INTRODUCTION

Fungal endophytes are those organisms which establish mutuality with the tissues of actively growing plants. It has been thought that all plants in natural ecosystems bear endophytes as their symbionts [1]. Endophytes play an important role in plants in overcoming stress condition and better living under variable environment. Fungal endophytes are capable to produce several bioactive compounds (phenol, saponin, flavonoid, etc.) which help the host plants to defend against pathogenic attack. Endophytes perform antioxidant activity against reactive oxygen species (ROS) like superoxide, hydroxyl radical and hydrogen peroxide produced in the metabolic process within the living cells of all organisms. The free radicals play a beneficial role in cell signaling at low or moderate concentration, become harmful when produced in excess causing oxidative stress leading to damage of DNA, RNA, proteins and lipids. In human body excess production of free radicals enhances the risk of cardiovascular disease, cancer, autism, etc. To scavenge such free radical endogenous production of an antioxidant has been found insufficient enough and therefore exogenous supply of antioxidants is earnestly needed to make a balance between the level of free radicals and antioxidants. Fungal endophytes may act as an alternative easily available low cost source of antioxidant.

Calotropis procera (L.) R. Br. belonging to the family Asclepiadaceae is a common wild shrub grown in tropical countries like India, Bangladesh and Pakistan. This medicinal plant is well known for its role in curing disease like arthritis, skin diseases, bronchial problems, muscular sprains and joint pain etc.

Catharanthus roseus (L.) G. Don. belonging to the family Apocynaceae is a commonly cultivated ornamental herb in tropical countries. It is well-known for its anticancer and anti-diabetic properties.

A few investigations on both Calotropis procera (L.) R. Br. and Catharanthus roseus (L.) G. Don. has been carried out in relation to their endophytic inheritance. It is, however clear that the endophytes and their metabolic activity vary greatly in the same plants depending on the ecological factors like geographical locations [2, 3], differences in site [4], microclimate [5] etc. Studies of endophytic inheritance and formation of silver nanoparticles on both Calotropis procera and Catharanthus roseus have been carried out by the present research group [6, 7]. Moreover, the lack of information on bioactive compounds, including antioxidant activities of fungal endophytes in those two plants from Eastern India has led the investigators to undertake the present studies.

MATERIALS AND METHODS

Collection of plant materials

Leaves and stem bits of healthy Calotropis procera and Catharanthus roseus was collected from different parts of Burdwan district (latitude and longitude: 23.4595° N, 87.6186° E) of West Bengal, India, in the month of July to August. The plant material was brought to the laboratory in sterile polythene bags and processed within a few hours after collection. Fresh plant materials were used for isolation of endophytic fungi to reduce the chance of contamination.

Both the plant material was authenticated at Department of Botany, Visva-Bharati, Santiniketan, West Bengal where two herbarium voucher specimen was deposited (Accession No. 1037 and 1038).

Chemicals and reagents

All ingredients for media, chemicals, reagents, the substrates used in this study are of the highest purity, i.e. AR, Extra pure grade and...
Isolation and pure culture development of endophytic Fungi from *Calotropis procera* and *Catharanthus roseus* were carried out using the standard protocol with little modification [8].

The leaf of two medicinal plants was washed under running tap water for 1 h followed by sterile double distilled water (dH₂O). The leaves were cut into small pieces with the help of sterilized cork borer. The samples were then immersed in ethanol (70%) for 1 min, followed by sodium hypochlorite (4%) for 2 min and then rinsed with sterile distilled water (2-3 times). The excess moisture was blotted with the help of sterile filter paper (Whatman No. 1). The surface sterilized 4 leaf segments were placed in petri plates containing potato dextrose agar (PDA) media supplemented with streptomycin 100 units/ml concentration. The petri plates were sealed using parafilm and incubated at 29±1 °C for 18 d. The petri plates were monitored regularly to check the growth of endophytic fungal colonies from the segments. The isolated hyphal tips of the organism were then subcultured in PDA slants to obtain a pure culture of the endophytic fungi.

**Identification of endophytic fungi**

The isolated fungi were identified based on cultural characteristics and available reproductive structures following mainly the protocol of Burnett and Hunter [9] Gilman [10], Whatnabe [11].

**Calculation of colonization frequency**

Colonization Frequency (CF %) of endophytic fungi was calculated by using the following formula [12, 6].

\[ \text{Colonizing frequency} = \frac{\text{Number of segment colonized by fungi}}{\text{Total number of segments analyzed}} \times 100 \]

**Fermentation and extraction**

Fungal inoculums obtained from pure culture were inoculated into 250 ml Erlenmeyer flask containing 100 ml potato dextrose broth and incubated for 18 d at 29±1 °C at 120 rpm/min with constant shaking. The fungal culture was filtered; the filtrate was extracted with ethyl acetate (1:1 ratio) for three times. The organic phase was evaporated to dryness and stored at 4 °C for further use. The crude extract was dissolved in dimethyl sulfoxide (DMSO) to obtain different a concentration.

**Qualitative detection of bioactive compounds**

The ethyl acetate extract of the dominant endophytic fungi was used to detect the presence of the secondary metabolites such as alkaloids, phenols, flavonoids, saponins, cardiac glycosides, terpenoids and tannins following standard procedures.

**Quantitative determination of total phenol content**

The concentration of phenolics in fungal extracts was determined using spectrophotometric methods [13]. A methanolic solution of the extract (1 mg/ml) was used for the analysis. The reaction mixture was prepared by mixing 0.5 ml of methanolic extract with 2.5 ml of 10% Folin-Ciocalteu’s reagent dissolved in water and 2.5 ml 7.5% NaHCO₃. Blank was concomitantly prepared, containing 0.5 ml methanol, 2.5 ml 10% Folin-Ciocalteu’s reagent (prepared with water) and 2.5 ml of 7.5% of NaHCO₃. The samples were then incubated in a thermostat at 37 °C for 45 min. The absorbance was checked using UV-VIS spectrophotometer at a wavelength of 765 nm. The samples were prepared in triplicate for each analysis to obtain the mean value of absorbance. The same procedure was followed for the standard curve of gallic acid. The phenol content in the fungal extracts was derived from the standard curve; the results expressed as mg of gallic acid eq. per g dry weight of the extract. The values were expressed as mean±SD.

**Quantitative determination of total flavonoid content**

Total flavonoid content was measured using the standard protocol of aluminium chloride assay [14]. Sample (1 ml) was mixed with 4 ml of distilled water and 0.3 ml of sodium nitrite solution (5% w/v), allowed to stand for 5 min. Aluminum chloride solution (10%, 0.3 ml) was added to the sample mix followed by the addition of 0.2 ml of NaOH (1 M) after 1 min. The volume was made up to 10 ml with distilled water and mixed thoroughly. The absorbance was measured at a wavelength of 510 nm in UV-VIS spectrophotometer. Different concentration of quercetin (100-1000 μg/ml) was used for preparing the standard curve. The experiment performed in triplicates and the standard curve was then plotted using the optical density 0.0 values obtained for quercetin. The total flavonoid content was calculated from the standard curve, the result expressed as mg quercetin equivalent per g dry weight of the fungal extract. The values were expressed as mean±SD.

**Antioxidant assay**

(a) Determination of antioxidant activity of phosphomolybdenum (total antioxidant capacity) assay

The antioxidant activity of endophytic fungi was determined by a colourimetric method using ascorbic acid as standard [15]. An unknown sample (1 ml) was mixed with ammonium molybdate (4 mmol, 1 ml) followed by disodium hydrogen phosphate (28 mmol, 1 ml). Then the mixture was incubated at 37 °C for 30 min after the addition of 2 ml of sulfuric acid (0.6 M). A standard set of ascorbic acid was prepared by taking a concentration of 20 μg-500 μg. A standard curve was used to calculate the antioxidant concentration in the fungal extract.

\[ \text{Percent Inhibition} = \frac{A_c - A_s}{A_c} \times 100 \]

Where "Ac" is the absorbance of control, and "As" is the absorbance of a solution containing sample extracts.

**Statistical analysis**

All assays were carried out in triplicate and the results were expressed as a mean ± standard deviation (SD).

**RESULTS AND DISCUSSION**

**Isolation and identification of endophytic fungi**

All the isolated endophytic fungi were identified on the basis of cultural characteristics, reproductive structure and molecular basis. Three dominant endophytic fungi out of ten (higher CF %) were selected from *C. roseus* for further studies. Among eight isolates of *C. procera* three dominant endophytic fungi (higher CF %) were selected for further studies. Endophytic fungi selected were *Penicillium singorense* Visage, Selbert and Samson; *Aspergillus neoflavipes* Hubka, Novakova, Kolarik and Peterson; *Curvularia geniculata* (Tray and Earle) Boedijn; *Alternaria alternata* and *Nigrospora* sp. *DRC4 MH021686 Alternaria alternata* roseus, which were subjected to plate culture showed growth of fungal endophytes in most of the sample. A total of eight fungal endophytes from *C. procera* and ten fungal endophytes from *C. roseus* were obtained. Out of eight fungal isolates of *C. procera* three (all from leaf discs) isolates namely *Penicillium singorense* (CF% = 25%), *Curvularia geniculata* (CF% = 12.5%) and *Aspergillus neoflavipes* (CF% = 21.87%) were found to be dominant. Out of ten fungal isolates of *C. roseus* three (from leaf discs) namely *Alternaria alternata* (CF% = 18.7%), *Nigrospora* sp (CF% = 12.5%) and *Penicillium singorense*
(CF%=18.75%) were dominant (fig. 1). The occurrence of Aspergillus neoflavipes, Penicillium singorense and Curvularia geniculata as fungal endophytes in C. procera has been reported by several researchers [17-20]. The occurrence of Alternaria alternata, Penicillium singorense and Nigrospora sp as fungal endophytes has also been investigated [17, 19, 21, 22].

Qualitative detection of secondary metabolites

It has been revealed that Penicillium singorense isolated from both plants and A. neoflavipes (isolated from C. procera) were found to be able to produce all the functional metabolites so far tested (table 1). Curvularia geniculata (from C. procera) and Alternaria alternata (from C. roseus) produced all the secondary metabolites except saponins. The inability for the production of saponin as well as terpenoids was also observed in Nigrospora sp (isolated from C. roseus). Although all the isolates showed more or less efficient (as observed from intensity of color) for the production of alkaloids, flavonoids and tannins, terpenoids were found to be produced in highest extent in P. singorense and A. alternata, saponins (highest extent of frothing) by P. singorense and phenols (dark bluish green coloration) by P. singorense, Curvularia geniculata and Nigrospora sp. Ability to produce bioactive compound of the fungal extract has also been reported in recent times [20, 23-25].

| Endophytic fungi       | Phenol | Flavonoids | Saponins | Terpenoids | Alkaloids | Tannins |
|------------------------|--------|------------|----------|------------|-----------|---------|
| Penicillium singorense | ++     | +          | ++       | +          | +         | +       |
| Aspergillus neoflavipes| +      | +          | +        | -          | +         | +       |
| Curvularia geniculata  | +      | +          | -        | -          | +         | +       |
| Alternaria alternata   | +      | +          | -        | ++         | +         | +       |
| Nigrospora sp          | ++     | +          | -        | -          | +         | +       |
| Penicillium singorense | ++     | +          | ++       | +          | +         | +       |

"+=present"-="absent"++="present in high conc.

Total phenol and flavonoid content

Total phenol content was estimated using Folin-Ciocalteau reagent; it is a simple, convenient and reproducible method. It is routinely used for the estimation of phenolic compounds [26]. Phenol content of fungal endophytes was estimated both in culture filtrate and mycelium. It is evident that the phenol content in the culture filtrate of all the fungi was higher than that of in the mycelium. Considering all the fungal isolates both from C. procera and C. roseus it is confirmed that C. geniculata is the best producer of phenol followed by Nigrospora sp. It is evident that the flavonoid content was also found to be higher (fig. 3) in the culture filtrate of the fungal endophyte than that of in the mycelium. Highest extent of flavonoid content in the culture filtrate was observed in C. geniculata (2.06±0.1 mg/g) isolated from C. procera followed by Nigrospora sp (1.55±0.28 mg/g) isolated from C. roseus.

Fig. 1: Graphical representation of colonization frequency of isolated endophytic fungi from the leaf of Calotropis procera and Catharanthus roseus

Fig. 2: Graphical representation of the phenol content of the culture filtrate and mycelium, all assays carried out in triplicate; mean data were plotted to the graph. "I" on top of the bar denote the value of standard deviation (±SD)

Fig. 3: Graphical representation of the flavonoid content of the fungal culture filtrate and mycelium, all assays carried out in triplicate; mean data were plotted to the graph. "I" on top of the bar denote the value of standard deviation (±SD)
Antioxidant activity

The antioxidant activity of culture filtrate and mycelium of the dominant fungal endophyte was determined following two different methods viz. phosphomolybdenum method and DPPH scavenging activity. The phosphomolybdenum method has been routinely used to evaluate the total antioxidant activity of different samples. In the presence of culture filtrate or mycelium extract, the molybdenum Mo (IV) is reduced to Mo (V) and as a result a green coloured phosphomolybdenum–V complex formed which showed maximum absorbance at 695 nm. All the tested fungal endophytes isolated from C. procera and C. roseus showed their hydrogen donating efficiency and as a result, high inhibition percentage was obtained (fig. 4). It is evident that the total antioxidant activity in the case of the culture filtrate of all the samples was higher than that of the mycelium. Highest antioxidant activity in the culture filtrate was noted in Curvularia geniculata (2.46±0.11) isolated from C. procera followed by A. Neoflavipes (0.62±0.05) and P. singorense (0.39±0.04). The culture filtrate of fungal isolates of C. roseus, Nigrospora sp (2.03±0.09) showed the highest extent of antioxidant activity followed by A. alternata (0.80±0.11 %) and P. singorense (0.39±0.04) (fig. 4). It is further evident that the culture filtrate of C. geniculata isolated from C. procera was exhibiting total antioxidant activity than Nigrospora sp from C. roseus.

The DPPH assay is considered as a basic and most widely used assay. DPPH free radical scavenging assay is considered as a most accurate screening method used to evaluate the antioxidant activity of different samples. The DPPH free radical scavenging potential of both the culture filtrate and mycelium of each fungal endophytes was determined. It is evident that the culture filtrate of fungal isolates in all cases showed higher antioxidant activity than the mycelium extracts. Among the dominant isolates of C. procera and C. roseus highest free radical scavenging activity showed by Curvularia geniculata (94.55±0.015 %) followed by Aspergillus neoflavipes (30.02±0.02 %) and P. singorense (24.29±0.03 %). Considering the isolates from C. roseus, Nigrospora sp proved to be the highest efficient endophyte in free radical scavenging activity (89.48±0.03 %) followed by A. alternata (74.21±0.32 %) and P. singorense (26.17±0.048 %) (fig. 5). Considering the efficacies of the isolates, C. geniculata from C. procera proved to be the best, followed closely by Nigrospora sp from C. roseus, followed closely by Nigrospora sp. Antioxidants act as free radical scavengers, inhibit lipid peroxidation and other free radical-mediated metabolic processes. In this way antioxidant is able to protect the human body from several diseases attributed to the reaction of radicals such as aging, cancer, neurodegenerative disorders, atherosclerosis and inflammations. Consumption of synthetic antioxidant has been reported to show toxic side effects, thus demanding the search for natural antioxidants and free radical scavengers. DPPH assay is widely accepted in natural product antioxidant studies, because antioxidant donate a proton to this radical to decrease the absorption. The antioxidant effect is proportional to the DPPH free radical conversion to DPPH by anti-oxidant compound [27].

Endophytic fungi isolated from mangrove plants, namely Phomopsis amygdala, Trichoderma sp and Alternaria sp have shown high antioxidant activities against various free radicals which conforms the present study [28-30]. Studies on antioxidant activity, including the production of phenol, flavonoids by endophytic fungi Penicillium sp and Aspergillus sp from C. procera have shown similar result [28]. Antioxidant properties of endophytes from C. procera and C. roseus have also been investigated and showed that Aspergillus sp has a good response in terms of total phenolic content and antioxidant properties [31]. Recently the antiplasmodial activity of endophytic fungi strains namely IP-2 and IP-isolated from Artemisia annua L. been reported [32]. The active metabolites of endophytic fungi, Aspergillus flavus isolated from the infected cadavers of butterfly (Delias eucharis) have been successfully used as an eco-friendly, reducing agent to generate AgNPs and synthesized nanoparticles showed antimicrobial properties against several human pathogens [32]. Production and anticancer activity of, camptothecin, a novel compound isolated from the Betel vine (Piper betel L.) endophyte fungus Aspergillus niger against colon cancer cell line has studied very recently [34]. The search and application of bioactive compound from endophytic fungi is gaining momentum, so our study probably might be helpful for further investigation in relation to such compound.

CONCLUSION

The present study, therefore, highlights the growing concept that the bioactive compounds produced by the endophytes not only established host endophyte relationship but also have an immense chance of an application in the field of medicine, agriculture and industry. Because of their dual role in providing the ability to the host plant to overcome stress conditions and acting as a source of pharmaceutical important secondary metabolites, they are expected to become an important component of fungal biology. Moreover, they have drawn recognition as an important area of natural product research. From the perusal of literature, it is revealed that few or little attention has been made for the study of fungal endophytes of medicinal plant origin especially from the Eastern part of India. Our study anticipating providing an immense focus for further related study in this region and abroad also.

ACKNOWLEDGEMENT

Authors are thankful to the Secretary, Oriental Institute of Science and Technology for providing the facilities to perform this work. The authors are grateful to Dr. Adani Lokho, Taxonomy Laboratory,
Department of Botany, Visva-Bharati, Santiniketan, West Bengal for identification of plant and deposit the herbarium in departmental herbarium facilities. Authors are also thankful to Dr. Arpita Samanta Chanda of BIMS to perform the statistical analysis.

**ABBREVIATION**

North-N, East-E, Temperature- °C, Milliliter-ml, Microgram-µg, Millimeter-mm, Percentage-%, Milligram-mg, Gram-g, Sodium Bicarbonate-NaHCO₃, Sodium Hydroxide-NaOH, Standard Deviation-SD, Mo [IV]-Molybdenum [IV], Mo [V]-Molybdenum [V], UV-VIS spectrophotometer-Ultrasiolet spectrophotometer, OD-Optical Density, SRL-Cisco Research Laboratory, AR-Analytical Grade Reagent.

**AUTHORS CONTRIBUTIONS**

The experimental part and manuscript were done by Debjani Roy-Chowdhury. Writing, correction and revision of the manuscript were done by Dr. Swapan Kumar Chattopadhyay and Dr. Subhash Kanti Roy.

**CONFLICT OF INTERESTS**

Authors declares there is no conflict of interest

**REFERENCES**

1. Rodriguez R, Redman R. More than 400 million years of evolution and some plants still can’t make it on their own: plant stress tolerance via fungal symbiosis. *J Exp Bot* 2008;59:1109-14.

2. Fisher PJ, Petrini O, Petrini LE, Sutton BC. Fungal endophytes from the leaves and twigs of *Quercus ilex* L. from England, Majorca and Switzerland. *New Phytol* 1994;127:133-7.

3. Collado J, Patas G, Gonzales P. Geographical and seasonal influence on the distribution of fungal endophytes in Quercus ilex. *New Phytol* 1999;145:525-32.

4. Okane I, Nakagiri A, Ito T. Endophytic fungi in leaves of *Abies balsamea* with a view to plant stress tolerance via fungal symbiosis. *J Exp Bot* 1995;46:384-90.

5. Roy Chowdhury D, Roy A, Chatterjee SK, Roy SK. Endophytic fungi from medicinal plant *Calotropis gigantea* (L.) R. Br. a medicinally important plant in India. *Biodiversity conservation and sustainable Development: issues and Approaches*. Vol. I. Editor-Dr. Prithwiraj Jha by Raignjour Banga Res Forum; 2014.p. 147-56.

6. Roy Chowdhury D, Chatterjee SK, Roy SK. Studies on diversity and beneficial aspects of endophytic fungi in *Calotropis procera* (L.) R. Br. A medically important plant in India. *Indian Journal of Microbiology* 2011;51:93-7.

7. Strobel G, Ford E, Worapong J, Harper JK, Arif AM, Grant DM, et al. Isopestacin, a unique isobenzofuranone from *Pestalotiopsis microsora* possessing antifungal and antioxidant properties. *Phytochemistry* 2002;60:179-83.

8. Burnett HL, Hunter BB. Illustrated genera of Imperfect fungi. 4th edn. The American Phytopathol Society; 1998.

9. Gilman CJ. A manual of soil fungi. Oxford and IBH publishing Company Calcutta; 1957.

10. Whatanabe T. Pictorial atlas of soil and seed fungi. 3rd edn. CRC Press, Boca Raton, New York; 2010.

11. Hata K, Futaki K. Endophytic fungi associated with healthy pine needles and needles infested by the pine needle gall midge, *Thecodiplosis japonensis*. Can J Bot 1995;73:384-90.

12. Murthy K, Pushapalatha KC, Joshi GG. Antioxidant activity and phytochemical analysis of medicinal plant *Catharanthus roseus* (L.) R. Br. A medicinally important plant in India. *Arch Pharm Res* 2008;31:374-9.

13. Sathishkumar T, Baskar R, Shanmugam S, Rajasekaran P, Sadasivam S, Manikandan V. Optimization of flavonoid extraction from the leaves of *Tabernaeontana heyneana* Wall. Using L16 orthogonal design. Nat Sci 2008;6:110-21.

14. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of total antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Anal Biochem* 1999;269:377-41.

15. Babu D, Rao GN. Antibacterial activity and electrochemical behavior of cultivated commercial Indian edible mushrooms. *J Food Sci Technol* 2013;50:301-8.

16. Rani R, Sharma D, Chaturvedi M, Yadav VP. Antifungal activity of 20 different endophytic fungi isolated from *Calotropis procera* and Time-kill assay. *Clin Microbiol* 2017;6:280.

17. Hemamalini V, Mukesh Kumar DJ, Immaculate Nancy Rebeca A, Srímathy S, Muthumary J, Kabichelvan PT. Isolation and characterization of taxol-producing endophytic fungi *Phoma sp.* from *Calotropis gigantea* and its antiproliferative studies. *J Academia Industrial Res* 2015;3:645-9.

18. Selvanathan S, Indra Kumar I, JohnPaul M. Biodiversity of the endophytic fungi isolated from *Calotropis gigantea* (L.) R. Br. Recent Res Sci Technol 2011;3:94-100.

19. Nagda V, Gajbiyeh A, Kumar D. Isolation and characterization of endophytic fungi from *Calotropis procera* for their antioxidant activity. *Asian J Pharm Clin Res* 2017;10:254-8.

20. Kharwar RN, Verma VC, Strobel G. The endophytic fungal complex of *Catharanthus roseus* (L.) G. Don. *Curr Sci* 2008;95:228-33.

21. Gayathri P, Chandra M. Screening of phytochemicals and isolation of endophytic fungi from medicinal plant *Helicteres isora* L. *IJSR* 2017;7:1-5.

22. Tan RX, Zon WX. Endophytes: a rich source of functional metabolites. *Nat Prod Rep* 2001;18:448-59.

23. Yadav M, Yadav A, Yadav JP. In vitro antioxidant activity and total phenolic content of endophytic fungi isolated from *Eugenia jambolana* Lam. *Asian Pacific J Trop Med* 2014;7:256-61.

24. Desire MH, Bernard F, Forsah MR, Assang CT, Denis ON. Enzymes and qualitative phytochemical screening of endophytic fungi isolated from *Lantana camara* Linn. *Leaves*. *J Appl Biol Biotechnol* 2014;2:1-6.

25. Radulovic N, Stunkov Jovanovic V, Stojanovi C, Smelcerovic A, Spitlettler M, Asakawa Y. Screening of *in vitro* antimicrobial and antioxidant activity of *nine Hypericum species* from the Balkans. *Food Chem* 2007;103:15-21.

26. MacDonald Wicks LK, Wood LL, Garg ML. Methodology for the determination of biological antioxidant capacity in *in vitro*: a review. *J Sci Food Agric* 2006;86:2046-56.

27. Bharathidasan R, Panwarvelselvan A. Antioxidant activity of the endophytic fungi isolated from mangrove environment of Karankadu, Ramanathapuram district. *Int J Pharm Sci* 2012;3:2866-9.

28. Kandasamy SK, Kandasamy K. Antioxidant activity of the mangrove endophytic fungus (Trichoderma sp.). *Coastal Life Med* 2015;8:701-4.

29. Purwanti I, Wahyono, Mustofa, Susidarti RA, Sholikhah EN, Hestiyani RAN. Antiplasmodial activity of endophytic fungi isolated from *Artemisia annua* L. *Int J Pharm Clin Res* 2016;8(5), Suppl:341-4.

30. Wilson A, Prabakumar S, Sathishkumar G, Sivaramakrishnan S. *Aspergillus flavus* mediated silver nanoparticles synthesis and evaluation of its antimicrobial activity against different human pathogens. *Int Appl Pharm* 2016;8:43-6.

31. Aswini A, Soundhari C. Production of camptothecin from endophytic fungi and characterization by high-performance liquid chromatography and anticancer activity against colon cancer cell line *Asian J Pharm Clin Res* 2018;11:166-70.