



Response of some pathogenic microorganisms to crude alkaloid of endophytic fungi isolated from *Catharanthus roseus*

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Abstract

Thirteen isolates of endophytic fungi were isolated from leaves and roots of *Catharanthus roseus*, these fungal isolates had been collected and identified by scientific laboratories of College of Science / Al-Mustansiriyah University in the period from 3rd to 7th of November, 2019. These fungal isolates were *Aspergillus niger*, *Fusarium solani*, *Cladosporium cladosporioides*, *Fusarium oxysporum*, *Mycelia sterilia*, *Curvularia* sp., *Alternaria alternate*, *Aspergillus parasiticus*, *Aspergillus flavus*, *Penicillium* sp., *Aspergillus ochraceus*, and two isolates of *Aspergillus terreus*, the first one was isolated from leaves and the second isolate from stems. In addition to these isolates, eight isolates of common bacterial isolates (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, *E. coli*, *Salmonella typhi*, *Shigella* sp. *Klebsiella* Sp.) as well as one yeast isolate (*Candida albicans*) were obtained from the same laboratories. The antimicrobial ability of the whole fungal extracts were tested separately against the nine selected pathogens by using well diffusion method. The results showed variable response and antimicrobial actions of endophytic fungal extracts against the selected bacteria and yeast. The ranges of actions were ranging between high ability of inhibition to no action against some isolates, which may be due to the difference between the cell wall of gram negative and positive.

Keywords: Endophytic fungi, *Catharanthus roseus*, Alkaloid extract, Antimicrobial actions.

Introduction

The term “Endophyte” is meaning endo or within, and *Phyton* means plant, and by that it means vegetable plants. Many microorganisms have known that they have the ability to colonize the tissues of plants, from these organisms are fungi, bacteria and actinomycetes, but the most efficient in this action are the fungi (Staniek *et al.*, 2008). Endophytic fungi are microorganisms that live in plant tissues without causing apparent harm to the host, therefore, harboring of the endophytic fungi by plants is asymptomatic, these fungi are part of the plant microbes and are ubiquitously found across plant species and ecosystems. (Lixiang and *et al.*, 2019). Endophytic fungi play important vital roles in various aspects of life through their effects on host plants to their effects on the environment and then their effects on human life. Many studies have shown the positive importance of endophytic fungi towards their plant hosts, including stimulating them to grow through different mechanisms, as the metabolic products of these fungi increase the resistance of direct plants. The production of plant hormones (growth regulators) is also stimulated by plants such as auxins, absesins, gibberellins, and indole acetic acid (IAA),

which known their importance to the plants (Malinowski and Belesky, 2000; Bodde *et al.*, 2003; Firakova *et al.*, 2007; Dai *et al.*, 2008).

Endophytic fungi have shown efficacy in stimulating plant resistance against disease through several mechanisms mainly due to the nutritional status of the host and increasing its efficiency through its resistance to abiotic stresses (Redman *et al.*, 2002; Bae *et al.*, 2008).

This paper aims to determine the antimicrobial activities of different endophytic fungi which were isolated from *Catharanthus roseus* against most common pathogens.

Material and Methods

Fungal Samples: Thirteen isolates of endophytic fungi which were isolated from leaves and roots of *Catharanthus roseus*, the fungal isolates were collected in the period from 3rd to 7th of November, 2019, and then identified in scientific laboratories of college of Science / Al-Mustansiriyah University, table (1) illustrate the endophytic fungal isolates.

Table (1): Endophytic fungi isolates.

No.	Species
1	<i>Aspergillus niger</i>
2	<i>Fusarium solani</i>
3	<i>Cladosporium cladosporioides</i>
4	<i>Fusarium oxysporum</i>
5	<i>Aspergillus terreus</i> (1)
6	<i>Myceli sterilia</i>
7	<i>Aspergillus terreus</i> (2)
8	<i>Curvularia sp</i>
9	<i>Alternaria alternata</i>
10	<i>Aspergillus parasiticus</i>
11	<i>Aspergillus flavus</i>
12	<i>Penicillium sp.</i>
13	<i>Aspergillus ochraceus</i>

Pathogenic Samples: The identified of eight pathogenic bacterial isolates and one yeast *Candida albicans* were obtained from the scientific laboratories of microorganisms at college of Science / Al-Mustansiriyah University. isolated from patients infected.

Table (2): Pathogenic microorganism isolates.

No.	Microorganisms
1	<i>Salmonella typhi</i>
2	<i>Shigella sp.</i>
3	<i>Escherichia coli</i>
4	<i>Staphylococcus aureus</i>
5	<i>Staphylococcus epidermidis</i>
6	<i>Streptococcus pneumoniae</i>
7	<i>Pseudomonas aeruginosa</i>
8	<i>Klebsiella sp.</i>
9	<i>Candida albicans</i>

Preparation of endophytic fungi extracts: The filtrates of selected endophytic fungal isolates which was prepared in the previous item were separated by using a separating funnel with a volume of (1000) ml by adding the ethyl acetate solvent in a ratio of (1: 1) volume: volume (filtrate: solvent), (Pharamat *et al.*, 2013). The separation funnel was shaken well for (5-10) minutes, the separation process was repeated for the filtrate layer three times, the top layer of ethyl acetate was collected and considered the crude alkaloids extract then distributed in finely weighed glass dishes, and placed in an incubator with temperature (37) C° until complete drying, then

the dry extract weight was estimated and a stock solution was prepared until using (Purwestri *et al.*, 2016).

Determine the response of pathogens to the fungal extracts: The extract of crude alkaloids extracted from endophytic fungi (ethyl acetate extract) of thirteen selected endophytic fungal isolates were prepared as mentioned in item (2.3.) and then followed by using well diffusion method against the nine pathogens.

Statistical analysis: The Statistical analysis was performed with SPSS Version 11.0 statistical software package. Data were expressed as Least Significant Difference (LSD). Comparisons between treatments were performed with analysis of ANOVA two ways.

Results and Discussion

The antimicrobial results showed that, as in table (3), figures (1), (2) and (3), there was a difference and contrast between in the antimicrobial activity of crude alkaloid of endophytic fungi against some pathogenic microorganisms. Generally, the most antimicrobial activity against the isolated isolates was by the fungus *Aspergillus*. *Aspergillus niger* showed the greatest inhibition activity for the growth of *Salmonella typhi*, *Streptococcus pneumoniae*, *Candida albicans*, and *Staphylococcus epidermidis* with inhibition diameter 24.33mm, 19.33mm, 19mm and 16.66mm respectively. The weaker inhibition activity was against *Escherichia coli*, with the rate of inhibition diameter 13.33mm and no inhibition activity was shown against *Pseudomonas aeruginosa* and *Klebsiella sp.*. The other species, *Aspergillus terreus* (1) showed an effect on the growth of *Candida albicans* yeast, *Staphylococcus aureus* and *Streptococcus pneumoniae* with inhibition diameter 21mm, 20.66mm and 20.33mm respectively, whereas weaker inhibition activity was shown against *Shigella sp.* with an inhibition rate 10.33mm, but did not show any inhibition activity against *Pseudomonas aeruginosa* and *Klebsiella sp.* *Aspergillus flavus* showed a good inhibition activity against *Candida albicans* yeast, *Shigella sp*, *Streptococcus pneumoniae* and *salmonella typhi* with inhibition diameter 21.66mm, 20.33mm, 19.66mm and 19.66mm respectively, other results ranged between 12.33 to 10mm. and no effect was shown against *Klebsiella sp.*

Table (3): Antimicrobial activity of crude alkaloids of fungal endophytes isolates against test pathogens

Endophytic fungus	(Inhibition zone in mm ± SD)										LSD _{0.05}
	<i>S. typhi</i>	<i>Shigella</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>S. epidermidis</i>	<i>P. aeruginosa</i>	<i>S. pneumoniae</i>	<i>Klepsiella Sp.</i>	<i>Candida albicans</i>		
<i>A. niger</i>	24.33±1.15	14.66±0.57	13.33±2.08	14.33±0.57	16.66±1.15	0	19.33±1.15	0	19±1	1.808	
<i>F. solani</i>	20.66±1.15	11±1.0	8.66±1.52	12.66±1.52	14.33±0.57	0	14.33±1.52	0	18.66±1.15	1.896	
<i>C. cladosporioides</i>	20.66±0.57	15.66±0.57	11.33±1.15	14.66±0.57	16.33±0.57	0	21±1.0	0	19.33±0.57	1.144	
<i>F. oxysporum</i>	14.33±0.57	12±0	12.66±0.57	14.66±1.52	13.66±1.52	0	18.66±1.52	0	20.33±0.57	1.617	
<i>A. terreus (1)</i>	17.33±1.15	10.33±0.57	14±0.57	20.66±1.15	14.66±1.15	0	20.33±1.52	0	21±1.73	2.682	
<i>M. sterilia</i>	17.66±1.52	13.33±1.52	12.33±0.57	15.33±0.57	15.33±0.57	4.33±3.78	17.33±1.15	0	14.33±1.15	2.722	
<i>A. terreus (2)</i>	20.33±1.52	15.66±0.57	11.33±1.0	14.33±0.57	16.66±1.52	8.66±0.52	16.33±1.15	0	19±1.73	1.896	
<i>Curvularia</i>	19.33±0.57	17±1.73	8.66±1.15	11±1.0	13.66±0.57	6.33±0.57	19±0	0	13.66±1.52	1.683	
<i>A. alternata</i>	19.33±1.15	18.66±1.52	8±1.73	21±2.64	12.66±1.52	6.66±0.57	11.33±1	0	14.66±1.52	2.557	
<i>A. parasiticus</i>	17.33±2.51	18.66±1.15	9.66±0.57	10.33±1.52	14.66±1.15	9.66±1.52	13.33±2.08	0	12.66±0.57	2.470	
<i>A. flavus</i>	19.66±2.51	20.33±0.57	10.33±0.57	9.33±1.52	12.33±2.08	10.33±0.57	19.66±0.57	0	21.66±2.30	2.536	
<i>Penicillium</i>	16.33±1.52	18±1.73	8.66±0.57	19.66±0.57	9.66±1.15	10.33±1.52	17.33±0.57	0	13.66±0.57	2.008	
<i>A. ochraceus</i>	16.66±0.57	17±1.73	9.66±1.52	17.33±1.15	14.66±0.57	12.33±1.15	15.66±1.15	0	16.66±1.52	2.018	
+ve control	34.33±0.57	30±0	25.66±0.57	31±1.73	28.2±2.0	25.33±1.52	33.66±1.15	10.66±1.15	20.66±1.15	2.139	
-ve control	0	0	0	0	0	0	0	0	0	0	
LSD _{0.05}	2.223	1.810	1.827	2.138	2.035	2.109	2.019	1.572	2.095		

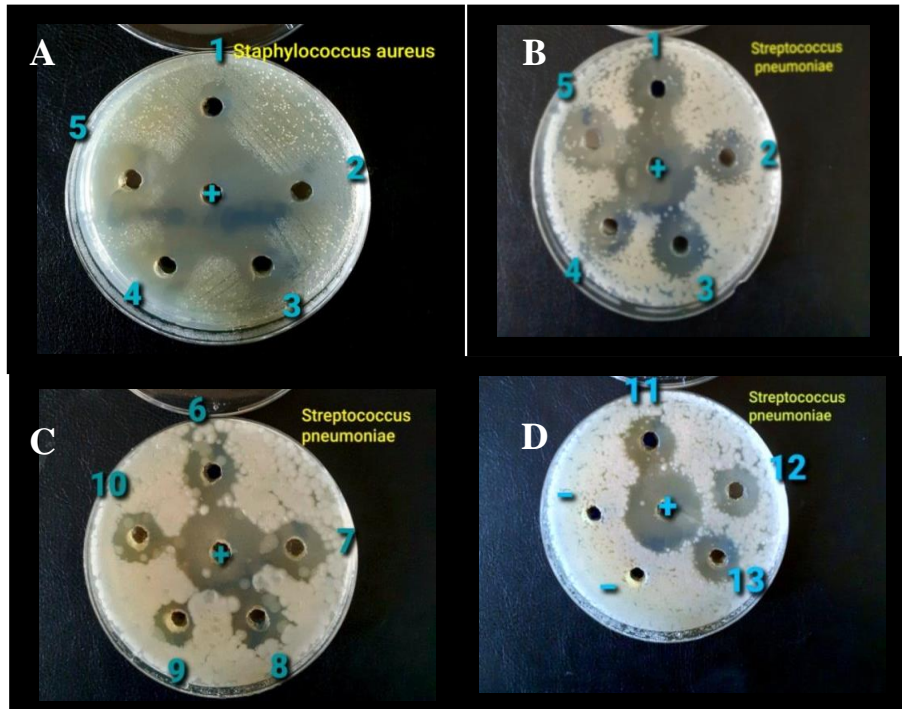


Figure (1): Antimicrobial activity of crude ethyl acetate extracts of endophytic fungi against pathogens (A: *Staphylococcus aureus*; B, C and D: *Streptococcus pneumoniae*). Where the numbers refer to Crude alkaloid of *Aspergillus niger* (1), *Fusarium solani* (2), *Cladosporium caldosporirides* (3), *Fusarium oxysporum* (4), *Aspergillus terreus* (isolate 1) (5), *Mycelia sterilia* (6), *Aspergillus terreus* (isolate 2) (7), *Curvularia sp* (8), *Aspergillus alternate* (9), *Aspergillus parasiticus* (10), *Aspergillus flavus* (11), *Penicillium sp* (12), and *A. ochraceus* (13), while (+) means: positive control and (-) means: negative control.

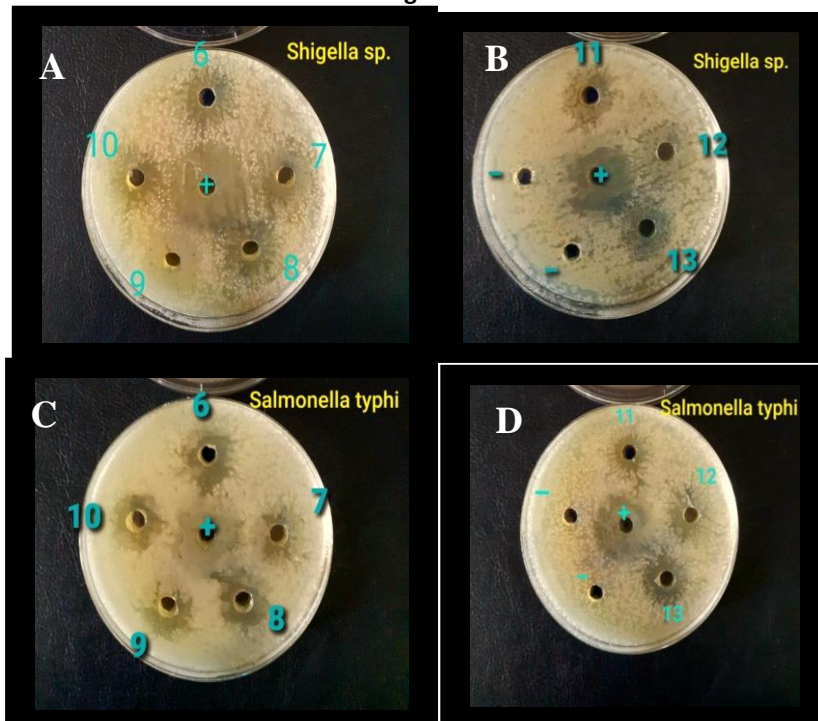


Figure (2): Antimicrobial activity of crude ethyl acetate extracts of endophytic fungi against pathogens (A and B: *Shigella sp.*; C and D: *Salmonella typhi*). Where the numbers refer to Crude alkaloid of *Aspergillus niger* (1), *Fusarium solani* (2), *Cladosporium caldosporirides* (3), *Fusarium oxysporum* (4), *Aspergillus terreus* (isolate 1) (5), *Mycelia sterilia* (6), *Aspergillus terreus* (isolate 2) (7), *Curvularia sp* (8), *Aspergillus alternate* (9), *Aspergillus parasiticus* (10), *Aspergillus*

flavus (11), *Penicillium* sp (12), and *A. ochraceus* (13), while (+) means: positive control and (-) means: negative control.

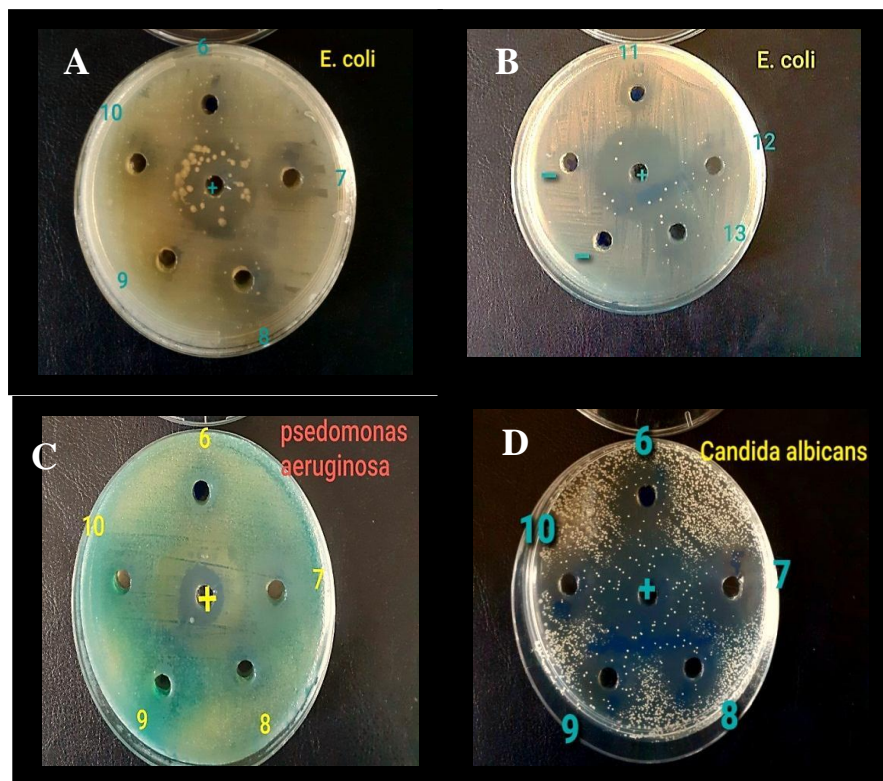


Figure (3): Antimicrobial activity of crude ethyl acetate extracts of endophytic fungi against pathogens (A and B: *Escherichia coli*; C and D: *Pseudomonas aeruginosa*).

Where the numbers refer to Crude alkaloid of *Aspergillus niger* (1), *Fusarium solani* (2), *Cladosporium caldosporioides* (3), *Fusarium oxysporum* (4), *Aspergillus terreus* (isolate 1) (5), *Mycelia sterilia* (6), *Aspergillus terreus* (isolate 2) (7), *Curvularia* sp (8), *Aspergillus alternate* (9), *Aspergillus parasiticus* (10), *Aspergillus flavus* (11), *Penicillium* sp (12), and *A. ochraceus* (13), while (+) means: positive control and (-) means: negative control.

Aspergillus ochraceus showed highest inhibition activity against *Staphylococcus aureus* with 18.33mm and weakest inhibition activity against *Escherichia coli* with 9.66mm, other results ranged between 17 to 12.33mm and no effect was shown against *Klebsiella* sp. too.

The inhibition rates of *Aspergillus parasiticus* ranged from 18.66 to 9 mm, whereas no effect was shown against *Klebsiella* sp.. *Cladosporium cladosporioides* showed the highest effect against both *Streptococcus pneumoniae* and *Candida albicans* yeast with inhibition zones 21mm and 20.66mm respectively, on the other hand, the weakest inhibition activity was recorded against *Escherichia coli* with 11.33mm. Other results ranged between 20.33 to 14.66mm, whereas no effect was shown against *Klebsiella* sp. and *Pseudomonas aeruginosa*. In addition, *Fusarium oxysporum* showed effect against pathogens, as it had the highest inhibition activity against *Candida albicans* yeast and *Streptococcus pneumoniae* with inhibition zones equal to 20.33mm and 18.66 mm respectively, whereas weakest inhibition activity

was against *Shigella* sp. with 12mm, and other results ranged between 14.66 to 12.66 mm. *Klebsiella* sp. and *Pseudomonas aeruginosa* did not affected. In *Fusarium solani* the results ranged between 20.66 to 8.66 mm whereas no effect was shown against *Klebsiella* sp. and *Pseudomonas aeruginosa*. The results of the efficacy of *Mycelia sterilia* showed the highest inhibition rate against *Streptococcus pneumoniae* with 18.66mm and the lowest against *Pseudomonas aeruginosa* with 7.33mm, whereas the other results ranged between 15.33 to 12.33mm, *Klebsiella* sp. not affected. Finally, *Penicillium* sp. showed the highest rate of inhibition against *Staphylococcus aureus* with 19.66mm and the lowest was against *Escherichia coli* with 8.66mm, while other results ranged between 16.33 to 9.66mm, but *Klebsiella* sp. not affected.

Through the results of the current study, it was observed that the *Candida albicans* yeast and gram-positive bacteria in general, were more susceptible to crude ethyl acetate extract of endophytic fungi, while the gram-negative bacteria

samples were more resistant and less sensitive. This study matched the results of many other studies, which were carried out on many types and various species of endophytic fungi in different plants against various groups of gram-positive and negative bacteria, in addition to the *Candida albicans* yeast. (Handayani *et al.*, 2015; Bisht *et al.*, 2016; Yin *et al.*, 2017; Kalyani and Hemalatha, 2017; Xin *et al.*, 2018 and Deepthi *et al.*, 2018).

It was also observed from the results of this study that *salmonella typhi* was affected by considerable proportion compared to other gram-negative pathogens; it ranged between 14.33 to 20.33mm. This result matches with other study (Rani *et al.*, 2017) which found the extracts of *Aspergillus* sp, *Fusarium solani*, and *Curvularia* sp, which were endophytic in *Calotropis procera*, showed the highest effect against *Salmonella typhi*.

The ethyl acetate extracts of endophytic fungi of *Catharanthus roseus* and *Euphorbia hirta* showed antimicrobial activity against different species of pathogenic bacteria and *Candida* yeast. As these fungi produced many compounds which have antimicrobial property such as paxilline, nigricinol, citreoisocoumarin, sceptrin, desmethyldichlorodiaportin, N-5 (3- (5-Isopropyl-3,6-dioxo-piperazin-2-yl) -propyl) -2-phenyl-acetamide, fatty acid, cladosporin and desmethyldiaportinol (Mark *et al.*, 2017).

The antimicrobial activity obtained from the ethyl acetate extract is attributed to the production of endophytic fungi many bioactive compounds against pathogens. The fungi crude alkaloid obtained from Spikes of *Pinus roxburghii* showed presence of alkaloids, flavonoids, saponins, phenols, tannins, steroids and terpenoids, and also was observed premium antimicrobial activity was obtained from crude ethyl acetate extracts against pathogenic bacteria *E. coli*, *Salmonella typhimurium*, *Staphylococcus aureus*, and *Candida albicans* yeast (Bhardwaj *et al.*, 2015). So, the extracts of endophytic fungi isolated from *Hybanthus enneaspermus*, *Ziziphus mauritiana* and *Mukia maderaspatna* plants, contained many bioactive compounds such as terpenoids, tannins, flavonoids, phenols, coumarins, anthraquinones and alkaloids (Sheeba *et al.*, 2019).

The difference in the activity of the antimicrobial against the microorganisms was also observed in other studies of the same species or funji isolates against the same pathogens, and it was found that this activity is affected by the difference of the host plant, the plant's geographical location and the local environment (Selim *et al.*, 2011). In addition to the internal effects of the host and its components, which are considered more influential on the activity or the compounds

produced by the endophytic fungi (Firakova *et al.*, 2007 and Selim *et al.*, 2011).

Conclusion

The results of the current study showed deferent activities of endophytic fungi isolates against the tested pathogens.

References

- Bae, H.; Kim, S. H.; Kim, M. S.; Sicher Jr., R. C.; Strem, M. D.; Natarajan, S. & Bailey, B. A. (2008). The drought response of *Theobroma cacao* (cacao) and the regulation of genes involved in polyamine biosynthesis by drought and other stresses. *Plant & Biochem.*, 46:174-188.
- Bhardwaj, A.; Sharma, D.; S.; Jadon, N. and Agrawal, P. K. (2015). Antimicrobial and Phytochemical Screening of Endophytic Fungi Isolated from Spikes of *Pinus roxburghii*. *Archives of Clinical Microbiology*, 6 (3)
- Bisht, R., Sharma, D. and Agrawal, P. K. (2016). Antagonistic and antibacterial activity of endophytic fungi isolated from needle of *cupressus torulosad* D. Don. *Asian Journal of Pharmaceutical and Clinical Research*, 9 (3): 282-288.
- Bodde, E. W. H.; Visser, E.; Duysens, J. E. & Hartman, E. H. (2003). Donor-site morbidity after free vascularized autogenous fibular transfer: Subjective and quantitative analysis. *Plastic & Reconstr. Surg.*, 111(7): 2237-2242.
- Dai, C. C.; Yu, B. Y. & Li, X. (2008). Screening of endophytic fungi that promote the growth of *Euphorbia pekinensis*. *Afric. J. Biotechn.*, 7(19): 3505-3510.
- Deepthi, V. C.; Seepana ,S.; Faisal M.; and Elyas, K. K. (2018) . Isolation and identification of endophytic funji with antimicrobial activates from the leaves of *Elaeocarpus sphaericus* (Gaertn.) K. schum. and *Myristica fragrans* houtt. *International Journal of Pharmaceutical Sciences and Research* , 9 (7): 2783-2791.
- Firakova, S.; Sturdikova, M. & Muckova, M. (2007). Bioactive secondary metabolites produced by microorganisms associated with plants. *Biology*, 62(3): 251-257.
- Handayani, D.; Ahdinur, R. F. and Rustini, R. (2015). Antimicrobial activity of endophytic fungi from marine Sponge *Haliclona fascigera* article info abstract. *Journal of Applied Pharmaceutical Science*, 5(10): 154-156.
- Kalyani, P. and Hemalatha, K.P.J. (2017) . In Vitro Antimicrobial Potential of *Aspergillus niger* (MTCC-961). *International Journal of ChemTech Research* , 10(4): 430-435
- Lixiang W.; Lili, R.; Chunchun L.; Chenglong, G.; Xiaobo, L. ;Ming, W. & Youqing, L. 2019 . Effects of endophytic fungi diversity in different coniferous species on the

- colonization of *Sirex noctilio* (Hymenoptera: Siricidae) Scientific Reports , 9(1):5077: 1-11.
- Malinowski, D. P. & Belesky, D. P. (2000). Adaptations of endophyte-infected cool-season grasses to environmental stresses: Mechanisms of drought and mineral stress tolerance. *Crop Sci.*, 40(4): 923-940.
- Mark, O. A.; Peter M. E.; Chika C. A.; Charles U. N., Festus B. C. O. and Charles O. E. (2017). Metabolites of Endophytic Fungi Isolated from *Euphorbia hirta* growing in Southern Nigeria. *Chemical Science Review and Letters*, 6(21): 12 – 19
- Ran, X., Zhang, G., Li, S., & Wang, J. (2017). Characterization and antitumor activity of camptothecin from endophytic fungus *Fusarium solani* isolated from *Camptotheca acuminata*. *African health sciences*, 17(2), 566–574.
- Rani, R.; Sharma, D.; Chaturvedi, M. and Yadav, JP (2017). Antibacterial Activity of Twenty Different Endophytic Fungi Isolated from *Calotropis procera* and Time Kill Assay. *Clin Microbiol.* 6: 280
- Redman, R. S.; Sheehan, K. B.; Stout, R. G.; Rodriguez, R. & Henson, J. M. (2002). Thermotolerance conferred to plant host and fungal endophyte during mutualistic symbiosis: *Science*, 298: 1581.
- Selim, K.A.; Elkhateeb, W.A.; Tawila, A.M.; El-Beih, A.A.; Abdel-Rahman, T.M.; El-Diwany, A.I.; Ahmed, E.F.(2018). Antiviral and Antioxidant Potential of Fungal Endophytes of Egyptian Medicinal Plants. *Fermentation* , 4, 49
- Sheeba, H., Ali, M. S., & Anuradha, V. (2020). In-vitro Anti-cancer Activity of Endophytic Fungi Isolated from *Ziziphus mauritiana* in Cervical Cancer Cell Line. *European Journal of Medicinal Plants*, 31(4), 38-48
- Sheeba, H.; Syed, M. A. and Anuradha, A. (2019). Bioactive compounds and antimicrobial activity of fungal crude extract from medicinal plants. 11(5): 1826-1833.
- Staniek, A.; Woerdenbag, J.W. & Kayser, O. (2008). Endophytes exploiting biodiversity for the improvement of natural product-based drug discovery. *J. Plant Tract.*, 3(2): 75-93.
- Xian, Q. S.; Xin, Z.; Qiu, J. H.; Xiao, B. L.; Gang, L.; Rui, J. L.; Yang, J.; Jin, C. Z. and Hong, X. L. (2013). Xanthone derivatives from *Aspergillus sydowii*, an endophytic fungus from the liverwort *Scapania ciliata* S. Lac and their immunosuppressive activities. *Science Direct*, 6 (3): 218-321
- Xin, T.; Xiao, Y. and Cheng, L. H. (2018). Antimicrobial Activity of Fungal Endophytes from *Vaccinium dunalianum* var. *urophyllum* . *Sains Malaysiana*, 47 (8): 1685–1692
- Yin, O.; Ibrahim, D. and Chong, C.L. (2017). Bioactive Compounds from *Aspergillus Terreus* MP15, an Endophytic Fungus Isolated from *Swietenia Macrophylla* Leaf. *Malaysian Journal of Medical and Biological Research*, 2 (3) : 262-272.