Annals of Phytomedicine 5(1): 110-115, 2016

Journal homepage: www.ukaazpublications.com

ANNALS OF PHYTOMEDICINE
An International Journal

ISSN: 2393-9885

Antibacterial and antioxidant properties of *Argyreia osyrensis* Roth.

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Received April 15, 2016: Revised April 30, 2016: Accepted May 5, 2016: Published online June 30, 2016

Abstract

A study was designed to determine the phytochemical constituents, antibacterial and antioxidant properties of different solvent extracts of *Argyreia osyrensis* Roth. which is one of the important ethnomedicinal plants of Western Ghats. The preliminary phytochemical screening of the extract showed the presence of alkaloids, sterols, saponins, triterpenoids, flavonoids, tannins, carbohydrates, resins and glycosides, while proteins were absent in all the solvent extracts. Antibacterial activity of the plant extract offered potential antibacterial properties with a maximum zone of inhibition 26 mm in ethyl acetate extract of fruit against *P. aeruginosa*, followed by 25 mm inhibition observed against *E. coli* by the methanolic leaf extract. It was evident from the results that the plant possessed antibacterial activity against all the test pathogens. The MIC of the plant extracts revealed that a minimum of 0.156 mg/ ml and a maximum of 0.625 mg/ ml of the extracts were required to inhibit the test pathogens. Further, the studies on DPPH radical scavenging activity confirmed that the plant extracts contained natural antioxidants, which was dose dependent. The results obtained in this study justify the use of *A. osyrensis* by traditional medical practitioners for various ailments.

Key words : *Argyreia osyrensis* Roth., phytochemical screening, antibacterial activity, antioxidant activity, DPPH

1. Introduction

Natural products have gained considerable attention in the 21st century as they are known to posses greater biological activities with minimum side effects. Plants have been the main source for isolation of natural products due to its vast availability and considered as the oldest existing complete medical system in the world (Dias *et al*., 2012). In India, it is estimated that 17,000 plant species exist and among these, 2000 species of medicinal plants are used by several ethnic communities in India for their medicinal properties. The failures of chemotherapeutics in addition to antibiotic resistance exhibited by pathogenic microbial infectious agents, have led to the isolation of various novel metabolites from plants for their potential biological activities.

It has been noted that, the present day antibiotics have been losing their effectiveness against pathogens and to maintain these, utilization of various medicinally important plants for their biological activities is the need of the hour and challenging (Van der Waaij and Nord, 2000; Brahmachari, 2011; Singh *et al*., 2012). Natural antioxidants from plants have been known to reduce the risk of certain diseases including cancer, stroke and heart (Prior and Cao, 2000). The oxidative stresses in the body generate reactive oxygen species (ROS) which have the ability to damage the pathophysiology leading to several disease including cardiovascular dysfunction, atherosclerosis, inflammation, carcinogenesis, *etc*. (Aruoma, 1998).

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The harmful effects caused due to the oxidative stress can be blocked by the antioxidants which scavenge the free radicals and nullify their damaging effect on cellular constituents (Praveena and Pradeep, 2012).

Plants are known to possess a wide variety of free radical scavenging molecules like phenolics, flavonoids and other secondary metabolites that are rich in antioxidant activity (Cai *et al*., 2003). Antioxidants are considered as possible protective agents reducing oxidative damage to the human body (Yam *et al*., 2008). Many of these phenolic compounds also possess other functional attributes like antimutagenic, hypocholestemic, antimicrobial, antiplatelet aggregation and anti-inflammatory properties (Praveena and Pradeep, 2012; Hakkim *et al*., 2013).

The species of *Argyreia* are known for their medicinal properties and the genus includes around 90 species which are distributed around tropical and subtropical regions of the world (Shu, 1995). There are various reports on different species of *Argyreia* having medicinal properties. The whole plant of *A. nervosa* is used traditionally in Indian medicine for treating stomach complaints, sores on foot, small pox, syphilis, dysentery and diarrheal, antifertility, antirheumatic, antifungal, cerebral disorders, diseases of nervous system, *etc*. (Das, 2003; Krishaveni and Thakur, 2009). Similarly, *A. spesioca* is well known for antidiabetic, antifungal, antioxidant, anti-inflammatory, immunomodulatory and hepatoprotectivity (Gokhale *et al*., 2003; Galani and Patel, 2009). To the best of our knowledge, there have been no published reports on biological activities of *A. osyrensis*. Hence, the present study was carried out to find out the presence of secondary metabolites, antibacterial and antioxidant properties of leaf and fruit extracts of *A. osyrensis*.

2. Materials and Methods

2.1 Collection of plant materials

The fresh plant material (leaves and fruits) of *A. osyrensis* Roth. was collected from Western Ghats of Karnataka, India. The collected plant was identified with the help of Flora of Presidency of Madras (Gamble, 1935) and a Voucher Specimen No. 1142 is deposited in the Herbarium, Department of Studies in Botany, University of Mysore, Manasagangotri, Mysore, Karnataka, India.

2.2 Preparation of plant extract

The collected leaves and fruits were washed in running tap water and then shade dried to complete dryness and powdered using wearing blender. 50 g of dried leaf and fruit powder was filled in the thimble separately and successively extracted with petroleum ether, chloroform, ethyl acetate and methanol based on their polarity with the help of Soxhlet extractor. After extraction, each of the collected extract was subjected to flash evaporation and the concentrated extracts were stored at 4°C in air tight vials (Harborne, 1973).

2.3 Phytochemical screening

The collected plant extracts were subjected to qualitative phytochemical screening for the identification of various classes of active chemical constituents, using the methods described by Harborne (1973) and Trease and Evans (1987).

2.4 Evaluation of antibacterial activity

2.4.1 *Test organisms*

All the extracts of the plant were screened for antibacterial activity against indicator bacteria including both Gram-positive (*Staphylococcus aureus* MTCC 7443 and *Bacillus subtilis* MTCC 121) and Gram-negative (*Escherichia coli* MTCC 7410 and *Pseudomonas aeruginosa* MTCC 1688) bacteria obtained from Microbial Type Culture Collection and Gene Bank (MTCC), India and used throughout the study. All the bacterial cultures were adjusted to 0.5 McFarland standards containing approximately $1.5x10^8$ cfu/ ml (Schwalbe *et al*., 2007).

2.4.2 *Disc diffusion method*

The antibacterial screening of the plant extracts was carried out by disc diffusion method (Elecyinimi, 2007). The test bacteria ($1.5 x10^8$ cfu/ ml) were seeded onto the surface of nutrient agar (NA) media and uniformly spread using sterile glass spreader. Each sterile disc (6 mm) was loaded with 50 µl of plant extract (1 mg/ disc) and 50 µl of respective solvent and equidistantly placed on NA plates, while streptomycin discs (10 µg/disc) were served as control. The plates were incubated at 37° C for 24 h and zone of inhibition around the disc were measured. The experiment was carried out in three replicates.

2.4.3 *Determination of minimum inhibitory concentration*

Minimal inhibitory concentration (MIC) was determined according to the method of Sarker *et al.* (2007). The crude plant extracts were diluted to a concentration of 40 mg/ ml, which served as a stock solution. 100 µl of broth was dispensed into each well and a two-fold serial dilution was carried out (2 - 0.0009 mg/ ml). A 10 µl inoculum suspension was added to each well and incubated at 37° C for 24 h and absorbance was measured at 517 nm using ELISA plate reader. MIC was also detected by adding 10 µl/ well of TTC (2,3,5-triphenyl tetrazolium chloride at 2 mg/ ml) and incubated for 30 min. The MIC value taken at the lowest concentration at which the color change was noticed. All MIC tests were carried out in triplicates.

2.5 DPPH radical scavenging activity

The DPPH free radical scavenging activity (RSA) of plant extracts were determined by DPPH method (Sultanova *et al*., 2001). All the collected plant extracts were subjected for DPPH free RSA at 200, 400, 600, 800 and 1000 µg/ ml. The reaction mixture containing 5 µl of extract and 95 µl of DPPH (300 µM) in methanol was incubated at 37±2º C for 30 min and absorbance was measured at 517 nm. The reaction mixture containing ascorbic acid and only DPPH served as positive and negative controls, respectively. The experiment was carried out in triplicates and per cent RSA was calculated using the formula :

$$\% \text{ Radical Scavenging Activity} = \frac{\text{Absorbance of Control} - \text{Absorbance of Test Sample}}{\text{Absorbance of Control}} \times 100$$

2.6 Statistical analysis

All the experiments were conducted in three replicates and the obtained data was subjected to arcsine transformation and analysis of variance (ANOVA) using SPSS Inc. 16.0 by Tukey's Honestly Significant Differences (HSD) test with F value ($p \leq 0.05$).

3. Results

3.1 Phytochemical screening

The collected plants material were extracted sequentially with solvents, *viz.*, petroleum ether, chloroform, ethyl acetate and methanol based on polarity index. The solvent extracts of both leaf and fruit of *A. osyrensis* were subjected to qualitative phytochemical screening. The results of the present study revealed that both leaf and fruit extracts of *A. osyrensis* showed the presence of alkaloids, sterols, saponins, flavonoids, glycosides, tannins, triterpenes, carbohydrates, while resins were present only in leaf extracts. Likewise, proteins were absent in all the extracts of both leaf and fruit of the selected plant (Table 1).

3.2 Evaluation of antibacterial activity

3.2.1 *Disc diffusion method*

The crude solvent extracts of *A. osyrensis* were further subjected for their potential to inhibit test bacterial pathogens. The results of antibacterial activity showed a significant zone of inhibition against test pathogens in methanol and ethyl acetate extracts when compared to other solvent extracts (Table 2). Among the test pathogens, methanolic extract of the leaf showed a maximum zone of inhibition of 25 and 21 mm against *E. coli* and *Staph aureus* (Figure 1), while 22 and 26 mm of zone of inhibition to *B. subtilis* and *P. aeruginosa*, respectively was observed with ethyl acetate extract of fruit. There was no zone of inhibition against *E. coli* in chloform and against *E. coli* and *B. subtilis* in petroleum ether extract of leaf, respectively.

3.2.2 *Minimum inhibitory concentration* (MIC) *by micro broth dilution assay*

The results of the minimum inhibitory concentration (MIC) of *A. osyrensis* against the test bacteria are presented in Table 3.

Table1: Preliminary phytochemical analysis of *A. osyrensis* Roth.

Phytochemical Tests	Leaf				Fruit			
	PE	CH	EA	ME	PE	CH	EA	ME
Alkaloids								
a. Mayer's test	+	-	+	+	-	+	+	-
b. Dragendroff's test	-	-	+	+	-	+	+	-
c. Wagner's test	+	-	-	-	-	+	-	-
d. Hager's test	-	-	-	-	-	-	+	-
Carbohydrates								
a. Molisch's test	+	-	-	+	-	-	+	+
b. Fehling's test	+	-	-	+	-	-	-	+
c. Benedict's test	-	-	-	+	-	-	-	+
Flavonoids								
a. Shinado test	+	+	+	+	+	-	+	-
b. $FeCl_3$ test	+	+	+	+	+	-	+	-
c. Lead Acetate test	-	+	+	-	-	-	+	-
Glycosides								
a. Keller-Killiani test	+	-	+	+	+	+	+	+
Proteins								
a. Biuret test	-	-	-	-	-	-	-	-
b. Ninhydrin test	-	-	-	-	-	-	-	-
Resins								
a. Turbidity test	+	-	-	+	-	-	-	-
b. Acetic anhydride test	+	-	-	-	-	-	-	-
Saponins								
a. Foam test	+	+	+	+	+	+	+	+
Sterols								
a. Salkowski test	-	+	-	+	-	+	-	+
b. Libermann-Burchardts test	-	+	-	+	-	+	-	+
Tannins								
a. $FeCl_3$ test	-	+	-	+	+	-	+	-
b. Gelatin test	-	+	-	+	+	-	+	-
Triterpenes								
a. Salkowski test	+	-	+	-	+	-	+	+
b. Libermann-Burchardts test	+	-	+	-	+	-	+	+

Note : '+' Present and **'–'** Absent**, Pe**-Petroleum Ether**, Ch**-Chloroform**, Met**-Methanol**, Ea**-Ethyl Acetate

Table 2: Antibacterial activity of different solvent extracts of *A. osyrensis* Roth. (inhibition zone measured in mm)

Extract	Solvent	Zone of inhibition (mm)			
		E. coli	***B. subtilis***	***Staph. aureus***	***P. aeruginosa***
Leaf	Petroleum ether	0.00 ± 0.00^{g}	0.00 ± 0.00^{g}	14.12 ± 0.31^{f}	16.37 ± 0.23^{e}
	Chloroform	0.00 ± 0.00^{g}	12.00 ± 0.29^{e}	10.20 ± 0.14^{h}	10.05 ± 0.29^{h}
	Ethyl acetate	20.25 ± 0.17^{c}	23.10 ± 0.23^{a}	22.25 ± 0.13^{b}	21.87 ± 0.38^{b}
	Methanol	25.00 ± 0.51^{a}	21.05 ± 0.09^{c}	21.37 ± 0.23^{c}	20.35 ± 0.22^{c}
Fruit	Petroleum ether	12.02 ± 0.17^{f}	10.00 ± 0.18^{f}	10.35 ± 0.15^{h}	14.30 ± 0.15^{g}
	Chloroform	13.20 ± 0.14^{e}	10.10 ± 0.12^{f}	11.17 ± 0.15^{g}	15.25 ± 0.12^{f}
	Ethyl acetate	19.02 ± 0.30^{d}	22.00 ± 0.21^{b}	19.15 ± 0.17^{e}	26.00 ± 0.24^{a}
	Methanol	20.95 ± 0.09^{c}	18.20 ± 0.14^{d}	20.00 ± 0.12^{d}	19.05 ± 0.17^{d}
Control (Streptomycin)		22.05 ± 0.15^{b}	21.07 ± 0.14^{c}	24.02 ± 0.06^{a}	19.10 ± 0.10^{d}

Values are means of three independent replicates ± indicate standard error. Means followed by the same letter(s) within the same column are not significantly different according to Tukey's HSD.

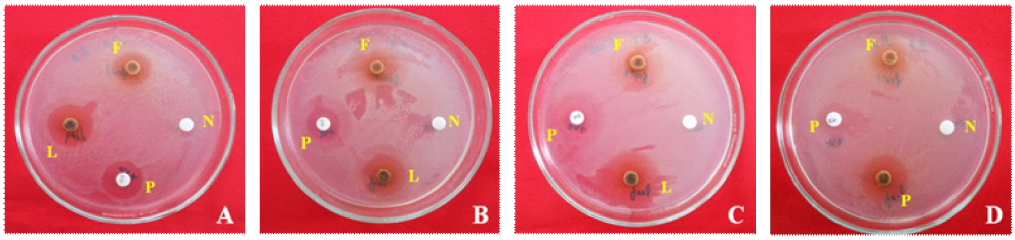

Figure 1: Antibacterial activity of *A. osyrensis* Roth. methanol extract against test pathogens by disc diffusion method. A: *B. subtilis*; B: *S. aureus*; C: *E. coli*; D: *P. aeruginosa*; N: Negative control; P: Positive control; L: Leaf extract; F: Fruit extract

Table 3: MIC of different solvent extracts of *A. osyrensis* Roth. against test bacteria (mg/ ml)

Extract	Solvent	Test organisms			
		E. coli	*B. subtilis*	*Staph. aureus*	*P. aeruginosa*
Leaf	Petroleum ether	00	00	0.156	0.156
	Chloroform	00	0.156	0.312	0.312
	Methanol	0.156	0.156	0.078	0.156
	Ethyl acetate	0.312	0.156	0.156	0.156
Fruit	Petroleum ether	0.625	0.625	0.625	0.625
	Chloroform	0.312	0.312	0.312	0.312
	Methanol	0.312	0.312	0.156	0.156
	Ethyl acetate	0.625	0.312	0.625	0.312
Control	(Streptomycin)	0.015	0.015	0.007	0.015

Values are means of three independent replicates

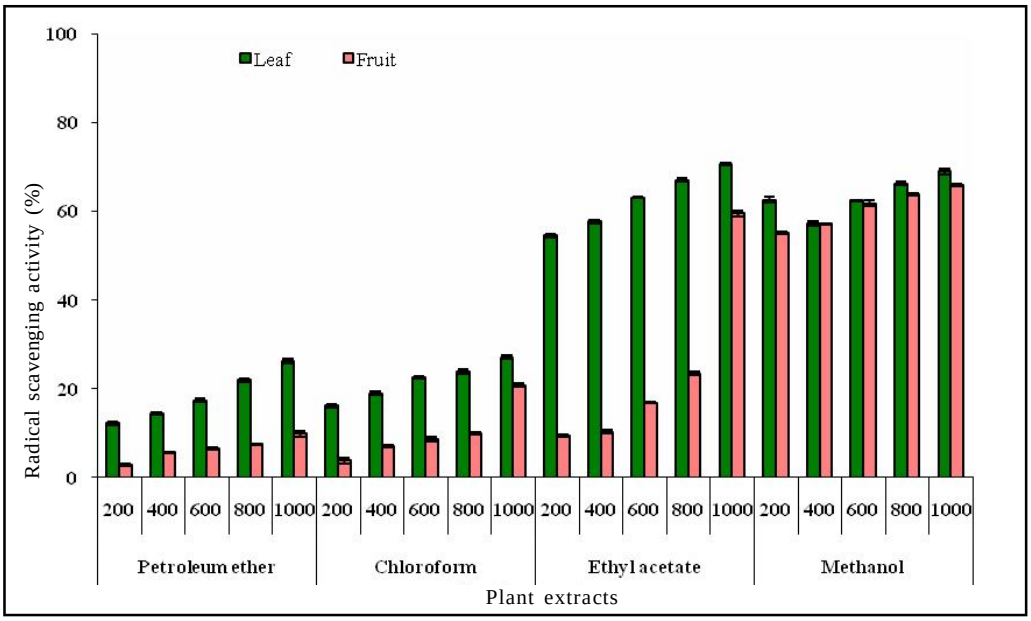

Figure 2: DPPH radical scavenging activity of different solvent extracts of *A. osyrensis* Roth. at different concentrations. Values are means of three independent replicates ± standard error

The results of the MIC revealed that irrespective of the solvent extract (fruit or leaf) used in the study, except for petroleum ether and chloroform extracts of leaves inhibited the growth of test bacteria to a varying degree. Among the different extracts tested, methanolic extract of leaves offered minimum MIC values ranging from 0.078 (against *Staph. aureus*) to 0.156 mg/ ml (to all other test

bacteria), followed by ethyl acetate (leaf) and methanol (fruit) extracts. It was evident from the study that a minimum of 0.078 mg/ ml and a maximum of 0.625 mg/ ml were sufficient to inhibit the growth of test bacteria.

3.3 DPPH radical scavenging activity

Antioxidant activity of leaves and fruit extracts of *A. osyrensis* was measured as free radical scavenging activity (RSA) is presented in Figure 2. The results of the study revealed that the RSA of the extracts was dose dependent as it increased with increase in the concentration of the extract. Among the extracts tested, ethyl acetate and methanol extracts of leaf and fruit offered more than 50% inhibition while the same was not observed with petroleum ether and chloroform extracts. The ethyl acetate extract of leaf showed a significant RSA of 54, 57, 63, 67 and 70% at 200, 400, 600, 800 and 1000 µg/ ml concentration, respectively followed by methanol leaf extract. Reference standard ascorbic acid showed 75% of inhibition at 50 µg/ml which was also dose dependent.

4. Discussion

The presence of phytochemical constituents in plants is of great importance as they contribute to its medicinal value as well as exhibit physiological activity (Sofowora 1993). The phytochemical constituents like alkaloids, tannins, flavonoids, saponins, sterols, triterpenes, *etc*., are synthesized or metabolized in plants for defense purpose which may be toxic or useful to man (Usman and Osuji, 2007). Likewise, these phytochemical constituents possess various protective and therapeutic effects (Mir *et al*., 2013).

The preliminary phytochemical screening of *A. osyrensis* leaf and fruit extracts showed the presence of alkaloids, carbohydrates, flavonoids, glycosides, saponins, resins, sterols, tannins and triterpenes were present at least in any one of the solvent extracts of both leaf and fruit of the plant. It was also noticed from the results that, the presence of proteins were absent in all the extracts of leaf and fruit of the plant. Likewise, Das *et al*. (2015) have reported the presence of lignans, protein, carbohydrate, flavonoids and alkaloids in the ethanolic extract of *Taxus wallichiana*. It is noted from the literature that, the presence of secondary metabolites in plant extracts is of great importance and contribute to its medicinal value as well as exhibiting physiological activity (Sofowora, 1993). Among the secondary metabolites detected, alkaloids and tannins are known for its various pharmacological activities, including spasmolytic activity in smooth muscle cells (Cragg and Newman, 2005). Likewise, flavonoids have been reported to have excellent free radical scavenging properties, disease prevention and therapeutic properties (Annegowda *et al*., 2010). Similarly, the presence of phenols and flavonoids in crude plant extracts has been linked to their activities against disease causing bacteria (Ruiz-Teran *et al*., 2008). Akharaiyi *et al*. (2012) have reported that flavonoid, phenols, alkaloids and saponin contents in *Gliricidia sepium* and *Spathodea campanulata* extracts for their antibacterial potentials.

Based on the preliminary phytochemical analysis, the solvent extracts of leaf and fruit of *A. osyrensis* was further subjected to their antibacterial and antioxidant potential. The results of antibacterial studies showed that except for petroleum ether and chloroform extracts of leaf, all the other solvent extracts exhibited antibacterial activity for test pathogens. The highest inhibition exhibited on the test bacteria was 25 mm (against *E. coli*) by the methanolic leaf extract of the plant; in contrast a maximum of 21 mm of inhibition against test pathogen was obtained from the same solvent extract of fruit. Likewise, Chattopadhyay *et al*. (2001) reported that the crude extracts of *Alstonia macrophylla* showed the presence of flavonoids, triterpenes and steroids and in turn also offered significant activity against various strains of *Staph aureus* and *E. coli*. In the earlier study by Akharaiyi *et al*. (2012), *Spathodea campanulata* crude extract was effective against *E. coli, S. aureus, B. cereus* and *P. aeruginosa*. Similarly, the crude methanol and chloroform extracts of *Piper betle* recorded significant antibacterial activity and also showed the presence of various phytochemicals. Further, MIC of the studied extracts also showed inhibition to test pathogens in the range of 625 to 156 µg/ ml (Jayalakshmi *et al*., 2015). In addition, the ethanolic extract of *L. intermedia* leaf and stem of the plant showed the MIC at 195 µg/ ml against *M. aurum* and 780 µg/ ml against *S. aureus* (Madikizela *et al*., 2013). The results of MIC of the present study have pointed out the least concentration at which the leaf and fruit extracts of the studied plant were bactericidal to test bacterial isolates. It may be noted that the antibacterial activity of the extracts tested in the study may be due to the presence of secondary metabolites and very importantly, due to the presence of saponins. The findings of the present study corroborate with the work of Sparg *et al*. (2004) and Negi *et al*. (2010) where they have reported the antimycobacterial effect of plant extracts is due to the presence of saponins in the extracts.

Further, the leaf and fruit extracts of the selected plant were subjected to DPPH radical scavenging activity. Similarly, Riddhi and Yogesh (2012) and Sravanthi *et al*. (2013) have also carried out DPPH radical scavenging activity for aromatic herbs and *Ocimum sanctum*, respectively. The results of the present study showed that among the extracts tested, methanol and ethyl acetate extracts of both leaf and fruit offered potent radical scavenging activity. It was also noted from the results that the antioxidant activity of the extract was dose dependent and these results are in good agreement with the findings of Moukette *et al*. (2015). Likewise, Puneetha *et al*. (2013) have also reported dose dependent antioxidant activity in the leaf extracts of *Dendrophthoe trigona*, a parasitic angiosperm. The results of the study may be attributed to the presence of flavonoids and phenolic compounds, wherein phenolic compounds are recognized for their act as free radical terminators and also exhibit physiological functions in plants (Shahidi and Wanasundara, 1992; Safowara, 1993). Likewise, flavonoids are known to possess hydroxyls which are responsible for radical scavenging effects in most plants (Das and Pereira, 1990).

5. Conclusion

From the above results, it could be stated that the plant selected in the present study offered good inhibition against test bacterial isolates which are in importance in the present day situation. The plant extracts apart from offering antibacterial activity, also comprised radical scavenging activity which was dose dependent. In conclusion, it can be stated that the plant *A. osyrensis* used in the present study include a large number of secondary metabolites which in turn lead to antibacterial and antioxidant activities. However, further studies involving isolation and purification of the active principle(s) are warranted for its better utilization as a therapeutic agent.

Acknowledgement

The authors are grateful to the University of Mysore, Mysore for financial support through Institution of Excellence (IOE) Project and DST-FIST of the department.

Conflict of interest

We declare that we have no conflict of interest.

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