Print ISSN : 0972-8813
e-ISSN : 2582-2780

[Vol. 21(1) January-April 2023]

Pantnagar Journal of Research

(Formerly International Journal of Basic and Applied Agricultural Research ISSN : 2349-8765)

G.B. Pant University of Agriculture & Technology, Pantnagar

PANTNAGAR JOURNAL OF RESEARCH

Vol. 21(1) January-April 2023

CONTENTS

Quantitative estimation of chlorophyll and caretenoid contents in endangered medicinal plants *Gentiana kurroo* Royle and *Swertia chirayita* (Roxb.) H. Karst

NEETIKA NAUDIYAL and VANDANA A. KUMAR

Department of Biochemistry, College of Basic Sciences and Humanities, G.B. Pant University of Agriculture and Technology, Pantnagar-263145 (U. S. Nagar, Uttarakhand)
Corresponding author's email id: 52522.neetika.naudiyal@gbpuat-cbsh.ac.in

ABSTRACT: *Gentiana kurroo* Royle and *Swertia chirayita* (Roxb.) H. Karst., commonly found in the Indian Himalayan Region have been listed into the category of critically endangered medicinal plants. Considering the important role of chlorophyll and carotenoid contents in plant's photosynthetic ability, the present study focuses on solvent extraction methods to compare photosynthetic pigment content in *G. kurroo* Royle and *S. Chirayita* (Roxb.) H. Karst. A higher chlorophyll and carotenoid contents in *S. chirayita* (Roxb.) H. Karst. was observed than that of *G. kurroo* Royle. Extraction using DMSO and methanol showed a higher content than that with water. A variation in values was observed in both the plants as they grow at different elevations implying a difference in biochemical components of the plants and requirement of different solvents for extraction.

Key words: Carotenoid, chlorophyll, extraction, *Gentiana kurroo* Royle, *Swertia chirayita* (Roxb.) H. Karst.

Abbreviations: Chl a= Chlorophyll a, Chl b= Chlorophyll b

Human beings have been interested in medicinal herbs due to their pharmacological relevance and have been using therapeutic plants growing in their natural environments since the beginning of mankind (Shukla *et al.*, 2010). It has been incredibly common to look for natural products possessing bioactive components and, therefore, the search of therapeutic plants has led to novel medication discoveries and has well been evidenced by vast literature. The use of herbals, particularly therapeutic plants, has long served as a general gauge of ecosystem health (Singh, 2002). *Gentiana kurroo* Royle, a critically endangered medicinal plant often known as "Karu" (due to the bitter nature of its roots), grows between 1500 and 5000 meters above sea level in the mid Himalayas (Skinder *et al.*, 2017). *Swertia chirayita* (Roxb.) H. Karst. commonly known as "Chiretta", is a critically endangered plant very well documented since ages growing at high elevations in the sub-temperate parts of the Himalayas between 1200 and 2100 metres from Kashmir to Bhutan (Clarke, 1885) in moist shady patches (Gaur,1999). The primary bioactive elements responsible for pharmacological relevance to both these plants, include Iridoids (gentiopicroside, swertiamarin, and sweroside), Xanthones (isogentisin, bellidifolin and mangiferin) and Flavonoids like isovitexin and isoorientin (Menkovic *et al.*, 2010; Mustafa *et al.*, 2015; Öztürk *et al.*, 2006; Schaufelberger and Hostettmann, 1988; Wu *et al.*, 2017; Zhao *et al.*, 2013).

Plants have an inherent photosynthetic capacity to convert solar energy into chemical energy in the presence of chlorophylls and accessory pigments. Chlorophylls found in higher plants, cyanobacteria, various photosynthetic algae, dinoflagellates are categorized as chlorophyll a, b, c, d, e, and f; are prenyl lipids having a porphyrin head and a phytol tail. The primary pigment chlorophyll a is bluish green (chl-a; $C_{55}H_{72}N_4O_5Mg$; λ_{max}= 647 nm), whereas the auxiliary pigment chlorophyll b is yellowish green (chl-b; $C_{55}H_{70}N_4O_6Mg$; λ_{max}= 664 nm) (Hartzler *et al.*, 2014; McCartney-Melstad *et al.*, 2018; Rebeiz, 2014). Depending on the type of plant, the habitat, the age of leaves, these two pigments are present in a ratio ranging between 2 to 5 (Parry *et al.*, 2014; Prasann *et al.*, 2018; Rebeiz, 2014). Due to its sandwiching between protein and lipid layers of the chloroplast lamellae where porphyrin is protein bound and tail is lipid bound, the chlorophyll molecule is insoluble in water but soluble in organic solvents (Palta, 1990). The

dynamic balance of photosynthetic pigments aids in the cell's ability to maintain photostasis (Huner *et al.* 1998). The chl-a to chl-b ratio in terrestrial plants has been used as a measure of their sensitivity to light or shade conditions (Porra, 1991; Vicas *et al.* 2010). Photosynthetic ability of a plant is determined by the total chlorophyll content (chl-a+chl-b) and chl-a/b ratio (Croft *et al.*, 2017). The chlorophylls are recognized as antioxidants, anti-inflammatory, and anti-cancer agents because of their positive effects on health and are also used for food colourings and in cosmetics (Feng *et al.*, 2017; Viera *et al.*, 2019). The low fraction of chl-a/b is a sensitive biomarker of pollution and environmental stress (Tripathi and Gautam, 2007). An important group of accessory pigments in photosynthesis *i.e.*, carotenoids transmit the absorbed light energy to chlorophyll pigments (Palett and Young, 1993). Carotenoids are non-polar pigments which are essential for photoprotection as they inactivate the reactive oxygen species (ROS) generated on light exposure. Structurally they belong to the terpenoid-class pigments, and have heavily conjugated polyene chains which gives them distinctive colours like red, orange, purple, or yellow. Carotenoids absorb visible light primarily between 400 and 500 nm (Britton, 2008). They can be divided into two categories: one carotenes (carotene and lycopene), which are unsaturated hydrocarbons and secondly xanthophylls (lutein, zeaxanthin, and fucoxanthin), which essentially possess at least one oxygen containing functional group on a structure similar to carotenes (Poojary *et al.*, 2016). Carotenoids, due to their antioxidant nature, also have a role in prevention of cancer and cardiovascular diseases (Sangeetha and Baskaran, 2010). The plant's photosynthetic ability is correlated with the amount of carotenoid and chlorophylls **(**Gamon and Surfus, 1999**)**.

Various methods have been employed for chlorophyll extraction from plants. The extraction of pigments is routinely done using a mortar and pestle with organic solvents like acetone or dimethyl formamide and is quantified using well-established formulae (Arnon 1949; Porra *et al.*, 1989). Polar aprotic solvents like acetone, dimethyl sulfoxide (DMSO), and N, N-Dimethylformamide (DMF) as well as polar protic solvents like ethanol and methanol have been used extensively in solvent extraction techniques (Lichtenthaler and Buschmann, 2001). Numerous researchers have thoroughly examined the chlorophylls' absorption coefficients in various organic solvents and proposed concurrent equations to estimate the chlorophyll concentrations (Cole *et al.*, 2019; Masaló and Oca, 2020; Negrão *et al.*, 2017; Özkan and Bilek, 2015; Porra and Scheer, 2019; Ritchie, 2006). Pure chl-a and chl-b absorption coefficients have been calculated for a variety of solvents, and these coefficients have been found to be solvent dependent (Prasann *et al.*, 2018; Saito *et al.*, 2018). Standardization of chlorophyll extraction becomes cumbersome due to content difference within leaves and other parts. Other factors like temperature, light solvent polarity and solution's pH make the standardization protocol more complex (Campbell *et al.*, 2019).

Considering the important role of chlorophyll and carotenoid contents in plant's photosynthetic ability, the present study focuses on solvent extraction methods to compare photosynthetic pigment content in the critically endangered multi utility therapeutic plants *G. kurroo* Royle and *S. Chirayita* (Roxb.) H.Karst.

MATERIALS AND METHODS

Plant Material: Present investigation was conducted in the Department of Biochemistry, College of Basic Sciences and Humanities, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand. The plant material of *G. kurroo* Royle and *S. chirayita* (Roxb.) H. Karst. was collected from mid Himalayan hills at Gaurikund and High-Altitude Nursery of Herbal Research and Development Institute (HRDI), Mandal, Gopeshwar, respectively, situated in Chamoli district, Uttarakhand. The herbarium specimen of the plant was submitted at the G.B. Pant University of Agriculture and Technology, Pantnagar. Dr. D.S. Rawat, Assistant Professor and Plant Taxonomist, Department of Biological Science, C.B.S.H., G.B.P.U.A.&T, helped in establishing the identity

of *Gentiana kurroo* Royle (voucher number GBPUH- 1512/28-12-2022) and *Swertia chirayita* (Roxb.) H. Karst. (voucher number GBPUH- 1511/ 28-12-2022).

Chlorophyll estimation

a) Chlorophyll estimation using dimethyl sulphoxide (Hiscox and Israelsham, 1979)

The fresh leaves (50mg) of *G. kurroo* Royle and *S. chirayita* (Roxb.) H. Karst. after cutting into small pieces were suspended in 10 mL DMSO and kept in water-bath for 3h at 60^{0}C. This mixture after keeping at room temperature for 1h was decanted and absorbance of the solution (leaf extract) was taken at 663 and 645nm against DMSO blank. The carotenoid content was estimated using the same leaf extract by measuring the absorbance at 480nm and quantified as per formulas in Table 1. All the quantifications were carried out in triplicate.

b) Chlorophyll estimation using methanol and water (Longjam *et al.*, 2018)

The fresh leaves (0.5g) of *G. kurroo* Royle and *S. chirayita* (Roxb.) H. Karst. were cut into small pieces and ground in mortar pestle with 10 mL of different solvents (methanol and water). The samples were kept in the freezer for 2h. The samples were again ground and then kept for centrifugation at 10,000 rpm for 20min at 4^{0}C. The supernatant was separated and 0.5mL of it was mixed with 4.5mL of the respective solvent. The absorbance of this mixture was measured at different wavelengths for estimation of Chl a, Chl b and carotenoids using the formula as given in Table 2. All the quantifications were carried out in triplicate.

RESULTS AND DISCUSSION

It was observed in the present study that the leaves of *S. chirayita* (Roxb.) H. Karst. possessed higher contents of Chl a, b and carotenoid in comparison to those of *G. kurroo* Royle in all the three solvents (Table 3). Li *et al.* (2018) reported that latitude, climate, soil, and phylogeny were some factors that caused a variation in chlorophyll content in plant species.

The efficacy of extraction of Chl a, which was more non-polar than other pigments followed the order of DMSO>methanol>water for both species. The extraction efficacy for Chl b in *S. chirayita* (Roxb.)

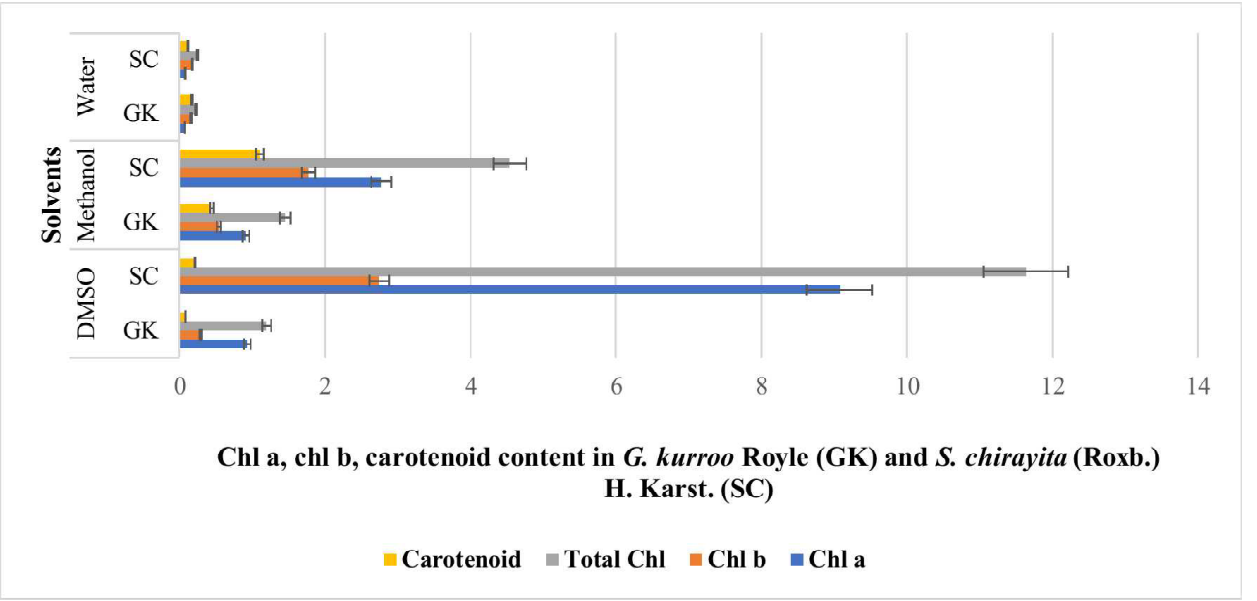

Fig. 1: Chl a, Chl b and carotenoid content in different leaf extracts of *G. kurroo* Royle and *S. chirayita* (Roxb.) H. Karst. [Units: DMSO: (mg g^{-1} fresh wt.); methanol and water: (µg ml^{-1})]

Table 1: Formula to quantify Chl a, Chl b and carotenoid (mg g^{-1} fresh wt.) by DMSO

Chl a (mg g^{-1} fresh wt.)	$(12.7 \times A_{663} - 2.69 \times A_{645})\ V \div 1000 \times W$
Chl b (mg g^{-1} fresh wt.)	$(22.9 \times A_{645} - 4.48 \times A_{663})\ V \div 1000 \times W$
Total chl (mg g^{-1} fresh wt.)	$(20.2 \times A_{645} + 8.02 \times A_{663})\ V \div 1000 \times W$
Carotenoid (mg g^{-1})	$(A_{480} + 0.11 \times A_{663} - 0.638 \times A_{645})\ V \div 1000 \times W$

where, A = Absorbance of chlorophyll extract at specific indicated wavelengthV = Final volume of the sample; W = Weight of tissue extracted on fresh weight basis

Table 2: Formula to quantify Chl a, Chl b and carotenoid (µg mL^{-1}) by different extraction solvents

Extraction in methanol	Extraction in water
Chlorophyll a = $15.65 \times A_{666} - 7.340 \times A_{653}$	Chlorophyll a=$12.7 \times A_{663} - 2.69 \times A_{645}$
Chlorophyll b = $27.05 \times A_{653} - 11.21 \times A_{666}$	Chlorophyll b=$22.9 \times A_{646.8} - 4.68 \times A_{663}$
Carotene = $(1000 \times A_{470} - 2.860$ Chl a $- 129.2$ Chl b$) \div 245$	Carotene= $(1000 \times A_{470} - 1.82$Chl a $- 85.02$ Chl b$) \div 198$

where, A = Absorbance of chlorophyll extract at specific indicated at wavelength; Chl a = Chlorophyll a, Chl b = Chlorophyll b

Table 3: Chl a, Chl b and carotenoid content in different leaf extracts of *G. kurroo* Royle and *S. chirayita* (Roxb.) H. Karst.

Solvents/	DMSO (mg g^{-1} fresh wt.)		Methanol (µg ml^{-1})		Water (µg ml^{-1})	
Contents	*G. kurroo* Royle	*S. chirayita*	*G. kurroo* Royle	*S. chirayita*	*G. kurroo* Royle	*S. chirayita*
Chl a	0.9275	9.0722	0.9133	2.7727	0.0707	0.0782
Chl b	0.288	2.7395	0.537	1.7694	0.1565	0.1686
Total Chl	1.1978	11.6388	1.4503	4.5421	0.2272	0.2468
Carotenoid	0.0823	0.2088	0.4394	1.1039	0.1611	0.112

Note: Polarity of solvents: DMSO=0.444; Methanol=0.762; Water=1.0

H. Karst. was similar to that of Chl a but for *G. kurroo* Royle it was methanol>DMSO>water. Highest extraction efficacy was observed in methanol for carotenoid in both the plants (Fig. 1). Earlier studies on several plant species reported that different solvents would extract different amounts of chlorophyll due to biotic factors (variation in leaf shapes, plant age, physiological status), abiotic factors (light, temperature, growth season) and different equations or formulae used for content calculation. Besides the efficacy of solvents, the pigment quantification would also depend on technical factors like spectrophotometric resolution, range, and absorbance parameters (Almomani and Örmeci, 2018; Lichtenthaler,1988; Martín-Tornero *et al.*, 2020; Masaló and Oca, 2020).

Evidently, DMSO being most non-polar amongst the three solvents (Table 3) was the best solvent for Chl a extraction. Castle *et al.* (2011) compared different solvents for chlorophyll extraction from soil crusts and found ethanol and DMSO being the best solvents for extractions. Martín-Tornero *et al.* (2020) also reported DMSO being used for increased Chl a and b extraction in a variety of plant species. It was interesting to note that the ratio of Chl a: Chl b contents extracted in DMSO was 3.22 and 3.31 in case of *G. kurroo* Royle and *S. chirayita* (Roxb.) H. Karst., respectively, which was very similar. The ratio of Chl a: Chl b was fairly comparable in methanol extraction *i.e.*, 1.70 and 1.56 in case of *G. kurroo* Royle and *S. chirayita* (Roxb.) H. Karst., respectively. In the case of water, the ratio of Chl a: Chl b was 0.45 and 0.46 in *Gentiana* and *Swertia*, respectively which clearly indicated that water did not prove to be a good solvent.

G. kurroo Royle and *S. chirayita* (Roxb.) H. Karst. show an altitudinal variation in their growth *i.e.*, *Gentiana* grows between 1500 and 5000 meters while *Swertia* grows between 1200 and 2100 metres. Several reports proclaimed that photosynthetic pigments involved in light absorption and transformation would directly impact photosynthetic capacity and were also vulnerable to environmental stresses. Chlorophyll content would decrease due to slow down in its synthesis, increased decomposition, or damaged chloroplast structures during water, light, and temperature stresses (Ashraf and Harris, 2013; Hazrati *et al.*, 2016). In present

study also, the pigment content in *G. kurroo* Royle decreased with increasing elevation and was an adaptive response to prevent oxidative damage by reducing light absorption and thereby preventing ROS production. Armstron *et al*. (1996) and Demmig-Adams *et al*. (1992) reported that carotenoids were multifunctional complex playing an important role in light-harvesting complexes (LHCs) construction, affecting photosynthesis as well as efficient removal of accumulated ROS thus preventing photooxidative damage to chloroplast (Armstron *et al.*, 1996; Demmig-Adams *et al.*, 1992). Ahmad *et al.* (2016) also reported a decrement in carotenoid content with increasing elevation. In the present study, the chlorophyll and carotenoid contents depicted a large variation in their values for leaves of both the plants (Table1).

CONCLUSION

Chlorophyll and carotenoid from *G. kurroo* Royle and *S. chirayita* (Roxb.) H. Karst. were extracted and estimated using different solvents. A higher chlorophyll and carotenoid content in *S. chirayita* (Roxb.) H. Karst.was observed than that of *G. kurroo* Royle. Extraction using DMSO and methanol showed a higher content than that with water. A variation in values was observed in both the plants as they grew at different elevations implying a difference in biochemical components of the plants and requirement of different solvent for extraction. This led to the conclusion that photosynthetic pigment requirements varied between plant species and were directly related to the activity and rate of photosynthesis. Further investigations regarding the other biochemical parameters like total soluble protein and sugar content along with chromatographic techniques would be suggestive to have a greater understanding to examine the valuable attributes of bioactive pigments.

ACKNOWLEDGEMENTS

The work was supported by M/s Directorate of Research, GBPUA&T (Code-6001) funded project entitled 'Phytochemical characterization of endangered medicinal Himalayan plants *Gentiana kurroo* and *Nardostachys jatamansi* for their therapeutic potential'. Dr. D.S. Rawat, Assistant Professor Biological sciences at G.B.P.U.A.&T is thankfully acknowledged for plant species identification.

REFERENCES

Ahmad, K.S., Hameed, M., Fatima, S., Ashraf, M., Ahmad, F., Naseer, M. and Akhtar, N. (2016). Morpho-anatomical and physiological adaptations to high altitude in some Aveneae grasses from Neelum Valley, Western Himalayan Kashmir. *Acta Physiologiae Plantarum*, 38: 1-14.

Almomani, F.A. and Örmeci, B. (2018). Monitoring and measurement of microalgae using the first derivative of absorbance and comparison with chlorophyll extraction method. *Environmental Monitoring and Assessment*, 190: 1-19.

Armstrong, G.A. and Hearst, J.E. (1996). Genetics and molecular biology of carotenoid pigment biosynthesis. *The FASEB Journal*, 10(2): 228-237

Arnon, D.I., 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. *Plant Physiology*, 24(1):1

Ashraf, M.H.P.J.C. and Harris, P.J. (2013). Photosynthesis under stressful environments: an overview. *Photosynthetica*, 51: 163-190

Britton, G. (2008). Functions of intact carotenoids. *Carotenoids: Natural Functions*, (4): 189-212.

Campbell, P.K., Huemmrich, K.F., Middleton, E.M., Ward, L.A., Julitta, T., Daughtry, C.S., Burkart, A., Russ, A.L. and Kustas, W.P. (2019). Diurnal and seasonal variations in chlorophyll fluorescence associated with photosynthesis at leaf and canopy scales. *Remote Sensing*, 11(5): 488.

Castle, S.C., Morrison, C.D. and Barger, N.N. (2011). Extraction of chlorophyll a from biological soil crusts: a comparison of solvents for spectrophotometric determination. *Soil Biology and Biochemistry*, 43(4), 853-856.

Clarke, C.B. (1885). Verbenaceae. In: 'J.D. Hooker (Eds.). The Flora of British India'. L. Reeve and Co, London, 4: 560–604.

Cole, J.M., Pepe, G., A. l. Bahri, O.K. and Cooper, C.B. (2019). Cosensitization in dye-sensitized solar cells. *Chemical Reviews*, 119(12): 7279-7327.

Croft, H., Chen, J.M., Luo, X., Bartlett, P., Chen, B. and Staebler, R.M. (2017). Leaf chlorophyll content as a proxy for leaf photosynthetic capacity. *Global Change Biology*, 23(9): 3513-3524.

Demmig Adams, B. and ADAMS III, W.W. (1992). Carotenoid composition in sun and shade leaves of plants with different life forms. *Plant, Cell & Environment*, 15(4): 411-419.

Feng, Y., Cheng, H., Han, J., Zheng, X., Liu, Y., Yang, Y. and Zhang, L. (2017). Chlorophyll sensitized BiVO4 as photoanode for solar water splitting and CO_2 conversion. *Chinese Chemical Letters*, 28(12): 2254-2258.

Gamon, J.A. and Surfus, J.S. (1999). Assessing leaf pigment content and activity with a reflectometer. *The New Phytologist*, 143(1):105-117.

Gaur, R.D. (1999). *Flora of the District Garhwal, North West Himalaya*. Transmedia. Flora of the District Garhwal. Transmedia, North-West Himalaya, 811 p.

Hartzler, D.A., Niedzwiedzki, D.M., Bryant, D.A., Blankenship, R.E., Pushkar, Y. and Savikhin, S. (2014). Triplet excited state energies and phosphorescence spectra of (bacterio) chlorophylls. *The Journal of Physical Chemistry B*, 118(26): 7221-7232.

Hazrati, S., Tahmasebi-Sarvestani, Z., Modarres-Sanavy, S.A.M., Mokhtassi-Bidgoli, A. and Nicola, S. (2016). Effects of water stress and light intensity on chlorophyll fluorescence parameters and pigments of *Aloe vera* L. *Plant Physiology and Biochemistry*, 106: 141-148.

Hiscox, J.D. and Israelstam, G.F. (1979). A method for the extraction of chlorophyll from leaf tissue without maceration. *Canadian Journal of Botany*, 57(12): 332-1334.

Huner, N.P., Öquist, G. and Sarhan, F. (1998). Energy balance and acclimation to light and cold. *Trends in Plant Science*, 3(6): 224-230.

Li, Y., He, N., Hou, J., Xu, L., Liu, C., Zhang, J., Wang, Q., Zhang, X. and Wu, X., 2018. Factors influencing leaf chlorophyll content in natural forests at the biome scale. *Frontiers in Ecology and Evolution*, 6: 64.

Lichtenthaler, H.K. and Buschmann, C. (2001). Extraction of phtosynthetic tissues: chlorophylls and carotenoids. *Current Protocols in Food Analytical Chemistry*, 1(1): F4-2.

Lichtenthaler, H.K. (1988). *In vivo* chlorophyll fluorescence as a tool for stress detection in plants. In: Applications of Chlorophyll Fluorescence in Photosynthesis Research, Stress Physiology, Hydrobiology and Remote Sensing: An introduction to the various fields of applications of the in vivo chlorophyll fluorescence also including the proceedings of the first International Chlorophyll Fluorescence Symposium held in the Physikzentrum, Bad Honnef, FRG, 6–8 June 1998 (129-142). Springer Netherlands.

Longjam, K.D., Ng, A., Keisam, S. and Oinam, P.C. (2018). Quantitative Estimation of Total Chlorophyll and Carotenoid Content in *Oreopanax Xalepensis*. *International Journal of Pharmacy and Biological Sciences*,8(4): 410-415.

Martín-Tornero, E., de Jorge Páscoa, R.N.M., Espinosa-Mansilla, A., Martín-Merás, I.D. and Lopes, J.A., (2020). Comparative quantification of chlorophyll and polyphenol levels in grapevine leaves sampled from different geographical locations. *Scientific Reports*, 10(1): 6246.

Masaló, I. and Oca, J. (2020). Evaluation of a portable chlorophyll optical meter to estimate chlorophyll concentration in the green seaweed *Ulva Ohnoi*. *Journal of Applied Phycology*, 32(6): 4171-4174.

McCartney-Melstad, E., Gidiş, M. and Shaffer, H.B.,

2018. Population genomic data reveal extreme geographic subdivision and novel conservation actions for the declining foothill yellow-legged frog. *Heredity*, 121(2): 112-125.

Menkovic, N., Juranic, Z., Stanojkovic, T., Raonic Stevanovic, T., Šavikin, K., Zdunić, G. and Borojevic, N., 2010. Radioprotective activity of *Gentiana lutea* extract and mangiferin. *Phytotherapy Research*, 24(11): 1693-1696.

Mustafa, A.M., Caprioli, G., Ricciutelli, M., Maggi, F., Marín, R., Vittori, S. and Sagratini, G., 2015. Comparative HPLC/ESI-MS and HPLC/DAD study of different populations of cultivated, wild and commercial *Gentiana lutea* L. *Food Chemistry*, 174: 426-433.

Negrão, S., Schmöckel, S.M. and Tester, M.J.A.O.B., 2017. Evaluating physiological responses of plants to salinity stress. *Annals of botany*, 119(1): 1-11.

Özkan, G. and Bilek, S.E., 2015. Enzyme-assisted extraction of stabilized chlorophyll from spinach. *Food chemistry*, 176: 152-157.

Öztürk, N., Korkmaz, S., Öztürk, Y. and Başer, K.H., 2006. Effects of gentiopicroside, sweroside and swertiamarine, secoiridoids from gentian (*Gentiana lutea* ssp. symphyandra), on cultured chicken embryonic fibroblasts. *Planta Medica*, 72(04): 289-294.

Palett, K. E. and Young, A. J. (1993). Carotenoids. In: R. G. Alscher, J. L. Hess (Eds.): Antioxidants in higher plants, 60-89. CRC Press, Boca Raton.

Palta, J.P. (1990). Leaf chlorophyll content. *Remote Sensing Reviews*, 5(1): 207-213.

Parry, C., Blonquist Jr, J.M. and Bugbee, B. (2014). *In situ* measurement of leaf chlorophyll concentration: analysis of the optical/absolute relationship. *Plant, Cell & Environment*, 37(11): 2508-2520.

Poojary, M.M., Barba, F.J., Aliakbarian, B., Donsì, F., Pataro, G., Dias, D.A. and Juliano, P. (2016). Innovative alternative technologies to extract carotenoids from microalgae and seaweeds. *Marine Drugs*, 14(11): 214.

Porra, R.J., 1991. Recent advances and re-assessments in chlorophyll extraction and assay procedures for terrestrial, aquatic and marine organisms, including recalcitrant algae. *Chlorophylls*, 31-57.

Porra, R.J. and Scheer, H., 2019. Towards a more accurate future for chlorophyll a and b determinations: the inaccuracies of Daniel Arnon's assay. *Photosynthesis Research*, 140(2): 215-219.

Porra, R.J., Thompson, W.A.A. and Kriedemann, P.E. (1989). Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, 975(3): 384-394.

Prasann, K., Mandala, H., Kumar, P.S., Johnson, Y., Nada, J., Mohit, N., Lamneivah, M. and Sunil, K. (2018). Effect on chlorophyll a/b ratio in cadmium contaminated maize leaves treated with putrescine and mycorrhiza. *Annals of Biology*, 34(3): 281-283.

Rebeiz, C.A. (2014). *Chlorophyll Biosynthesis and Technological Applications* (pp. 359-408). Berlin/Heidelberg, Germany: Springer.

Ritchie, R.J. (2006). Consistent sets of spectrophotometric chlorophyll equations for acetone, methanol and ethanol solvents. *Photosynthesis Research*, 89: 27-41.

Saito, K., Suzuki, T. and Ishikita, H. (2018). Absorption-energy calculations of chlorophyll a and b with an explicit solvent model. *Journal of Photochemistry and Photobiology A: Chemistry*, 358: 422-431.

Sangeetha, R.K. and Baskaran, V., 2010. Carotenoid composition and retinol equivalent in plants of nutritional and medicinal importance: Efficacy of β-carotene from Chenopodium album in retinol-deficient rats. *Food Chemistry*, 119(4):1584-1590.

Schaufelberger, D. and Hostettmann, K. (1988). Chemistry and pharmacology of *Gentiana lactea*. *Planta Medica*, 54(03): 219-221.

Shukla, S., Mehta, A., Mehta, P., Vyas, S.P., Shukla, S. and Bajpai, V.K. (2010). Studies on anti-inflammatory, antipyretic and analgesic properties of *Caesalpinia bonducella* F. seed oil in experimental animal models. *Food and Chemical Toxicology*, 48(1): 61-64.

Singh, J.S. (2002). The biodiversity crisis: a multifaceted review. *Current Science*, 638-647.

Skinder, B.M., Ganai, B.A. and Wani, A.H. (2017). Scientific study of *Gentiana kurroo* Royle. *Medicines*, 4(4): 74.

Tripathi, A.K. and Gautam, M. (2007). Biochemical parameters of plants as indicators of air pollution. *Journal of Environmental Biology*, 28(1):127.

Vicas, S.I., Laslo, V., Pantea, S. and Bandici, G. (2010). Chlorophyll and carotenoids pigments from Mistletoe (*Viscum album*) leaves using different solvents. *Analele Universitatii din Oradea, Fascicula Biologie*, 17(2): 213-218.

Viera, I., Pérez-Gálvez, A. and Roca, M. (2019). Green natural colorants. *Molecules*, 24(1):154.

Wu, T., Li, J., Li, Y. and Song, H. (2017). Antioxidant and hepatoprotective effect of swertiamarin on carbon tetrachloride-induced hepatotoxicity via the Nrf2/HO-1 pathway. *Cellular Physiology and Biochemistry*, 41(6): 2242-2254.

Zhao, Z.J., Song, Y.G., Liu, Y.L., Qiao, M., Zhai, X.L. and Xiang, F.N. (2013). The effect of elicitors on oleanolic acid accumulation and expression of triterpenoid synthesis genes in *Gentiana straminea*. *Biologia Plantarum*, 57(1): 139-143.

Received: February 1, 2023
Accepted: April 22, 2023