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ENDOPHYTIC MICROBES: AN EMERGING SOURCE OF ANTITHROMBOTIC AGENTS

SALINI G

Amrita School of Biotechnology, Amrita Vishwa Vidyapeetham, Amritapuri, Clappana P.O., Kollam, Kerala, India- 690525.

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Abstract: Thrombus (clot) in blood vessels is the result of fibrin accumulation under unbalanced physiological situations, which can lead to serious consequences such as myocardial and cerebral infarction .The currently available drugs for atherothrombotic diseases are constrained by various limitations and an ideal drug is thus far to be developed . Research has revealed microorganisms as vital resources for developing antithrombotics. Traditionally used medicinal plants possessing antithrombotic activity have also been reported before. Endophytic microorganisms that reside asymptomatically inside the living plant tissues have recently been recognized as a repository of novel metabolites of pharmaceutical significance. This review provides an insight into the feasibility and future prospects of plant endophytic microbes as an emerging source of anti thrombotics.

Keywords: Thrombus, Endophyte, Medicinal Plants, Bioactive Compounds, Antithrombotic Activity

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Corresponding Author: MS. SALINI G.

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INTRODUCTION

An antithrombotic agent can be used therapeutically for prevention or treatment of an acute thrombus. They include anti platelet aggregation drugs, anticoagulants and thrombolytic drugs, which strike on different events in the formation of a thrombus [1]. During thrombus formation, fibrinogen is converted to fibrin via proteolytic action of thrombin, resulting in the formation of insoluble fibrin clots. Thus, fibrin, fibrinogen and thrombin are important targets in the screening of antithrombotic agents [2].

Thrombolytic drugs are able to dissolve a thrombus and reopen an occluded blood vessel. They may be used to treat a heart attack, stroke, deep vein thrombosis or pulmonary embolism, which can be immediately life-threatening. Plasminogen activators, such as reteplase or recombinant tissue plasminogen activator (Retavase), alteplase or tissue plasminogen activator (Activase), urokinase or urokinase-type plasminogen activator (Abbokinase), anisoylated plasminogen streptokinase activator complex or APSAC (Anistreplase), streptokinase (Streptase) etc. are the frequently used thrombolytic agents. They are also called fibrinolytic agents as they are serine proteases and convert plasminogen to plasmin which breaks down fibrin and dissolves the clot. Direct acting thrombolytic agents like plasmin is also being developed [3]. Despite their wide spread use, all these agents are relatively expensive and have one or the other limitations like hemorrhagic side effects, short half-life in the body [4], low fibrin specificity, allergic reactions [5] and reperfusion injury [6]. Furthermore, the most widely prescribed antiplatelet agents like Aspirin, Clopidogrel, Ticlopidine and anticoagulants like Warfarin are also not efficient enough to prevent recurrent ischemic attacks [1]. Therefore, there is considerable interest worldwide to develop safer, efficient and less expensive antithrombotics.

Various fibrinolytic compounds have been successively discovered from microbial sources, including bacteria, actinomyces and fungi [7] , oriental fermented food products[8] , bat saliva [9] snake venom[10][11], earthworms [12][13], marine algae[14], mushrooms[[15][16]] etc. But most of them are still under study with added focus being given on microbes. Several plants used for the treatment of thromboembolic diseases in different systems of traditional medicine have also shown antithrombotic activity [17]. In this context, this review aims to focus on the prospects of plant endophytic microbes as a potential source of antithrombotic drugs.

ANTITHROMBOTICS FROM MEDICINAL PLANTS

In traditional culture, medicinal plants are used all over the world and they are becoming increasingly popular in modern society as natural alternatives to synthetic chemicals [18]. There are evidences that consuming herbs, natural food sources and their supplements having antithrombotic (anticoagulant and antiplatelet) effect helps in prevention of cardio vascular diseases and stroke [19][20][21][22] . A lot of plants claimed in the traditional system still remain to be scientifically investigated [17]. However, lately there are reports of thrombolytic activity of a variety of medicinal plants as well (Table 1).

Table 1: Plants with *in vitro* thrombolytic activity

Plant	Reference
Ocimum sanctum	Khan et al. 2011[23]
Curcuma longa	
Azadirachta indica	
Anacardium occidentale	
Alpinia conchigera	Sultana et al .2012[24]
Lannea grandis	
Aglaonema hookerianum	
Withania somnifera	Shahriar et al. 2012[25]
Terminalia arjuna	
Moringa olifera	
Asparagus racemosus	
Sida acuta	Bahar et al .2013[26]
Ficus glomerata	Kirankumar and Ramesh 2014 [27]
Kalanchoepinnata	Akanda et al .2014[28]
Nigella sativa	Ansari et al .2014 [29]

ANTITHROMBOTICS FROM MICROBES

Consequent to the purification of streptokinase from *Streptococcus hemolyticus* and staphylokinase from *Staphylococcus aureus*[30], a succession of microbes producing fibrinolytic enzymes from fermented food and other sources have been discovered (Table 2 and 3). Their isolation techniques, strain improvement methods, production parameters, purification and properties of fibrinolytic enzymes have also been documented [31].

Table 2: Microbes with *in vitro* fibrinolytic activity isolated from oriental fermented foods

Microorganism	Source	Reference
Bacillus sp.KA38	Korean Jeot-gal	Kim et al . 1997[32]
Bacillus sp. DJ-4	Korean doenjang	Kim and Choi 2000[33]

***Bacillus amyloliquefaciens* DC-4**	Douchi	Peng et al. 2003[34]
***Bacillus subtilis natto* B-12**	Natto	Feng et al . 2009[35]
Bacillus amyloliquefaciens	Chinese soybean paste	Wei et al. 2011[36]
***Bacillus subtilis* LD-8547**	Douchi	Yuan et al . 2012[37]
***Aspergillus oryzae* KSK-3**	Rice-koji	Shirasaka et al. 2012[38]
***Acetobacter* sp. FP1**	Fermented pine needle extract	Park et al. 2012[39]
Bacillus subtilis* and *B. amyloliquefaciens	Cheonggukjang	Choi et al. 2013[40]
***Paenibacillus* sp. IND8**	Cooked Indian rice	Vijayaraghavan and Vincent 2014[41]

Table 3: Other microorganisms with *in vitro* fibrinolytic activity

Microorganism	Reference
Fusarium oxysporum	Sun et al. 1998[42]
***Strptomyces megaspores* SD5**	Chitte and Dey 2000[43]
***Aspergillus ochraceus* 513**	Betomunkueva and Egrov 2001[44]
***Rhizopus chinensis*12**	Xiao-lan et al. 2005[45]
***Bionectria* sp.**	Rovati et al . 2010[46]
Marine Fungi FG216	Xing et al. 2012[47]
***Streptomyces* sp.**	Simkhada et al. 2012[48]
***Bacillus amyloliquefaciens* CH51**	Kim et al. 2013[49]
***Bacillus subtilis* B-2805**	Alekseev et al. 2014[50]

ENDOPHYTIC MICROBES

Endophytes are those microorganisms that inhabit the interior of plants, especially leaves, stems and roots and showing no apparent harm to host [51]. Microbial endophytes mimic bioactive compounds produced by the plant itself, thus making them a promising source of novel molecules [52]. The production of bioactive substances by endophytes is directly related to the independent evolution of these microorganisms, which may have incorporated genetic information from higher plants, allowing them to better adapt to plant host. Secondary metabolites produced by endophytes can be safe for human use as they may have reduced cell toxicity, otherwise host tissue will die [53]. Recently endophytic research has come out with evidences of bioactive compounds with antimicrobial, antineoplastic, antioxidant, immunosuppressive, antithrombotic, anti-inflammatory and anti Alzheimer's activity among others [54].

ANTITHROMBOTICS FROM ENDOPHYTIC MICROBES

Although only a few studies have been conducted on the antithrombotic activity of endophytes, the results are promising as they draw attention to the production of different antithrombotics such as fibrinolytic compounds, anticoagulants and antiplatelet agents.

Ueda et al. (2007) isolated a *Fusarium* sp. BLB, which produces a strongly fibrinolytic alkaline protease, from Hibiscus leaves [55]. The enzyme was purified with ammonium sulfate precipitation and column chromatography with CM-Toyopearl 650M and Superdex 75 and was homogeneous on SDS-PAGE. Maximum activity of the protease with a molecular weight of 27 kDa was observed at pH 9.5 and temperature 50^0C respectively. A serine protease with fibrinolytic activity named verticase was identified by Li et al. (2007) from *Verticillium* sp. Tj33 strains residing in *Trachelospermum jasminoides* [56]. It was subsequently purified by a combination of DEAE-52, Sephadex G-75 and hydrophobic column chromatography and was demonstrated to be homogeneous by SDS-PAGE and isoelectric focusing. Verticase, with a molecular mass of 31 kDa and optimum pH and temperature 9-10 and 50^0-60^0 C, was found to be an enzyme that hydrolyzes fibrin directly without activation of plaminogen. Lu et al. (2007) screened the endophytic strain *Paenibacillus polymyxa* EJS-3, which produces novel fibrinolytic enzyme from root tissues of *Stemona japonica* (Blume) Miq, a Chinese traditional medicine[57]. Lu et al. (2010) purified the fibrinolytic enzyme PPFE-I from *Paenibacillus polymyxa* EJS-3, which showed additional significant anticoagulant effect in vitro [4]. The enzyme with a molecular mass of 63.3 kDa and optimum temperature and pH 37 °C and 7.5 respectively was purified with ammonium sulfate precipitation, hydrophobic, ion exchange and gel filtration chromatography. An endophytic *Fusarium* sp.CPCC480097 was also found to show high anti thrombotic activity (Wu et al. 2009) [2] and a fibrinolytic enzyme with molecular weight 28 kDa was identified by LC-MS/MS. Four endophytic strains, *Rahnella aquatilis*, *Pantoea agglomerans*, *Rhodotorula* sp. and *Penicillium paxilli*, isolated from Taiwanese herbal plants, showed inhibitory effects on collagen-induced and thrombin-induced platelet aggregation (Hsieh et al. 2009)[58]. Six secondary metabolites identified as lumichrome, genistein, daidzein, cyclo-Pro-Val , cyclo-Pro-Phe and methyl 2,4,5-trimethoxybenzoate were isolated subsequently from *Rahnella aquatilis* using bioassay-directed fractionation. Ahmed et al. (2013) isolated endophytic fungus FH-3 with fibrinolytic activity from Hibiscus leaves of Selangor, Malaysia [16].

FUTURE PROSPECTS

Investigations on antithrombotic activity of endophytes are in its infancy. Most of the endophytic research focuses on screening bioactive compounds with antimicrobial and antineoplastic activity [54][59]. Acknowledging the fact that endophytes are able to produce antithrombotics and also considering the growing scenario of mortality due to thrombotic diseases worldwide, equal attention should be given by researchers to exploit antithrombotics from endophytes.

In general, the study of endophytic microorganisms represents a relatively new branch and therefore an unexploited field [60]. It is since the isolation of Taxol producing endophyte *Taxomyces andreanae* [61] that endophytic microorganism fermentation came forth as an alternative cheaper and sustainable approach. Recently in biotransformation process also, endophytes have received big attention as biocatalysts in the chemical transformation of natural products and drugs, due to their ability to modify chemical structures with a high degree of stereo specificity [62].

Most of the endophytes in medicinal plants still remain to be researched on. Among the hundred thousand species of medicinal plants on earth, no more than five hundred species had been investigated worldwide for endophytes [63]. Bioactive natural products of medicinal plants have long been an important source of medicinal raw materials. However, the natural habitats for wild medicinal plants are being threatened by overuse and environmental and geopolitical instabilities [64]. Research on endophytes has now been accelerated, as extinction of any single plant species will diminish the entire suite of associated potential endophytes [54].

Another unexploited endophyte reservoir which needs to be focused alongside is the seaweeds of the marine biome. Substances obtained from endophytes associated with seaweeds have been shown to have antimicrobial, cytotoxic, antioxidant and other properties [65].

Additional pharmacological studies like in vivo assays, isolation of active components and clinical studies are necessary before these antithrombotics are introduced to the market. For a successful commercial production, strain improvement techniques, production parameters, purification and characterization processes of the compound etc. also need to be optimized [4].

CONCLUSION

Despite the availability of most modern and highly developed procedures like angioplasty, thrombolysis still continues as a widely accepted, timely intervening rescue practice in acute myocardial infarction. For ischemic strokes also, giving thrombolytics within three hours of the first stroke symptoms can facilitate in restraining stroke impairment. In many cases, antithrombosis therapy may be continued to decrease the risks. But an affordable, safe and efficient antithrombotic is yet to be formulated. The feasibility of endophytic antithrombotics should be studied in the light of this ever mounting need of alternative antithrombotics. Endophytic microbes have the ability to produce antithrombotic metabolites. But there is need for further exploration of the scantily researched endophytic habitats. This implies that in the future, these hidden microbes may provide high quality drug candidates to improve thrombosis prevention and treatment.

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REFERENCES

1. Sikka P and Bindra VK: Newer antithrombotic drugs. Indian J Crit Care Med. 2010; 14(4): 188–195.

2. Wu B, Wu L, Ruan L, Ge M and Chen D: Screening of endophytic fungi with antithrombotic activity and identification of a bioactive metabolite from the endophytic fungal strain CPCC 480097. Curr. Microbiol.2009; 58:522–527.

3. Marder VJ, Jahan R, Gruber T, Goyal A and Arora V: Thrombolysis with plasmin: Implications for stroke treatment. Stroke 2010; 41:S45-S49.

4. Lu F, Lu Z, Bie X, Yao Z, Wang Y, Lu Y and Guo Y: Purification and characterization of a novel anticoagulant and fibrinolytic enzyme produced by endophytic bacterium Paenibacillus polymyxa EJS-3. Thrombosis Research 2010; 126(5): e349–e355.

5. Kunamneni A, Abdelghani T and Ellaiah P: Streptokinase-the drug of choice for thrombolytic therapy. J. Thromb Thrombolysis 2007; 23: 9-23.

6. Wang QD, John P, Sjoquist PO and Lars R: Pharmacological possibilities for protection against myocardial reperfusion injury. Cardiovasc.Res.2002; 55: 25-37.

7. Peng Y, Yang X and Zhang Y: Microbial fibrinolytic enzymes: an overview of source, production, properties, and thrombolytic activity in vivo. Appl Microbiol Biotechnol 2005; 69:126–132.

8. Mine Y, Wong AHK and Jiang B: Fibrinolytic enzymes in Asian traditional fermented foods. Food Res Int 2005; 38:243–250.

9. Schleuning WD: Vampire bat plasminogen activator DSPA-alpha-1(desmoteplase): a thrombolytic drug optimized by natural selection. Haemostasis 2001; 31(3-6):118-22.

10. Bello CA, Hermogenes ALN, Magalhaes A, Veiga SS, Gremski LH, Richardson M and Sanchez EF : Isolation and biochemical characterization of a fibrinolytic proteinase from Bothrops leucurus (white-tailed jararaca) snake venom. Biochimie 2006; 88:189–200.

11. Marunak SL, Acosta OC, Leiva LC, Ruiz RM, Aguirre MV and Teibler P: Mice plasma fibrinogen consumption by thrombin-like enzyme present in rattlesnake venom from the north-east region of Argentina. Medicina (Buenos Aires) 2004; 64: 509–517.

12. Nakajima N, Mihara H and Sumi H : Characterization of potent fibrinolytic enzymes in earthworm, Lumbricus rubellus. Biosci Biotechnol Biochem 1993; 57:1726–1730.

13. Park YD, Kim JW, Min BG, Seo JW and Jeong JM : Rapid purification and biochemical characteristics of lumbrokinase III from earthworm for use as a fibrinolytic agent. Biotechnol Lett 1998; 20:169–172.

14. Matsubara K, Hori K, Matsuura Y and Miyazawa K: Purification and characterization of a fibrinolytic enzyme and identification of fibrinogen clotting enzyme in a marine green alga, Codium divaricatum. Comp Biochem Physiol B 2000; 125:137–143.

15. Lee SY, Kim JS, Kim JE, Sapkota K, Shen MH, Kim S, Chun HS, Yoo JC, Choi HS, Kim MK and Kim SJ : Purification and characterization of fibrinolytic enzyme from cultured mycelia of Armillaria mellea. Protein Expr Purif 2005; 43:10–17.

16. Ahmad MS, Noor ZM and Ariffin ZZ: New Thrombolytic Agent from Endophytic Fungi and Lignosus rhinoceros. Bentham Sci. The Open Conference Proceedings Journal 2013; 4(Suppl-2, M22): 95-98.

17. Kumar S, Joseph L, George M and Sharma A: A review on anticoagulant / antithrombotic activity of natural plants used in traditional medicine. International Journal of Pharmaceutical Sciences Review and Research 2011; 8(1): 70-74.

18. Verma KR, Mishra G, Singh P, Jha KK and Khosa R.L: Alpinia galangal- An important medicinal plant: a review. Der Pharmacia Sinica 2011; 2(1): 142-154.

19. Bazzano LA, He J, Ogden LG, Loria CM, Vupputuri S and Myers L: Fruit and vegetable intake and risk of cardiovascular disease in US adults: The first National Health and Nutrition Examination Survey Epidemiologic Follow-up Study. Am J Clin Nutr 2002; 76:93-99.

20. Gillman MW, Cupples LA, Gagnon D, Posner BM, Ellison RC and Castelli WP: Protective effect of fruits and vegetables on development of stroke in men. JAMA 1995; 273: 1113-1117.

21. Joshipura KJ, Ascherio A, Manson JE, Stampher MJ, Rimm EB and Speizer FE: Fruit and vegetable intake in relation to risk of ischemic stroke. JAMA 1999; 282: 1233-1239.

22. Liu S, Manson JE, Lee IM, Cole SR, Hennekens CH and Willett WC: Fruit and vegetable intake and risk of cardiovascular disease: the Women's Health Study. Am J Clin Nutr 2000; 72:922-928.

23. Khan IN, Sarker MMI, Hawlader S, Habib MR ,Rahman MM and Mannan A : Thrombolytic potential of Ocimum sanctum L., Curcuma longa L., Azadirachta indica L. and Anacardium occidentale L. Journal of Basic and Clinical Pharmacy 2011; 2(3) :125–127 .

24. Sultana I, Noor M, Barua J, Mahmood A, Das MC and Islam M :In-vitro anti-atherothrombosis activity of four Bangladeshi plants. Int J Green Pharm 2012; 6:5-8.

25. Shahriar M, Sharmin FA, Islam SMA, Dewan I and Kabir S: Membrane stabilizing and anti-thrombolytic activities of four medicinal plants of Bangladesh. The Experiment 2012; 4 (4): 265-270.

26. Bahar E, Ara J, Alam M, Nath B, Bhowmik U and Runi N: In-vitro antioxidant and thrombolytic activity of methanol extract of Sida acuta. Journal of Pharmacognosy and Phytochemistry 2013; 2(2):125-133.

27. Kirankumar S and Ramesh L: Clot lysis and antimitotic study of Ficus glomerata Roxb fruit extracts. ISRN Pharmacology 2014 , Article ID 975303, 4 pages.

28. Akanda MRUZ, Tareq SM, Zaman S, Khoshnabish MAA, Huq I and Ullah HMA: Evaluation of antioxidant, cytotoxic and thrombolytic activity of Kalanchoepinnata leaf. World Journal of Pharmacy and Pharmaceutical Sciences 2014; 3(6):52-63.

29. Ansari VA, Siddiqui, HH and Dixit RK: Antithrombotic and thrombolytic activity of Nigella sativa seed extracts. Pharmanest 2014; 5(1):1829-1833.

30. Collen D and Lijnen HR: Staphylokinase, a fibrin specific plasminogen activator with therapeutic potential. Blood 1994; 84(3): 680-686.

31. Raju EVN and Divakar G: An overview on microbial fibrinolytic proteases. International Journal of Pharmaceutical Sciences and Research 2014; Vol. 5(3): 643-656.

32. Kim HK, Kim GT, Kim DK, Choi WA, Park SH, Jeong YK and Kong IS: Purification and characterization of a novel fibrinolytic enzyme from Bacillus sp KA38 originated from fermented fish. J Ferment Bioeng 1997; 84(4): 307-312.

33. Kim SH and Choi NS: Purification and characterization of subtilisin DJ-4 secreted by Bacillus sp strain DJ-4 screened from Doen jang. Biosci Biotechnol Biochem 2000; 64: 1722-1725.

34. Peng Y, Huang Q, Zhang RH and Zhang YZ: Purification and characterization of a fibrinolytic enzyme produced by Bacillus amyloliquefaciens DC-4 screened from douchi, a traditional Chinese soybean food. Comparative Biochemistry and Physiology Part B 2003; 134: 45-52.

35. Feng Y, Wang C, Zheng D, Kong F, Zu G and Du M : Purification and Characterization of Nattokinase from Bacillus subtilis Natto B-12. J. Agric. Food Chem 2009; 57 (20): 9722–9729.

36. Wei X, Luo M, Xu L, Zhang Y, Lin X, Kong P and Liu H : Production of fibrinolytic enzyme from Bacillus amyloliquefaciens by fermentation of chickpeas, with the evaluation of the anticoagulant and antioxidant properties of chickpeas. J. Agric. Food Chem. 2011; 59 (8): 3957–3963.

37. Yuan J, Yang J, Zhuang Z,Yang Y, Lin L and Wang S :Thrombolytic effects of douchi fibrinolytic enzyme from Bacillus subtilis LD-8547 in vitro and in vivo . BMC Biotechnology 2012; 12(1): p36.

38. Shirasaka N, Naitou M, Okamura K, Kusuda M, Fukuta Y and Terashita T : Purification and characterization of a fibrinolytic protease from Aspergillus oryzae KSK-3. Mycoscience 2012; 53(5): p354.

39. Park, Jaeyoung, Yoon S, Kim S, Lee B and Cheong H: Characterization and Fibrinolytic Activity of Acetobacter sp. FP1 isolated from fermented pine needle extract. J. Microbiol. Biotechnol.2012; 22(2): 215–219.

40. Choi YH, Lee JS, Bae SY, Yang KJ, Yeom KW, Jo DH, Kang O and Baik HS : Isolation of bacteria with protease activity from cheonggukjang and purification of fibrinolyticeEnzyme. Journal of Life Science 2013; 23(2); 259-266.

41. Vijayaraghavan P and Vincent SGP: Medium optimization for the production of fibrinolytic enzyme by Paenibacillus sp. IND8 using response surface methodology. The Scientific World Journal 2014; Article ID 276942, 9 pages.

42. Sun T, Liu BH, Li P, Liu DM and Li ZH: New solid state fermentation process for repeated batch production of fibrinolytic enzyme by Fusarium oxysporum. Process Biochem 1998; 33(4): 419-422.

43. Chitte RR and Dey S: Potent fibrinolytic enzyme from a thermophilic Streptomyces megaspores strain SD5. Lett Appl Microbiol 2000; 31(6): 405-410.

44. Batomunkueva BP and Egorov NS: Isolation, purification and resolution of the extracellular proteinase complex of Asperigillus ochraceus 513 with fibrinolytic and anticoagulant activities. Microbiology 2001; 70(5): 519-522.

45. Xiao-lan L, Lian-xiang D, Fu-ping L, Xi-qun Z and Jing X: Purification and characterization of a novel fibrinolytic enzyme from Rhizopus chinensis 12. Appl Microbiol Biotechnol 2005; 67(2): 209-214.

46. Rovati JI, Delgado OD, Figueroa LIC and Farina JI: A novel source of fibrinolytic activity: Bionectria sp., an unconventional enzyme-producing fungus isolated from Las Yungas rainforest (Tucuman, Argentina). World J Microbiol Biotechnol 2010; 26:55–62.

47. Xing W, Wen-hui W, Li-chun S, Zhi-hua C, Jie Z and Bin B: Isolation of fibrinolytic active compound from marine fungi and initial identification of the strain. Natural Product Research & Development 2012; 24(1): p57.

48. Simkhada JR, Cho SS, Mander P, Choi YH and Yoo JC: Purification, biochemical properties and antithrombotic effect of a novel Streptomyces enzyme on carrageenan-induced mice tail thrombosis model. Thrombosis Research 2012; 129: 176–182.

49. Kim J, Choi KH, Kim JH, Song YS and Cha J: Enhancement of the thermostability of a fibrinolytic enzyme from Bacillus amyloliquefaciens CH51. Journal of Life Science 2013; 23(1)15-23.

50. Alekseev A, Koutsenogiy P, Miroshnikov P and Shilova M. Isolation and properties of fibrinolytic subtilisin-like serine protease secreted by the Bacillus subtilis strain B-2805. Doklady Biochemistry & Biophysics 2014; 455(1): p72.

51. Azevedo JL, Maccheroni Jr W, Pereira JO and Luiz de Araujo W: Endophytic microorganisms: a review on insect control and recent advances on tropical plants. Electronic Journal of Biotechnology 2000; 3(1): 40-65.

52. Priti V, Ramesha BT, Singh S, Ravikanth G, Ganeshaiah KN, Suryanarayanan TS and Uma RS: Opinion: How promising are endophytic fungi as alternative sources of plant secondary metabolites? Current Science 2009; 97(4):477-478.

53. Strobel GA: Endophytes as sources of bioactive products. Microbes and Infection 2003; 5(6): 535–544.

54. Shukla ST, Habbu PV, Kulkarni VH, Jagadish KS, Pandey AR and Sutariya VN: Endophytic microbes: A novel source for biologically/pharmacologically active secondary metabolites. Asian Journal of Pharmacology and Toxicology 2014; 2(3): 1-16.

55. Ueda M, Kubo T, Miyatake K and Nakamura T : Purification and characterization of fibrinolytic alkaline protease from Fusarium sp. BLB. Applied Microbiology and Biotechnology 2007; 74 (2): 331-338.

56. Li Y, Shuang J, Yuan W, Huang W and Tan R: Verticase: a Fibrinolytic Enzyme Produced by Verticillium sp. Tj33, an Endophyte of Trachelospermum jasminoides. Journal of Integrative Plant Biology 2007 49(11): 1548-1554.

57. Lu F, Sun L, Lu Z,. Bie X, Fang Y, and Liu S: Isolation and identification of an endophytic strain EJS-3 producing novel fibrinolytic enzymes. Curr. Microbiol 2007; 54: 435-439.

58. Hsieh PW, Hsu LC and Lai CH: Evaluation of the bioactivities of extracts of endophytes isolated from Taiwanese herbal plants. World J Microbiol Biotechnol 2009; 25:1461-1469.

59. Joseph B and Priya RM: Bioactive compounds from endophytes and their potential in pharmaceutical effect: a review. American Journal of Biochemistry and Molecular Biology 2011; 1: 291-309.

60. Strobel G, Daisy B, Castillo U and Harper J: Natural products from endophytic microorganisms. J Nat Prod. 2004; 67(2):257-68.

61. Stierle A, Strobel G and Stierle D: Taxol and taxane production by Taxomyces andreanae, an endophytic fungus of Pacific yew. Science 1993; 260(5105):214–216.

62. Pimentel MR, Molina G, Dionisio AP, Marostica MR and Pastore GM: The use of endophytes to obtain bioactive compounds and their application in biotransformation process. Biotechnology Research International 2011; 576286 doi:10.4061/2011/576286 .

63. Zhang H., Ying C., and Bai X.: Advance in endophytic microbes from medicinal plants. Int J Pharm Sci Res 2014; 5(5): 1589-1600.

64. Balandrin MF, Kloche JA, Wurtele ES and Bollinger WH: Natural plant chemicals: sources of industrial and medicinal materials. Science 1985; 228: 1154-1160.

65. De Oliveira ALL, de Felício R and Debonsi HM. Marine natural products: chemical and biological potential of seaweeds and their endophytic fungi. Brazilian Journal of Pharmacognosy 2012; 22(4): 906-920.