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Antibacterial Activities of Different Solvent Extracts of *Centella asiatica* (L.) Urb. and its Endophytic Fungi

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Abstract: ***Evaluation of the antibacterial properties of fungal endophytes associated with Centella asiatica (L.) Urb., the ethnomedicinal plant widely used in traditional medicine practices, is part of the current study. Considering both molecular characterization and morphological characteristics, five endophytic fungi were isolated and identified namely Cladosporium sp. (F1), Penicillium sp. (F2), Aspergillus sp.(F3), Colletotrichum sp.(F4) and Curvularia sp.(F5). Escherichia coli, Staphylococcus aureus, Klebseilla pneumoniae, and Bacillus cereus are the four pathogenic bacterial strains that were examined for the antibacterial activity of plant extract and its endophytic fungus extract. The lowest concentration of the extract which inhibits any visual growth was considered to be Minimum Inhibitory Concentration(MIC).The zone of inhibition that different extracts produced against selected strains was measured. The results showed that among all endophytic fungal extracts studied F4,Colletotrichum sp. exhibited the highest zone of inhibition against E. coli, while the hydroalcoholic and aqueous extracts of the Centella asiatica leaf sample showed the maximum zone of inhibition against E. coli and S. aureus, respectively.***

Keywords: Endophytic fungi, *Centella asiatica*(L.) Urb., Antibacterial activities, Apiaceae, ethnomedicinal, etc.

1. Introduction

Centella asiatica (L.)Urb. is an herbaceous, perennial plant commonly known as Indian pennywort and Asiatic pennywort belongs to the family Apiaceae [1]. It is locally known as Jal Brahmi, Beng Saag,and grows in many temperate and tropical marshy places. It is native to Southeast Asia, the Indian subcontinent, and the wetland areas of the southeastern US [2, 3]. Due to the generation of various bioactive secondary metabolites, *Centella asiatica* is one of the most significant therapeutic herbs used in Indian Ayurvedic traditions. It is also known for its antibacterial, antifungal, antidiabetic, antidiuretic, and antioxidant [4] properties. Its diverse bioactive ingredients, which possess cytotoxic and anticancer [5], cardioprotective [6], anti-inflammatory [7], neuroprotective [8], and wound healing [9] properties, are essential for improving medical problems. *C. asiatica* prevents the oxidative damage that takes place in neuropathological disorders, including stroke, Parkinson's disease, and Alzheimer's disease, by improving the antioxidant neurological state related to aging.

Endophytic microorganisms are recognized as one of the most chemically promising groups of microorganisms in terms of diversity and pharmaceutical potential. These are microorganisms, primarily fungi and bacteria, that proliferate in the intercellular spaces of higher plants without evidently harming their hosts. Endophytic fungi form enduring associations with their host plants because endophytes have been shown to mediate the development of secondary metabolites in some plant species. A few endophytic fungi, such as *Colletotrichumgloeosporioides*, have been documented in various existing literature to produce asiaticoside and madecassoside as secondary metabolites [10]. Several bioactive secondary metabolites, such as steroids, alkaloids, peptides, terpenoids, tannins, quinone, flavonoids, and phenolics, are characteristic of endophytic microorganisms, particularly fungi [11]. It is now known that ethno-medicinal plants include a wealth of endophytes that may yield new metabolites with significant therapeutic value [12, 13, 14]. Certain natural compounds that are associated with endophytic fungi may have antibacterial, antioxidant, anti-tumour, and anti-inflammatory properties [15].

The exploration of plants for endophytic fungi can be of immense value in screening for potential metabolites. So, the present investigation has been designed to evaluate the antibacterial and antioxidant activities of endophytic fungi associated with *Centella asiatica* (L.) Urb.

2. Materials and Methods

2.1 Sampling

Centella asiatica plants with no visible symptoms of the disease were carefully selected and collected from the local area of Ranchi. The plant was identified and authenticated

by Botanical Survey of India, Kolkata with specimen no. RU/SJM-01.

Surface Sterilization and Isolation of the Endophytic Fungi-

The surface sterilization was done according to Tejovathi et al, (1996)[16]. The plantwas rinsed with tap water followed by a distilled water wash to remove adhered dust and debris and then washed with teepol and were cut into small pieces with 1.5mm segments which were surface sterilized with 70% ethanol for 1min. The segments were soaked in 0.2% (w/v) mercuric chloride solution for 1min. and finally rinsed thrice with sterile distilled water. The segments were then inoculated into Potato Dextrose Media under a Laminar Air Flow Cabinet and incubated at 25+2°C for 7 days. The growth of endophytic fungi from plant segments was observed once a day. Hyphal tips growing out of the plated segments were transferred into fresh PDA plates and incubated at 25+2°C for 5-7 days to obtain pure cultures.

2.2 Extract Preparation

Plant extract preparation-

Plant samples were collected and rinsed with double distilled water to remove dust and dirt particles then they were washed with 10% saline solution and then 2-3 times with distilled water, then shade dried and ground to powder form and sieved for uniformity [17]. In this experimentation, solvent extraction and Soxhlet extraction were carried out to get the plant extracts [18, 19]. The hydro-alcoholic extracts of *C. asiatica* were extracted using 70% aqueous and 30% ethanol upon gentle shaking at 120 rpm and 37°C for 72 hours. It was then filtered using Whatman No. 1 filter paper and the solvent was evaporated using a rotary evaporator to get the dried extracts. Then it is re-dissolve in two solvents that are aqueous and hydro-alcohol.

Fungal extract preparation-

For the mass cultivation of fungus, agar blocks of actively growing pure cultures (3mm in diameter) were placed on 100 ml Potato Dextrose Broth in a 250 ml Erlenmeyer flask. Each flask was incubated at 25=2°C for 3 weeks with periodical shaking at 150 rpm in an incubator shaker. The culture was then filtered through three layers of muslin cloth to remove fungal mycelia. The culture filtrate was then filtered thrice with equal volumes of solvent ethyl acetate. The organic phase was collected and the solvent was removed by evaporation under reduced pressure at 45°C using rotatory vacuum evaporator. The dry solid residue was re-dissolved in ethyl acetate and evaluated for its antibacterial activities.

Test Organisms

*Escherichia coli ATCC 13076, Staphylococcus aureus ATCC 25923, Klebseillapneumoniae ATCC 13883*and *Bacillus cereus ATCC 10876* were used during the present experiment were procured from Hi-media which are potential causative pathogen for different diseases.

Antibacterial Activity By Well-Diffusion Method

Antibacterial activity was done by agar well diffusion method[20] to determine the inhibitory activity of the tested extracts. The antibacterial activity of aqueous and hydro-alcoholic extract of *C. asiatica* and crude extracts of its five different endophytic fungi was carried out on Mueller Hinton Agar medium for test bacteria. 100µl of bacterial cell suspension culture was uniformly spread onto sterile Mueller Hinton Media by a sterile L-shaped spreader to prepare the test plates[21]. After solidifying the media, 7mm wells were punched by using a sterile cork borer in which different concentrations (2,4,8,16µl) of the test sample were loaded on inoculum-swapped plates. Afterwards, the plates were incubated for 24-48hr at 37°C for the zone of inhibition to be observed [22].

3. Result and Illustrations

Isolation of Endophytic fungi

A total of five endophytic fungi from *Centella asiatica* were isolated from different parts of plants. Based on microscopic and morphological features, the isolates were identified namely *Cladosporiumsp.* (F1), *Penicillium sp.* (F2), *Aspergillus sp.* (F3), *Colletotrichum sp.* (F4), and *Curvularia sp.*(F5).

Antibacterial Assay

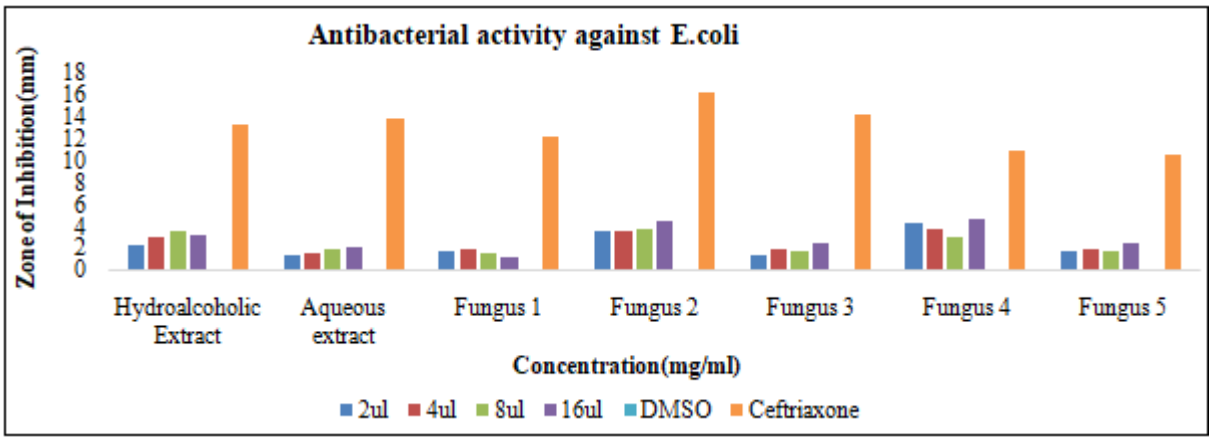
The current study used the ethyl acetate extract of five distinct endophytic fungi as well as the aqueous and hydroalcoholic extract of *Centella asiatica* leaves to test the antibacterial activity of these extracts against a range of pathogenic bacteria, including *S. aureus, E. coli, Klebseillapneumoniae*, and *Bacillus cereus*. The hydroalcoholic extract of a leaf sample from *Centella asiatica* displayed a zone of inhibition (2.3 mm) in antibacterial tests against *E. coli*, but the maximum zone of inhibition (4.3 mm) was observed in the case of endophytic fungal extracts, F4 (*Colletotrichum sp*.). While endophytic fungal samples 01, 03, and 05 do not exhibit any zone of inhibition, the aqueous extract of *Centella* leaf extract showed the maximum zone of inhibition (6.0 mm) in its efficacy against *Staphylococcus aureus*. Endophytic fungal sample 1 (*Cladosporium sp*.) had the highest zone of inhibition (5.6 mm) in comparison to the remaining samples against *Klebsiella pneumoniae*. For *Bacillus cereus*, hydroalcoholic extract exhibits the highest inhibitory zone (5.3 mm) among the other samples.

4. Discussion

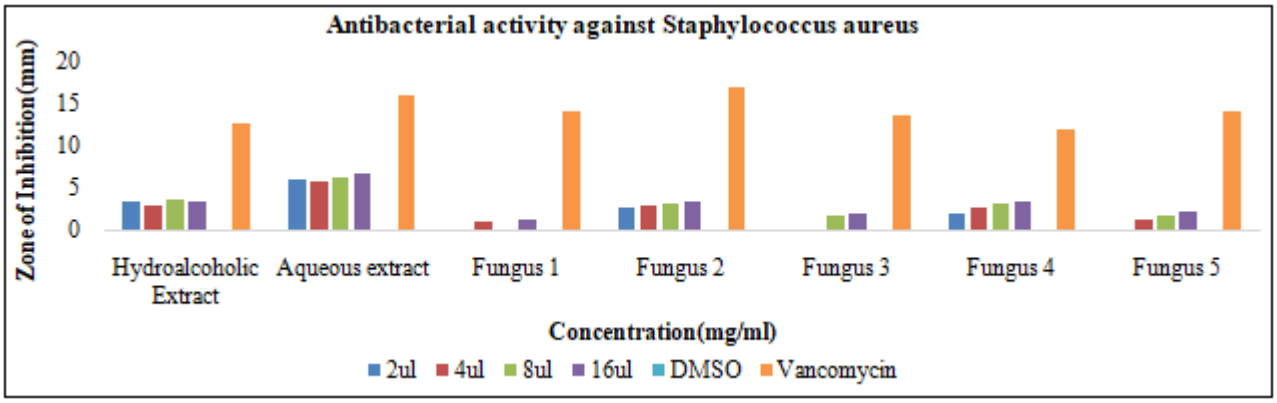
It has also been shown that fungal endophytes are a source of unique secondary metabolites, some of which have useful biological properties [23]. Plant endophytes are probably ubiquitous, their populations depending on the type of host and its geographic region [24]. Many studies have shown that the endophytes that are isolated from medicinal plants are highly effective in producing metabolites that are cytotoxic, fungicidal, and bactericidal [25]. The earlier study demonstrated the presence of several kinds of phytochemicals such as phenols, flavonoids, terpenoids, alkaloids, saponins, and cardiac glycosides in plant as well as in its endophytic fungal extract. Earlier studies of *Centella asiatica* L.(Urb.), its dichloromethane: methanolic extracts revealed substantial inhibition of *Escherichia coli* and *Staphylococcus aureus* growth[26] while during the present investigation, its hydroalcoholic and aqueous extract showed antibacterial effect against the same pathogens.

During the present investigation,the endophytic fungal extract of *Colletotrichum sp.*(F4) of *Centella asiatica* L.(Urb.) showeda zone of inhibition (Graph 2) against *Staphylococcus aureus* but during previous work of *Colletotrichum gloeosporioides*, no zone of inhibition was reported against the same pathogen [27].

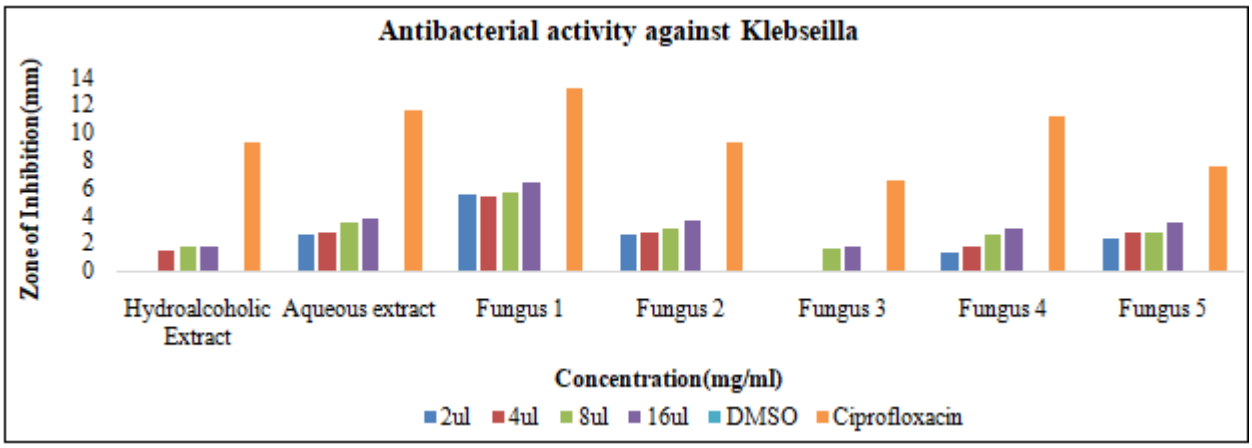
The endophytic fungal isolates from *C. asiatica* include *Cladosporiumsp.* (F1), *Penicillium sp.* (F2), *Aspergillus sp.* (F3), *Colletotrichum sp.* (F4) and *Curvularia sp.*(F5)which revealed antibacterial activities against some pathogenic and non-pathogenic bacteria.Antimicrobial metabolites from fungal endophytes have been investigated as one of the alternative approaches for reducing the issue of drug resistance among pathogens, which has been the area of more attention [28]. The endophytic fungus, *Colletotrichum sp.*, showed a better zone of inhibition against *Klebseilla pneumoniae*. The hydroalcoholic extract exhibits the highest inhibitory zone against *Bacillus cereus*. This could be due to their higher efficiency in metabolite production, indicating that these fungi have differing activity potential.A wide range of microorganisms exist that are not being utilized for the evaluation of the generation of metabolites that could have beneficial bioactivities, such as antibacterial activity [29].



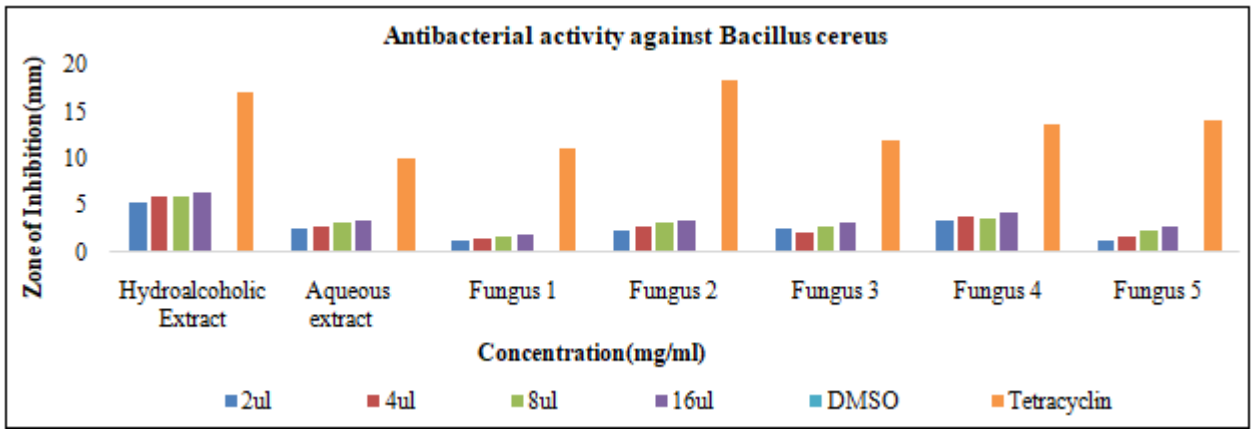
Graph 1: Antibacterial activities of plant extract and its endophytic fungal extract against *E. coli* showing zone of inhibition with different concentrations.



Graph 2: Antibacterial activities of plant extract and its endophytic fungal extract against *Staphylococcus aureus*showing zone of inhibition with different concentrations.



Graph 3: Antibacterial activities of plant extract and its endophytic fungal extract against *Klebsiellapneumoniae* showing zone of inhibition with different concentrations.



Graph 4: Antibacterial activities of plant extract and its endophytic fungal extract against *Bacillus cereus* showing zone of inhibition with different concentrations.

Conflicts of Interest

The authors declare no conflict of interest.

References

[1] "*Centella asiatica* (Asiatic pennywort) ". Invasive Species Compendium, CABI. 22 November 2017. Archived from the original on 23 July 2017. Retrieved 2 January 2018.

[2] "*Centella asiatica*". *Alabama Plant Atlas. Retrieved 17 August 2015.*

[3] "Centella asiatica". *Atlas of Florida Vascular Plants. Retrieved 17 August 2015.*

[4] Xu, M.-f., Xiong, Y.-y., Liu, J.-k., Qian, J.-j., Zhu, L., and Gao, J. (2012). Asiatic Acid, a Pentacyclic Triterpene in *Centella asiatica*, Attenuates Glutamate-Induced Cognitive Deficits in Mice and Apoptosis in SH-SY5Y Cells. *Acta Pharmacol. Sin.* 33 (5), 578–587. doi:10.1038/aps.2012.3

[5] Rizvi, I.; Celli, J.P.; Evans, C.L.; Abu-Yousif, A.O.; Muzikansky, A.; Pogue, B.W.; Finkelstein, D.; Hasan, T.: Synergistic enhancement of carboplatin efficacy with photodynamic therapy in a three-dimensional model for micrometastatic ovarian cancer. Can. Res. **70**(22), 9319–9328 (2010)

[6] Harwoko P. S., Nugroho A. E. (2014). Triterpenoid-rich Fraction of centella Asiatica Leaves and *In Vivo* Antihypertensive Activity. *Int. Food Res. J.* 21 (1), 149–154.

[7] Somchit MN, Sulaiman MR, Zuraini A, et al. Antinociceptive and antiinflammatory effects of *Centellaasiatica* . *Indian Journal ofPharmacology*. 2004;36(6):377–380

[8] Gray, N. E., Zweig, J. A., Caruso, M., Zhu, J. Y., Wright, K. M., Quinn, J. F., et al. (2018c). *Centella asiatica* Attenuates Hippocampal Mitochondrial Dysfunction and Improves Memory and Executive Function in β-amyloid Overexpressing Mice. *Mol. Cell Neurosci.* 93, 1–9. doi:10.1016/j.mcn.2018.09.002

[9] Saeidinia A., Keihanian F., Lashkari A.P., Lahiji H.G., Mobayyen M., Heidarzade A., Golchai J. Partial-Thickness Burn Wounds Healing by Topical Treatment: A Randomized Controlled Comparison between Silver Sulfadiazine and Centiderm. *Med. (United States)* 2017;**96**:e6168. doi: 10.1097/MD.0000000000006168.

[10] Gupta S., Bhatt P., Chaturvedi P, Determination and quantification of asiaticoside in endophytic fungus from Centella asiatica (L.)Urban, World Journal of Microbiology and Biotechnology (2018) 34: 111 https://doi.org/10.1007/s11274-018-2493-9

[11] Tan RX, Zou WX. Endophytes: A rich source of functional Metabolites. Nat. Prod. Rep. 2001; 18:448–459.

[12] Gunatilaka AAL. Natural products from plant associated microorganisms: Distribution, Structural diversity, bioactivity and implications of their occurrence. J. Nat. Prod. 2006; 69: 509–526.

[13] Strobel G, Daisy B, Castillo U, Harper J. Natural products from endophytic microorganisms. J. Nat. Prod. 2004; 67:257-268.

[14] Wiyakrutta S, Sriubolmas N, Panphut W, Thongon N, Danwiserkanjana K, Ruangrungsi N, Meevootisom V. Endophytic fungi with antimicrobial, anti-cancer, anti-malarial activities isolated from Thai medicinal plants. World J. Microb. Biotech. 2004; 20:265- 272.

[15] Verma VC, Kharwar RN, Strobel GA. Chemical and functional diversity of natural products from plant associated endophytic fungi. Nat. Prod. Commun. 2009; 4:1511-32.

[16] Tejovathi G., Vimla Y., Bhadauria R. (1996). A practical manual for plant biotechnology. CBS Publisher & Distributors, Daryagang, New Delhi.

[17] Wink, M. (1999) Function of secondary metabolites, in Functions of Plant Secondary Metabolites and Their Exploitation in Biotechnology (Wink, M., ed.), Annual Plant Reviews, vol. 3, Sheffield Academic Press, Sheffield, UK, 1–16.

[18] Farnsworth, N. R. (1994) Ethnopharmacology and drug development, in Ethnobotany and the Search for New Drugs, (Prance, G. T., Chadwick, D. J., and Marsh, J., eds.), Ciba Foundation Symposium Series, vol. 185, Wiley, New York, 42–51.

[19] Kinghorn, A. D. (2001) Pharmacognosy in the 21st century. J. Pharm. Pharmacol. 53, 135–148.

[20] Parekh, J., & Chanda, S. (2007). Antibacterial and phytochemical studies on twelve species of Indian medicinal plants. *African Journal of Biomedical Research*, *10*(2).

[21] Cockerill, Franklin R.; et al. (2012). *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria*

That Grow Aerobically; Approved Standard—Ninth Edition. CLSI. p. 12. ISBN 1-56238-784-7

[22] Nath Archana, PathakJyoti and JoshiSR. Bioactivity assessment of endophytic fungi associated with Centella asiatica and Murrayakoengii. J App Biol Biotech. 2014; 2 (05): 006- 011.

[23] Bills, G. F., &Polishook, J. D. (1991). Microfungi from Carpinus caroliniana. *Canadian Journal of Botany*, *69*(7), 1477-1482.

[24] Tan, R. X., & Zou, W. X. (2001). Endophytes: a rich source of functional metabolites. *Natural product reports*, *18*(4), 448-459.

[25] Wang, F. W., Jiao, R. H., Cheng, A. B., Tan, S. H., & Song, Y. C. (2007). Antimicrobial potentials of endophytic fungi residing in Quercus variabilis and brefeldin A obtained from Cladosporium sp. *World Journal of Microbiology and Biotechnology*, *23*, 79-83.

[26] Sieberi, B. M., Omwenga, G. I., Wambua, R. K., Samoei, J. C., & Ngugi, M. P. (2020). Screening of the Dichloromethane: Methanolic Extract of Centella asiatica for Antibacterial Activities against Salmonella typhi, Escherichia coli, Shigella sonnei, Bacillus subtilis, and Staphylococcus aureus. *The Scientific World Journal*, *2020*.

[27] Nath, A., Pathak, J., & Joshi, S. R. (2014). Bioactivity assessment of endophytic fungi associated with Centella asiatica and Murrayakoengii. *Journal of Applied Biology and Biotechnology*, *2*(5), 006-011.

[28] Strobel G, Daisy B, Castillo U, Harper J. Natural products from endophytic microorganisms. J. Nat. Prod. 2004; 67:257-268.

[29] Motta AS, Cladera-Olivera F, Brandelli A. Screening for antimicrobial activity among bacteria isolated from the Amazon basin. Braz. J. Microbiol. 2004; 35:307–10