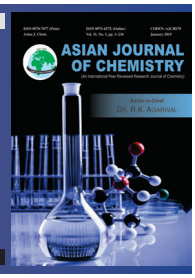
Asian Journal of Chemistry; Vol. 31, No. 3 (2019), 551-554

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2019.21738



Green Synthesis, Characterization and Antimicrobial Activity of Zinc Oxide Nanoparticles Using Root Extract of *Viola canescens* Wall. ex. Roxb.

ARUN KUMAR KHAJURIA[1,*], ABHA NEGI[2], N.S. BISHT[1], V. MAURYA[2] and ANUJ KANDWAL[3]

[1]Department of Botany, H.N.B. Garhwal University, Pauri Campus, Pauri-246001, India
[2]Department of Botany and Microbiology, H.N.B. Garhwal University, Chauras Campus, Srinagar-246174, India
[3]Department of Chemistry, H.N.B. Garhwal University, Pauri Campus, Pauri-246001, India

*Corresponding author: E-mail: arun.khajuria20@gmail.com

Received: 3 October 2018; *Accepted*: 17 October 2018; *Published online*: 31 January 2019; AJC-19243

Stable zinc oxide nanoparticles were synthesized by using green approach of nanotechnology. The present investigation aimed to synthesize, stable, cost effective, rapid, eco-friendly approach for the bio-reduction of zinc nitrate hexahydarate to their nano size using phytochemicals present in the root extract of *Viola canescens*. The synthesized nanoparticles were further characterized by UV-visible, XRD, FTIR, SEM techniques and tested against the Gram-negative and Gram-positive pathogenic cultures of bacteria. The average size of synthesized nanoparticles was less than 11 nm with hexagonal morphology. The clear zone of inhibition against tested bacteria showed their capability as antimicrobial agent.

Keywords: Zinc oxide nanoparticles, Root extract, XRD, FTIR SEM, Antimicrobial activity.

INTRODUCTION

Viola canescens Wall ex., Roxb., Himalayan white violet an important and preferred medicinal herb in different folk or ethnomedicinal preparations and use to cure wide spectra of diseases from common cold to life thrating diseases (neurological disorder, renal dis-functioning and cancer) [1-6]. Roots of the plants are known to have an alkaloid named violin along with some other important phytochemicals such as methyl silicates, phenols, steroids *etc*. [7]. Nanotechnology, one of the fastest emerging technique involves the synthesis and application of material in its nano sizes most preferably in 1-100 nm range. The techniques of science is basically based on the manipulation of individual atom or molecule which ultimately results in nano scale level by generally two approaches *i.e.*, "top down" approach or "bottom up". Top down approach of nanoparticle synthesis involves reducing or size reduction of starting material [8] by different physical and chemical treatments or by different physical measures. In bottom up method, nanoparticles are synthesized by joining smaller molecules and particles [9] mostly using chemical and biological methods. Green route a bottom up method of nanoparticle synthesis, includes the plant phytochemicals as a reducing, capping and stabilizing agent in synthesis of nanoparticles [10-15]. Nanoparticles synthesized *via* this route are in high demand due to its cost effectiveness, easy to perform, safe for human use, enhanced antimicrobial activity and stable structure, over the other physical and chemical methods [16-18]. Material at its nano level shows improved character over its source material such as small size, large surface area with increased reactivity over the bulky cousin and thus called as "Wonder of Modern Medicine" [19] or nano antibiotic because of their antimicrobial activities [20]. Zinc oxide is II-IV binary compound, semiconductor crystallize in either hexagonal wurtzite or cubic zinc blend structure. Green synthesis of ZnO nanoparticles are preferred and has drawn worldwide interest due to some of their facilitating properties *i.e.*, biocompatible, biodegradable, less hazardous, non-toxic, eco-friendly and wide range of applicability in different fields *i.e.*, agriculture, medical, electronic, optic and other material sciences. With the onslaught of modernization, the human manipulated its surrounding according to its own comfort and deteriorates the

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 (CC BY-NC-SA 4.0) International License which allows readers to freely read, download, copy, distribute, print, search, or link to the full texts of its articles and to use them for any other lawful non-commercial purpose as long as the original source is duly acknowledged.

nature and natural resources they have, which results in contaminating the essential resources (water and air) along with production of some new verity of organism, which are multidrug resistance. Thus, present work aimed to develop reliable protocol for green synthesis of nanoparticles using phytochemical present in the root of *Viola canescens* because plant have reducing capacity and at the same time possess good pharmaceutical property, which may used to encounter the problem of drug resistance and in future new drug may release by using the synthesized nanoparticle.

EXPERIMENTAL

Preparation of extract: Plants collected from wild were washed under tap water for 10 min to remove all adhering soil followed by washing with double distilled autoclaved water. Roots were then shade dry for 15 days or until the constant weight of the roots were obtained. These dried roots were used for extract preparation. The dried roots first homogenized using mortar and pestle, then 5 g of the powder used for preparing extract. Powder then soaked in 100 mL double distilled autoclaved water in Erlenmeyer flask and boiled at 60 °C for 15 min. Extracts were allowed to cool and then filtered using Whatman filter paper.

Green synthesis of zinc oxide nanoparticles: Green synthesis of ZnO nanoparticles was carried by mixing 100 mL of the root extract with 100 mL of $Zn(NO_3)_2 \cdot 6H_2O$ solution in 500 mL flask. Prepared root extract was heated at 60 °C with constant stirring using magnetic stirrer for 10 min, before adding zinc nitrate solution in the flask. After complete pouring of zinc nitrate solution in the flask having root extract, the mixture of both solutions was kept for vigorous stirring at 60 °C for 4 h. The change in colour of the solution was considered as a visual marker for the synthesis of nanoparticles. Further, the precipitate starts appearing after 3 h of vigorous stirring at 60 ºC. Precipitate formed in the reaction was allowed to settle at room temperature for 24 h. Supernatant was discarded and crude was centrifuged for 20 min at 5000 rpm. Finally, pellets were oven dried at 60 °C for 10 h followed by mashing in mortar and pestle to get a fine powder and stored in air tight bottles for further characterization and antimicrobial activities.

Characterization of green ZnO nanoparticles: Synthesized nanoparticles were subjected to UV-visible absorption spectra to study its optical properties. X-ray diffraction (XRD) analysis of nanoparticle was recorded in the range of 2θ from 0° to 65° using powder diffractometer, it is used to characterize crystallinity of the synthesized nanoparticles and their phase identification [21]. Fourier transform infrared spectroscopy (FTIR) in the range of 4000-500 cm^{-1} was used to identify various phytochemicals present in the reduction and capping of ZnO nanoparticles [22]. Surface morphology of ZnO nanoparticles was analyzed with scanning electron microscopy (SEM) [23].

Antibacterial assay: Antibacterial activity of ZnO nanoparticles was evaluated against *Staphylococcus aureus, Streptococcus pneumonia, Klebsiella pneumoniae* and *Escherichia coli,* by Agar well diffusion method. Muller Hinton Agar Medium (HI-Media) was used in bacterial assay and prepared by dissolving 33.9 g into 1000 mL of distilled water, this dissolved medium then subjected to the autoclave at 121 °C and 15 Pascal pressure for 15 min.

RESULTS AND DISCUSSION

Visual observation UV-visible spectra: Change in colour from brown to yellow evident the formation of ZnO nanoparticles using root extract. Further, confirmation for synthesis was carried out by using UV-visible analysis, which showed characteristic peak at 340 nm, which could be attributed to the ZnO nanoparticles.

X-ray diffraction (XRD): XRD spectra gave the insight of the synthesized nanoparticles, which includes crystallinity, structure and size of the nanoparticles. The XRD spectra in case of root as a plant source showed distinct peaks at 31.854, 34.450, 36.320, 47.580, 56.627, 62.840, 66.380 and 68.020, which corresponds to 100, 002, 101, 102, 110, 103, 200 and 112 lattice planes (Fig. 1). Confirming the hexagonal shape with average size of synthesized ZnO nanoparticles found to be < 11 nm (Table-1).

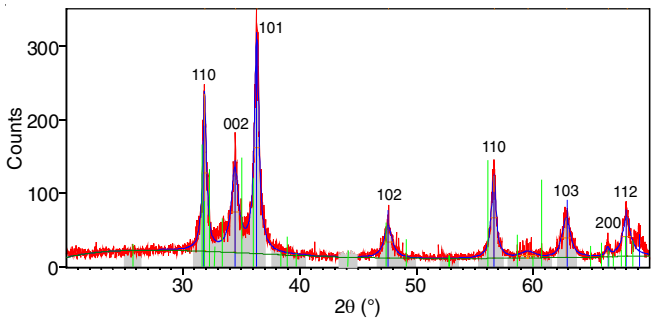


Fig. 1. XRD spectrogram of root base synthesis of ZnO nanoparticles

TABLE-1
PEAK LIST FOR AVERAGE SIZE
CALCULATION OF NANOPARTICLES

2θ (°)	hkl	FWHM left (2θ, °)	*d*-spacing (Å)	Rel. int. (%)
31.854	110	0.39	2.80707	74.11
34.450	002	0.74	2.60110	38.22
36.323	101	0.49	2.47132	100.00
47.580	102	0.81	1.90966	15.18
56.627	110	0.49	1.62409	38.70
62.840	103	0.84	1.47754	18.70
66.380	200	0.38	1.40709	03.51
68.020	112	0.82	1.37715	18.44

FTIR analysis: To find out the possible functional group of the plant involved in the reduction and capping of ZnO nanoparticle FTIR analysis of nanoparticles was recorded. FTIR spectrogram showed various peaks which corresponds to different organic groups. Broad band at 3400.18 cm^{-1} corresponds to the stretching vibrations –OH group (hydroxyl group). Besides, this peaks at 2427.75 cm^{-1}, 2096.55 cm^{-1} corresponds to stretching vibrations of phosphin (P–H) and C≡C functional groups. Further, peak at 1639.61 attributed to C=C aromatic stretching, peaks at 1384.12 cm^{-1}, 1149.46 cm^{-1}, 867.29 cm^{-1} corresponds to C–O and CH_2 stretching vibrations respectively. Further, presence of band at 443.74 cm^{-1} attributed to ZnO hexagonal structure Fig. 2.

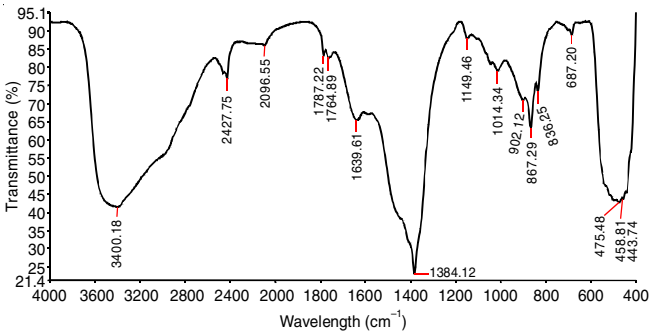


Fig. 2. FTIR of root based ZnO nanoparticles

SEM analysis: In order to visualize the surface morphology of ZnO nanoparticles, SEM analysis was carried out and obtained monograph clearly demonstrate the presence roots synthesized ZnO nanoparticles were appeared to rod shaped in morphology, moreover boundaries between each particle can easily be observed (Fig. 3).

Antimicrobial assay: Agar well diffusion method was used to perform the antimicrobial assay against four bacterial strain *i.e*., *Staphylococcus aureus, Streptococcus pneumonia, Klebsiella pneumoniae* and *Escherichia coli,* during the assay sodium chloride solution was used as positive control while amoxicillin was used as the drug for the comparative response and the results are summarized in Table-2. Results of present work clearly showed the dose dependent antimicrobial activity of nanoparticles and maximum zone of inhibition (13.50 ± 0.82 mm) was recorded for *Klebsiella pneumoniae* and least (11.20 ± 0.79 mm) was in the case of *E. coli.* Senthilkumar *et al.* [25] worked on antimicrobial activity of ZnO nanoparticles of green tea against four pathogenic strains of bacteria reported the same results *i.e.*, maximum zone of inhibition in *Klebsiella pneumoniae* and least in *E. coli,* the finding of Senthilkumar further support the present work. The possible mechanism for the antimicrobial activity of nanoparticles may involved the absorption of the nanoparticles by the host and inside the host organism, nanoparticles start the production of the free radical which interrupts the basic metabolism and some time may cause damage to the DNA of the host and results in the death of the host organism. Further, smaller the size of nanoparticles facilitates their easy entry into the cell wall of organism results in enhanced inhibition mechanism over the large sized nanoparticles [24] this concept valid for the present work because leave extract based ZnO nanoparticle of the same plant, which were larger in size than the root based nanoparticles showed inferior antimicrobial activity when compare with the present work [15]. Besides, results of the present work are in accord with several other researchers, worked on synthesis of zinc based nanoparticles using different plant extract (Table-3).

Conclusion

The plant based nanoparticles synthesis is based on the phytochemicals present in the aqueous plants extract. Roots of the *Viola canescens* successful in bio-reduction of zinc to its nanoparticles, functional groups (hydroxyl, phosphine and carbonyls) present in root extract effectively acted as reducing and capping agent, which lead to the synthesis of ZnO nanoparticles. X-ray diffraction pattern suggested a hexagonal structure with < 11 nm average size of nanoparticles. Moreover,

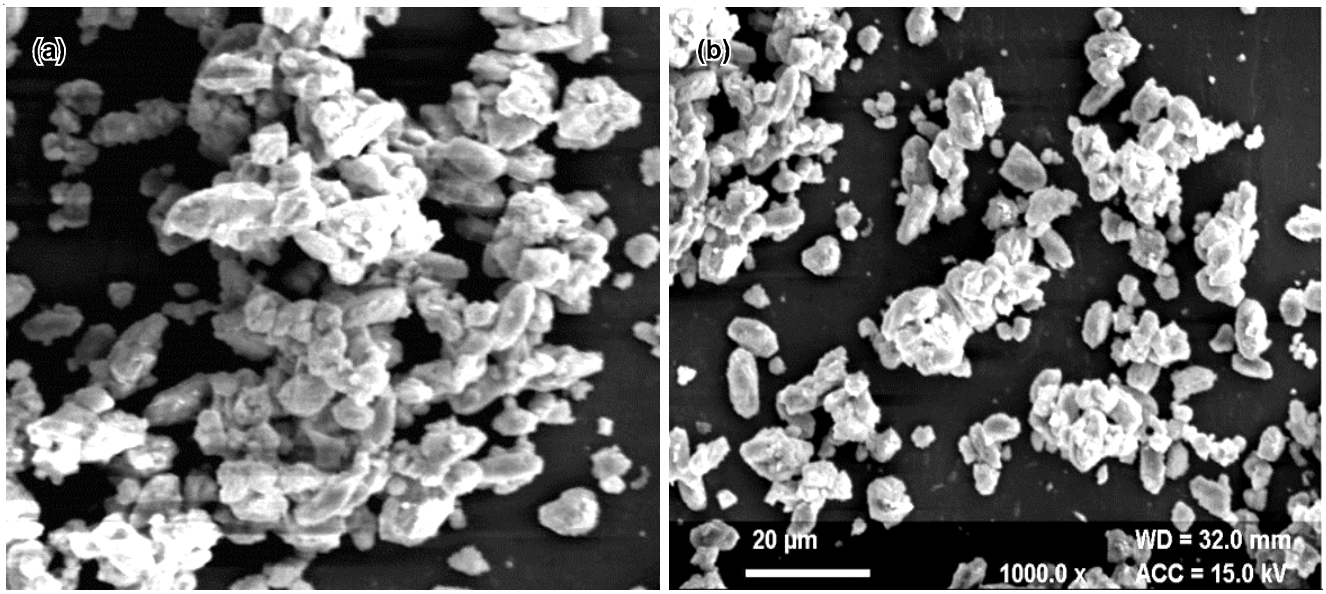


Fig. 3. (a&b) SEM monograph showing morphology of synthesized ZnO nano-particles by root extract of *Viola canescens*

TABLE-2
ANTIMICROBIAL ACTIVITY OF ROOT BASED ZnO NANOPARTICLES AGAINST DIFFERENT BACTERIAL STRAINS

S. No.	Test organisms	Control sodium chloride	Drug	50 µg	75 µg	100 µg
1	*Staphylococcus aureus*	0.00 ± 00	9.0	11.67 ± 0.90 mm	12.33 ± 0.58 mm	13.33 ± 0.83 mm
2	*Klebsiella pneumoniae*	0.00 ± 00	15.3	12.30 ± 0.92 mm	12.73 ± 0.83 mm	13.50 ± 0.82 mm
3	*Escherichia coli*	0.00 ± 00	11.7	9.17 ± 0.67 mm	10.17 ± 0.45 mm	11.20 ± 0.79 mm
4	*Streptococcus pneumoniae*	0.00 ± 00	15.0	9.53 ± 0.76 mm	12.67 ± 0.90 mm	13.10 ± 0.70 mm

TABLE-3
GREEN SYNTHESIS OF ZnO NANOPARTICLES OF SOME IMPORTANT MEDICINAL PLANTS

S. No.	Plant	Family	Material	Size (nm)	Ref.
1	*Aloe vera*	Liliaceae	Leaves	22.18	[26]
2	*Azadirachata indica*	Meliaceae	Leaves	18.00	[27]
3	*Calotropis gigantean*	Asclepiadaceae	Leaves	30-35	[28]
4	*Catharanthus roseus*	Apocynaceae	Leaves	23-57	[29]
5	*Coptidis rhizome*	Ranunculaceae	Rhizome	2.9-25.2	[30]
6	*Ocimum basilicum*	Lamiaceae	Leaves	14.28	[31]
7	*Ocimum teniflorum*	Lamiaceae	Leaves	13.68	[32]
8	*Rosa canina*	Rosaceae	Fruit	32-36	[33]
9	*Solanum nigrum*	Solanaceae	Leaves	20-30	[34]
10	*Vitex negundo*	Vitaceae	Leaves	38.17	[35]
11	*Viola canescens*	Violaceae	Roots	> 11	Present work

synthesized nanoparticles showed bactericidal activity against four different bacteria. These biological synthesized nanoparticles are free from toxic chemicals that are generally present when prepared by chemical method and less expensive, therefore may used in various industrial and biomedicinal application.

ACKNOWLEDGEMENTS

The authors are highly thankful to USIC Department of HNB Garhwal University and Punjab University Chandigarh for characterization help.

CONFLICT OF INTEREST

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

REFERENCES

1. M. Hamayun, S.A. Khan, H.Y. Kim, I.N. Chae and I.J. Lee, *Int. J. Bot.*, **2**, 205 (2006); https://doi.org/10.3923/ijb.2006.205.209.
2. C.S. Rana, A. Sharma, N. Kumar, L.R. Dangwal and J.K. Tiwari, *Nat. Sci.*, **8**, 9 (2010).
3. P.K. Rana, P. Kumar, V.K. Singhal and C. Rana, *The Scientific World J.,* **Article ID 753289** (2014); https://doi.org/10.1155/2014/753289.
4. A. Razzaq, F. Hadi, A. Rashid, M. Ibrar and U. Ali, *Euras. J. Agric. Enivron. Sci.*, **15**, 328 (2015).
5. N. Mann, A. Khajuria, P.L. Uniyal and S. Lakhanpaul, *The Botanica,* **66**, 58 (2016).
6. A.K. Khajuria, N.S. Bisht and R. Krishan, *Plant Arch.*, **17**, 833 (2017a).
7. M. Barkatullah, N. Ibrar, N.M. Ali and M. Ehsan, *Afr. J. Pharm. Pharmacol.*, **6**, 1142 (2012); https://doi.org/10.5897/AJPP12.061.
8. M.A. Meyers, A. Mishra and D.J. Benson, *Prog. Mater. Sci.*, **51**, 427 (2006); https://doi.org/10.1016/j.pmatsci.2005.08.003.
9. P. Mukherjee, A. Ahmad, D. Mandal, S. Senapati, S.R. Sainkar, M.I. Khan, R. Parishcha, P.V. Ajaykumar, M. Alam, R. Kumar and M. Sastry, *Nano Lett.*, **1**, 515 (2001); https://doi.org/10.1021/nl0155274.
10. J.Y. Song, H.K. Jang and B.S. Kim, *Process Biochem.*, **44**, 1133 (2009); https://doi.org/10.1016/j.procbio.2009.06.005.
11. K.N. Thakkar, S.S. Mhatre and R.Y. Parikh, *Nanomedicine*, **6**, 257 (2010); https://doi.org/10.1016/j.nano.2009.07.002.
12. J. Banerjee and R. Narendhirakannan, *Dig. J. Nanomater. Biostruct.*, **6**, 961 (2011).
13. S. Iravani, *Green Chem.*, **13**, 2638 (2011); https://doi.org/10.1039/c1gc15386b.
14. H.J. Lee, G. Lee, N.R. Jang, J.H. Yun, J.Y. Song and B.S. Kim, *Nanotechnology*, **1**, 371 (2011).
15. A.K. Khajuria, N.S. Bisht and G. Kumar, *J. Pharmacog. Phytochem.*, **6**, 1301 (2017).
16. A.T. Marshall, R.G. Haverkamp, C.E. Davies, J.G. Parsons, J.L. Gardea-Torresdey and D. van Agterveld, *Int. J. Phytoremed.*, **9**, 197 (2007); https://doi.org/10.1080/15226510701376026.
17. G.S. Dhillon, S.K. Brar, S. Kaur and M. Verma, *Crit. Rev. Biotechnol.*, **32**, 49 (2012); https://doi.org/10.3109/07388551.2010.550568.
18. A.K. Mittal, Y. Chisti and U.C. Banerjee, *Biotechnol. Adv.*, **31**, 346 (2013); https://doi.org/10.1016/j.biotechadv.2013.01.003.
19. B.H. Abbasi, S. Anjum and C. Hano, *RSC Adv.*, **7**, 15931 (2017); https://doi.org/10.1039/C7RA02070H.
20. H. Aggarwal, S.V. Kumar and S. Rajeshkumar, *Resource-Eff. Technol.*, **3**, 406 (2017); https://doi.org/10.1016/j.reffit.2017.03.002.
21. S. Sun, C. Murray, D. Weller, L. Folks and A. Moser, *Science*, **287**, 1989 (2000); https://doi.org/10.1126/science.287.5460.1989.
22. B.D. Chithrani, A.A. Ghazani and W.C.W. Chan, *Nano Lett.*, **6**, 662 (2006); https://doi.org/10.1021/nl052396o.
23. B. Schaffer, U. Hohenester, A. Trugler and F. Hofer, *Phys. Rev. B*, **79**, 041401(R) (2009); https://doi.org/10.1103/PhysRevB.79.041401.
24. J. Poozvishi and B. Krishnaveni, *Int. J. Biol. Pharm. Res*., **6**, 776 (2015);
25. S.R. Senthilkumar and T. Sivakumar, *Int. J. Pharm. Pharm. Sci.*, **6**, 461 (2014).
26. E. Varghese and M. George, *Int. J. Adv. Res. Sci. Eng*., **4**, 307 (2015).
27. K. Elumalia and S. Velmurugan, *Appl. Surf. Sci*., **345**, 329 (2015); https://doi.org/10.1016/j.apsusc.2015.03.176.
28. C. Vidya, S. Hiremath, M.N. Chandraprabha, M.L. Antonyraj, I.V. Gopal, A. Jain and K. Bansal, *Int. J. Curr. Eng. Technol.*, **1**, 118 (2013).
29. G. Bhumi and N. Savithramma, *Int. J. Drug Dev. Res*., **6**, 208 (2014).
30. P.C. Nagajyothi, T.V.M. Sreekanth, C.O. Tettey, Y.I. Jun and S.H. Mook, *Bioorg. Med. Chem. Lett.*, **24**, 4298 (2014); https://doi.org/10.1016/j.bmcl.2014.07.023.
31. S.H. Abdul, R. Sivaraj and R. Venckatesh, *Mater. Lett*., **131**, 16 (2014); https://doi.org/10.1016/j.matlet.2014.05.033.
32. S. Raut, P.V. Thorat and R. Thakre, *Int. J. Sci. Res*., **4**, 1225 (2015).
33. S. Jafarirad, M. Mehrabi, B. Divband and M. Kosari-Nasab, *Mater. Sci. Eng.: C*, **59**, 296 (2016); https://doi.org/10.1016/j.msec.2015.09.089.
34. M. Ramesh, M. Anbuvannan and G. Viruthagiri, *Spectrochim. Acta A: Mol. Biomol. Spectrosc.*, **136**, 864 (2015); https://doi.org/10.1016/j.saa.2014.09.105.
35. S. Ambika and M. Sundrarajan, *J. Photochem. Photobiol. B: Biology*, **146**, 52 (2015); https://doi.org/10.1016/j.jphotobiol.2015.02.020.