant activity. *Food Science and Technology*, 28(1): 25−30. https://doi.org/10.1016/S0023-6438(95)80008-5

Islam, R., Injamum-Ul-Hoque, M., Ashrafuzzaman, M., Uddin, M.N. 2018a. Leaf phenolics and pigments along with their antioxidative potential in some medicinal plants. Asian-Australasian Journal of Food Safety and Security. 2(2): 86-92.

Islam, R., Uddin, M. N., Ashrafuzzaman, M., Injamum-Ul-Hoque, M. 2018b. Phenolics and carotenoids contents and radical scavenging capacity of some selected solanaceous medicinal plants. Journal of Bangladesh Agricultural University. 16(1): 56–61. https://doi.org/10.3329/jbau.v16i1.36481

Jahan, R., Khatun, M.A., Nahar, N., Jahan, F.I., Chowdhury, A.R., Nahar, A., Seraj, S., Mahal, M. J., Khatun, Z., Rahmatullah, M., 2010. Use of Menispermaceae family plants in folk medicine of Bangladesh. *Advances in Natural and Applied Sciences,* 4(1): 1-9

Kapur, P., Jarry, H., Wuttke, W., Pereira, B.M., Seidlova-Wuttke, D. 2008. Evaluation of the antiosteoporotic potential of *Tinospora cordifolia* in female rats. *Maturitus*, 59(4): 329–338. https://doi.org/10.1016/j.maturitas.2008.03.006

Khoddami, A. Wilkes, M.A. Roberts T.H. 2003. Techniques for analysis of phenolic compounds. *Molecules,* 18: 2328- 2375. https://doi.org/10.3390/molecules18022328

Kingston, C., Nisha, B.S., Kiruba, S., Jeeva, S. 2007. Ethnomedicinal plants used by indigenous community in traditional healthcare system. *Ethnobotanical Leaflets*, 11: 32–7.

Kirubha, T.S.V., Senthamarai, R., Mariya, P. Mani, P. 2012. Pharmacognostical and phytochemical standards of *Cyclea peltata* (Lam) Hook.f & Thomson leaves. *Journal of Chemistry Pharmaceutical Research*, 4(3): 1465–1469.

Lalitha, S., Devi, V.K. 2011. Ethnomedicinal plants used by Kanikkars of Agasthiarmalai Biosphere Reserve, Western Ghats. *Journal of Ecobiotechnology*, 3(7): 16–25.

Leung, A.N. 1980. Encyclopedia of common natural ingredients used in food, drugs, and cosmetics. Wiley, New York.

Li, H.B., Wong, C.C., Cheng, K.W., Chen, F. 2008. Antioxidant properties in vitro and total phenolic contents in methanol extracts from medicinal plants. *LWT - Food Science and Technology*, 41(3): 385–390. https://doi.org/10.1016/j.lwt.2007.03.011

Lichtenthaler, H.K. 1987. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. *Methods in Enzymology*, 148: 350−382. https://doi.org/10.1016/0076-6879(87)48036-1

Makker, P.N., Tait, L. and Shekhar M.P.V. 2007. Inhibition of breast tumor growth and angiogenesis by a medicinal herb *Ocimum gratissimum*. *International Journal Cancer,* 121: 884–894. https://doi.org/10.1002/ijc.22733

Miguel, M.G. 2010. Antioxidant activity of medicinal and aromatic plants. *Journal of Flavour Fragrance*, 25: 291–312. https://doi.org/10.1002/ffj.1961

Nayampalli, S.S., Desai, N.K., Ainapure, S.S. 1986. Anti-allergic properties of *Tinospora cardifolia* in animal models. *Indian Journal of Pharmacology*, 18: 250–252.

Ngassoum, M.B., Ousmaila, H., Ngamo, L.T., Maponmetsem, P.M., Jirovetz, L., Buchbauer, G. 2004. Aroma compounds of essential oils of two varieties of the spice plant *Ocimum canum* Sims from northern Cameroon. *Journal Food Composition and Analysis,* 17: 197–204. https://doi.org/10.1016/j.jfca.2003.08.002

Nisha, P. Abdul Nazar, P., Jayamurthy, P. 2009. A comparative study on antioxidant activities of different varieties of *Solanum melongena. Food and Chemical Toxicology,* 47(10): 2640–2644. https://doi.org/10.1016/j.fct.2009.07.026

Prakash, P., Gupta, N., 2005. Therapeutic uses of *Ocimum sanctum* L. (Tulsi) with a note on eugenol and its pharmacological action. *Indian Journal of Physiology and Pharmacology,* 49(2): 125–131

Rahman, M.H., Alam, M.B., Chowdhury, N.S., Jha, M.K., Hasan, M., Khan, M.M., Rahman, M.S., Haque, M.E. 2011. Antioxidant, analgesic and toxic potentiality of *Stephania japonica International Journal of Pharmacology*, 7: 257–262. https://doi.org/10.3923/ijp.2011.257.262

Rege, N.N., Dahanukar, S.A., Karandikar, S.M. 1984. Hepatoprotective effects of *Tinospora cordifolia* against carbon tetrachloride induced liver damage. *Indian Drugs*, 21: 544–555.

Rice-Evans, C.A., Miller, N.J., Paganga, G. 1997. Antioxidant properties of phenolic compounds. *Trends in Plant Science*, 2(4): 152–159. https://doi.org/10.1016/S1360-1385(97)01018-2

Sanchez, C. 2002. Methods used to evaluate the free radical scavenging activity in foods and biological systems. *Food Science and Technology International*, 8: 121–137. https://doi.org/10.1177/1082013202008003770

Seraj, S., Jahan, F.I., Chowdhury, A.R., Monjur-E-Khuda, M., Khan, M.S.H., Aporna, S.A., Jahan, R., Samarrai, W., Islam, F., Khatun, Z., Rahmatullah, M. 2013. Tribal formulations for treatment of pain: a study of the *bede* community traditional medicinal practitioners of porabari village in Dhaka district *Bangladesh. African Journal of Traditional, Complement and Alternative Medicine*, 10: 26–34. https://doi.org/10.4314/ajtcam.v10i1.5

Sores, J.R., Dinis, T.C.P., Cunha, A.P., Almeida, L.M. 1997. Antioxidant activities of some extracts of *Thymus zygis. Free Radical Research*, 26: 469–478. https://doi.org/10.3109/10715769709084484

Vijayan, F.P., Rani, V.K., Vineesh, V.R., Sudha, K.S., Michael, M.M., Padikkala, J. 2007. Protective effect of *Cyclea peltata* Lam on cisplatin-induced nephrotoxicity and oxidative damage. *Journal of Basic and Clinical Physiology and Pharmacology.* 18**:** 101–114. https://doi.org/10.1515/JBCPP.2007.18.2.101

Wadood, N., Wadood, A., Shah, S.A. 1992. Effect of *Tinospora cordifolia* on blood glucose and total lipid levels of normal and alloxandiabetic rabbits. *Planta Medica*, 58: 131–136. https://doi.org/10.1055/s-2006-961414

Yucharoen, R. Anuchapreeda, S., Tragoolpua, Y. 2011. Anti-herpes simplex virus activity of extracts from the culinary herbs *Ocimum sanctum* L., *Ocimum basilicum* L. and *Ocimum americanum* L. *African Journal of Biotechnology*, 10: 860–866.