International Food Research Journal 25(3): 884-889 (June 2018)
Journal homepage: *http://www.ifrj.upm.edu.my*

Mini Review

# Metabolites of *Botryodiplodia theobromae* for therapeutic agent and food industry

*Purwandari, U.

*Department of Agroindustrial Technology, University of Trunojoyo Madura PO Box 2 Kamal, East Java, Indonesia 69162*

**Article history**

*Received: 5 March 2017*
*Received in revised form: 25 April 2017*
*Accepted: 29 April 2017*

**Keywords**

*Botryodiplodiatheobromae, Paclitaxel*
β-*glucan*
*Lasiodiplodins*
*Amylase*

**Abstract**

This paper reviews production of some metabolites by a soil fungus *Botryodiplodia theobromae,* including enzymes, hormones, exopolysaccharides, and paclitaxel/taxol, for therapeutic use, and for food industry.Paclitaxel, an anti-cancer agent, is produced when the fungus grows as an endophytic fungus. *B. theobromae* produces linear and branchedexoplysaccharide β-glucan, called lasiodiplodin, which shows various therapeutic activities: anti-tumor, hypoglycemic, anti-coagulant, anti-cancer, while protecting against DNA damage. Lasiodiplodins also show cytotoxicity against murine leukemia cell line. The fungus is known to produce amylase and glucoamylase to degrade starch into sugar for various process in food industry.



## Introduction

*Botryodiplodia theobromae* is well known as a high virulent plant pathogen in tropic and subtropic area. This soil-borne fungus infects important commodities such as mango (Khanzada *et al.*, 2004;Twumasi *et al*., 2014), cocoa pod (Twumasi *et al*., 2014), yam (Twumasi *et al.*, 2014), banana (Twumasi *et al.*, 2014), citrus (Zhao *et al.*, 2015), kenaff seed (Norhayati *et al*., 2016), rubber (Anh *et al.*, 2012), cashew (Adeniyi, 2013), and mulberry (Xieet al., 2014), leads to significant economic loss. For example, the fungus infected up 52%, and killed 8% of citrus plant,with around USD 3 million loss in a county in China (Xie *et al*., 2014). It affects around 280 species of plants, in various stages including postharvest time. Its high virulence resulted in cross infection with surrounding plants (Twumasi *et al*., 2014).

However, *B. theobromae* is utilised in the process of making gathot, a traditional fermented cassava originated from Java, Indonesia (Purwandari, 2000). Although various fungi grow during fermentation, *B. theobromae* is the dominant species growing inside cassava tuber and gives characteristic of black colour of gathot. *B. theobromae* has several synonyms. Some of them are *Botryodiplodia gossippii, Lasiodiplodia theobromae, L. tubericola, L. nigra, Diplodia theobromae, D. natalensis, D. gossypina,* and *Botryosphaeria rhodina (www.mycobank.org)*. A noodle made from gathot showed high antioxidant activity which is lacking in cassava (Purwandari *et al*., 2014).

Besides its pathogenicity, the fungus was reported to produce valuable natural products. This paper reviews some metabolites namely lasiodiplodin, paclitaxel, and amylaseproduced byB. theobromaewhich can be beneficial intherapeutic use and food industry.

### *Paclitaxel*

Paclitaxel has a brand name taxol, is a molecule capable of inhibiting tumor cell proliferation. The name of taxol is seemingly derived from yew tree *(Taxus brevifolia)* where it was isolated for the first time in the 60's. According to a traditional folk medicine, the substance of the bark can cure tumor (Zwawiak and Zaprutko, 2014). However, due to small concentration of the substance presents in the bark, the production of substance analogs to taxol from microorganism has been explored. A fungus, *Pestalotiopsis* sp., was successfully employed to produce taxol. The fungus was isolated from several medicinal plants: *Eucalyptus perriniana, Marchantia polymorpha, Rauwolfia serpentine, Nicotiana tabacum, Glycine max*, and *Jatropha curcas* (Zwawiak and Zaprutko, 2014).

Taxol is an important drug for treatment of

*Corresponding author.
Email: *umipurwandari@yahoo.com*

ovarian cancer, breast cancer, non-small cell lung cancer, and AIDS related Kaposi's sarcoma (Cancer Research UK, 2014). It is usually used in combination with other chemotherapy. There are several methods have been studied to improve the efficacy of taxol. Attaching nano particle of taxol with albumin is one of the methods, since albumin is a carrier of nutrition for tumor cells. As albumin absorb by the tumor cells, taxol attached to it is easily reach the target cells and inhibit its mitosis (Cancer Research UK, 2014). Combination of taxol and albumin is called abraxane or nab-paclytaxel which is designated to reduce side effects of taxol. Other method of improving the drug efficacy is by altering molecular structure. There are several alterations of the molecule to increase its activity against tumordevision, including replacement of phenyl, acetate, or acyloxy groups, and derivatisation of hydroxyl group (Zwawiak and Zaprutko, 2014). Method of drug administration also played important role, with low dose and frequent weekly administration gave better effect and less allergic reaction, than high dose with two weeks interval (Pandi *et al*., 2011; Mulcahy 2013).

Taxol was produced by endophytic *B. theobromae.* Endophytes are fungi grow in mutualistic way inside medicinal plants without showing any disease symptoms (Schulz *et al.,* 2002). Endophytic fungi produced exoenzymes to colonize the host plant, while utilizing apoplastic fluid of plant for their growth. On the other hand, host plant showed improve growth in the presence of endophytic fungi, presumably due to higher availability of minerals (Schulz *et al*., 2002). Endophytes demonstrated strong correlation with biological activity of host plants, and there was apparently a balance between fungal virulence and plant defence (Schulz *et al*., 2002). Due to its interaction with host and niche, endophytic fungi produced higher concentration of secondary metabolites than do pathogen or soil fungi (Schulz *et al*., 2002). Therefore, endophytic fungi are important in the production of natural products such as paclitaxel.

*Botryodiplodia theobromae* endophytic to some plants, showed significant production of taxol. The fungus isolated from *Morinda citrifolia* (Pandi *et al*., 2011), *Bidenspilosa* (Abdou *et al.*, 2010), and *Salacia oblonga* (Roopa *et al*., 2015), were among those tested for production of taxol. Taxol producing *B. theobromae* was isolated from various species and different parts of plant, such as leaf of *Morinda citrifolia* (Pandi *et al*., 2011), fresh aerial part of *Bidens pilosa* (Abdou *et al*., 2010), *Taxus baccata* (Venkatachalam *et al.*, 2008). Nevertheless, *B. theobromae* does not seem to be extensively explored for its taxol production, despite it infects broad types of plant species.

The taxol showed inhibition of proliferation of breast cancer cells. Giving the fungal taxolintraperitoneally at 7 mg/kg in 1 mL saline weekly for four weeks period increased some essential antioxidative enzymes in tumor-induced rats (Pandi *et al.*, 2010). Concentration of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) in serum or tissue of diseased rats treated with fungal taxol was also higher than those without taxol treatment. Meanwhile, concentration of non-enzymic antioxidants in the form of vitamin C, E, and glutathione (GSH) also increased in breast tissue of cancer-induced rats treated with fungal taxol. There was higher concentration of lipid peroxidase in breast tissue with fungal taxol treatment, while concentration of cyclooxygenase (COX) was lower, and cell proliferation in breast tissue was inhibited. The anti-cancer activity shown by fungal taxol was not different from that shown by commercial taxol at 1mg/kg concentration with the same frequency of treatment.

Several other biochemical parameters for anti-cancer activity, such as aspartate amino transaminase, alanine amino transaminase, and alkaline phosphatase reduced following *B. theobromaetaxol* treatment (Pandi *et al*., 2011). Decrease in expression of matrix metalloproteinase and proliferating cell nuclear antigen (PCNA) also happened in breast cancer bearing animals treated with fungal taxol. Taxol compounds called Botryorhodine A, and Botryorhodine B exhibited inhibition of breast cancer cell proliferation by more than 50%, at concentration as low as 0.1 µg/mL level (Abdou *et al*., 2010). The metabolites also showed toxicity to HeLa cells at level close to 50%. Whilst, when applied to MCF-7, a human breast cancer cellline, *B. theobromaetaxol* reduced cell viability up to 80% at concentration of 600 µg/mL (Pandi *et al.*, 2011).

Taxol production by *B. theobromae* is still low. The quantity of taxol produced by the fungus was around 280 µg/L medium (Venkatachalam *et al*., 2008; Gond *et al*., 2014), while the maximum taxol production by endophytic fungi isolated from *Taxus brevifolia* was over 800 µg/L (Gond *et al*., 2014). *Cladosporium cladosporioides* produced 800 µg/L, and *Metarhizium anisopliae* yielded 846 µg/L (Gond *et al*., 2014). There were several media used for taxol production by *B. theobromae,* including M1D medium (Venkatachalam *et al*., 2008; Pandi *et al*., 2010; Pandi *et al.*, 2011), malt extract, potato dextrose, casein-flesh-peptone, or corn steep-dextrose-yeast medium (Abdou *et al*., 2010). Fermentation was carried out

in 21 (Abdou *et al*., 2010), or 22 days (Pandi *et al.*, 2010) at 23°C (Abdou *et al.*, 2010).

Taxol is also an antifungal agent. Antimicrobial effect of taxol from *B. theobromae* (synonym of *Botryospheria rhodina*) was shown against some pathogens (Abdou *et al*., 2010). There were four types of taxol produced by the fungus, called Botryorhodine A, B, C, and D. Only Botryorhodine A and B exhibited inhibition of growth of *A. terreus, F. oxysporum,* and *Bacillus subtilis*. BotryorhodineC was the major product, but showed no antifungal effect. Similarly, Botryorhodine D also did not have antifungal activity. Fermentation method affects anti-fungal properties, with stationary cultures produced taxol of better anti-fungal effect than that produced by shaken cultures (Abdou *et al*., 2010).

*Lasiodiplodin*

Lasiodiplodins, also called lasiodiplodans, aremetabolites produced extra cellularly as exopolysaccharides by *B. theobromae.* The study about lasiodiplodins was apparently started back in 1998 whenthree lasiodiplodins were isolated from Lasiodiplodia theobromae ifo 31059(Matsuura *et al*., 1998). They consist of glucose monomer with β-(1→3)-D-linkage or β-(1→6)-D-linkage. Depending on type of isolate, the linear main chain of lasiodiplodin can have (1→3)-linkage at O-6 or (1→6)-linkage (Vasconcelos *et al.,* 2008). The exopolysaccharide had triple helix conformation (Vasconcelos *et al*., 2008). Production of exocellular polysaccharide owing β-(1→6)-D-glucans by fungi is rare.

Growth medium influenced type of branch oflasiodiplodins produced. Lasiodiplodins produced in glucose medium on a submerged fermentation, yielded up to 2.2 g/L (Oliveira *et al.*, 2015). Glucose was the only building block of the polysaccharide. Most of the lasiodiplodins (67%) was branched (1→ 3) (1→6)-β-glucans, which were insoluble in water. The linear lasiodiplodins have (1→6)-linkage, with two distinct molecular weight of 1.8 x $10^6$ Da and 7.0 x $10^7$ Da. Whengrown in a fructose containing medium, more branched lasiodiplodins were produced, than those produced in glucose medium (Queiroz *et al*., 2015).

β-glucan is related to antioxidant activity which was affected by molecular structure and water solubility. Lasiodiplodinof branched structure i.e. with (1→3; 1→6)-β-D-glucan showed high antioxidant effect, up to 80% total antioxidant activity (Giese *et al.*, 2015). Linear lasiodiplodin, on the other hand, showed very limited antioxidant activity (Giese *et al*., 2015). Some works had been done to improve its antioxidant activity by increasing its water solubility. Carboxymethylation of lasiodiplodin increased its water solubility, and consequently, improved antioxidant activity (Kagimura *et al*., 2015). There was a positive correlation between molecular size and antioxidant activity, as the result of carboxymethylation of lasiodiplodin (Kagimura *et al.*, 2015). However, there was only slight increase in antioxidant activity as shown by 36.28% ABTS radical removal, while DPPH scavenging acitivity was also negligible (Kagimura *et al*., 2015).

Exopolysaccharides of *B. theobromae* showed tumor inhibition activity. The activity is due to high antioxidant level, and was affected by branch type, growth medium, as well as dosage and duration of exposure. Linear (1→6)-β-D-glucan and branched (1→3; 1→6)-β-D-glucan showed inhibition of breast cancer cell proliferation (Quiroz *et al*., 2015). Branched lasiodiplodan produced from fructose medium had higher degree of branching and higher activity in reducing cell apoptosis and necrosis, while that produced in glucose medium showed lower activity. Direct effect of lasiodiplodan on cell death was demonstrated, while the indirect effect was shown by increasing oxidative stress of tumor cells, resulted in increasing tumorsuppressor and apoptotic genes (Quiroz *et al.*, 2015). The action of anti-proliferation was shown in time and dose dependent manner, with 1500 µg/mL as the most effective concentration. The longer exposure to lasiodiplodin, the more effective inhibition of cell proliferation.

Pro-inflammatory response was also induced by lasiodiplodins especially those highly branched with (1→3)-linkage chain (Oliveira *et al.*, 2015). Lasiodiplodin containing main chain with (1→6)-linkage did not induce anti-inflammatory response such as IL and TNF-α. Molecular weight, degree of branching, and water solubility positively correlated with the response. Moreover, it also prevented DNA damage induced by doxorubicin, and reduced inflammatory response (Mello *et al*., 2016).

Unlike endophytic fungi which are expected to produce health supporting substance(s), *B. theobromae* is a plant pathogen, thus it was not expected to produce such substances. Therefore, research on biosynthesis of lasiodiplodin by *B. theobromaeseems* lacking. However, there was a detail mechanism of EPS production by a plant pathogenic fungus *Aspergillus fumigatus* (Sheppard and Howell, 2016). The EPS was the main component of biofilm produced by the fungus, and categorized as galactosaminogalactan (GAG), a structure different from that of bacterial EPS. It is composed of galactose (Gal) and partially de-acetylated galactose (GalNAc), via several stages

started from synthesis of amino sugar within the cell, followed by polymerization of the sugars with α-1,4-linked and subsequently export of the polymer outside cells by transmembraneglycosyltransferase. The glycan polymer was then de-acetylated to form polycationic glycan which assisted attachment to surface of hyphae as well as host and inorganic material such as plastic. GAG also demonstrated prevention of recognition by innate immune receptor of host and resistance to killing by host neutrophil extracellular trap (Sheppard and Howell, 2016). There was no information whether lasiodiplodin was synthesized inside or outside cell. How this infection system of a plant pathogen turns into production of pharmacological substance does not seem to have been studied thoroughly. Nevertheless, EPS with moderate antioxidant effect was produced by a parasitic filamentous fungus *Cordyceps sinensis* (Leung *et al*., 2009). EPS is not only produced by pathogenic fungi, but also by endophytic species such as *Fusarium oxysporum* isolated from *Dioscorea zingiberensis* (Li *et al*., 2014).

*Amylase*

Enzymes belong to amylase group have important role in broad range of industries including food processing, fermentation, textile production, pharmacy, brewing, detergent production, bio-ethanol manufacturing, oligosaccharide mixture, and high branched dextrin for powdery food and cakes (Dey *et al*., 2016).

Broad range of fungi are amylase producers, including plant pathogens, endophytics, and post-harvest spoilage fungi. *Phytophtora* sp. is a plant pathogen produced amylase for carbohydrate degradation of host plant's cells (Brouwer *et al*., 2014). These carbohydrate-related enzymes were important for pathogenicity of plant pathogens (Brouwer *et al*., 2014). Amylase was also produced by spoilage fungi of agricultural commodities such tomato (Brindha *et al*., 2011) and legumes (Saleem *et al*., 2014).Moreover, endophytic fungi also capable of excreting amylase in considerable amount (Schulz *et al.*, 2002; Sunitha *et al*., 2012).

*B. theobromae* produces extracellular amylases (Ogundero 1987; Nwufo and Fajola 1988), as well other enzymes including cellulase,polygalacturonase (Salami and Akintokun, 2008), and pectin methyl esterase (Ogundero, 1987; Salami andAkintokun, 2008). Most microorganisms produces only α- and β-amylase (Adjuwon, 2011), however *B. theobromae* also produces extracellular glucoamylase (Umezurike, 1971). β-glucosidasewas produced by the fungus when grown in cellobiose medium (Umezurike, 1971). The enzyme hydrolyzes starch by releasing every single molecule of glucose from non-reducing end (Adjuwon, 2011). This type of enzyme is important for ethanolproduction.

In general, amylase production of *B. theobromae* grown in starch medium was low. Amylase of *B. theobromae* grown in cassava starch residue showed only3.25 U (mg glucose released/mL.h) (Ray, 2004). The production was increased by addition of inorganic ammonium salt, to reach 4.8 U (Ray, 2004). The fungus grown in cassava medium yielded 280 U/mL glucoamylase at 46 h incubation (Navaratnam *et al*., 1996). Upon addition of soybean powder, ($NH_3$) $PO_4$, peptone, $K_3PO_4$, and $CaCO_3$, glucoamylase production increased to 1950 U/mL (Navaratnam *et al.*, 1996).

*B. theobromae* is capable of producing amylase in raw starch at room temperature and neutral pH, to enable easy control of process. Maximum amylase production by the fungus was at around neutral pH 6 (Ray 2004), or 6-7 (Nwufo and Fajola, 1988), at 25°C (Nwufo and Fajola, 1988) or 30°C (Ray 2004). Enzyme production was positively correlated to starch concentration in the medium (Nwufo and Fajola, 1988), and soluble starch produced less amylase (Salami and Akintokun, 2008). Cassava starch residue was a substrate for amylase production. Complex starch-rich medium such as bread resulted in high amylase activity (Adjuwon, 2011). Contrary, lowest activity of the enzyme was resulted from glucose medium (Adjuwon, 2011). It was highlighted that crude enzyme even showed the highest activity as compared to purified enzyme (Adjuwon, 2011).

## Conclusion

*B. theobromae* shows great potential to be used in production of anti-microorganism and anti-tumor agent, antioxidant, and amylase. Its production needs to be improved, and its use for therapeutic use for other metabolic disorders such as hyperglycemia and prebiotic is worth exploring.

## Acknowledgement

Funding from Ministry of Research and Higher Education, the Government of Indonesia, is greatly acknowledged.

## References

Abdou, R., Scherlach, K., Dahsc, H-M., Sattler, I. and Hertweck, C. 2010. Botryorhodines A-D, antifungal and cytotoxic depsidones from *Botryosphaeriarhodina,* an endophyte of the medicinal plant Bidenspilosa.

Phytochemistry 71: 110-116.

Adeniyi, D. O, Adedeji, A. R, Oduwaye, O. F. and Kolawole, O. O. 2013. Evaluation of biocontrol agents against *Lasiodipodiatheobromae* causing inflorescence blight of cashew in Nigeria. IOSR Journal of Agriculture and Veterinary Disease 5(3): 46-48.

Adjuwon, A. O. 2011. Nutritional factors affecting the production of α-amylase in *Lasiodiplodiatheobromae* Pat. Biotechnology, Bioinformatics and Bioengineering 1(1): 131-135.

Brindha, J. R., Mohan, T. S., Immanuel, G., Jeeva, S. and Lekshmi, N. C. J. P. 2011. Studies on amylase and cellulose enzyme activity of the fungal organisms causing spoilage in tomato. Pelagia Research Library 1(3): 90-96.

Brouwer, H., Countinho, P. M., Henrissat, B. and de Vries R. P. 2014. Carbohydrate-related enzymes of important *Phytophtora* plant pathogens. Fungal Genetics and Biology 72: 192-200.

Cancer Research UK. February 2014. What paclitaxel is. Retrieved on August 28, 2016 from Cancer Research UK Website: *http://www.cancerresearchuk.org/about-cancer/cancers-in-general/treatment/cancer-drugs/paclitaxel.*

Dey, J. B., Kumar, A., Banerjee, R., Chandna, P. and Kuhad, R. C. 2016. Improvement of microbial α-amylase stability: Strategic approaches. Process Biochemistry. [In Press].

Giese, E. C., Gascon, J., Anzelmo, G., Barbosa, A. M., da Cunha, M. A. A. and Dekker, F. H. 2015. Free-radical scavenging properties and antioxidant activities of botryosphaeran and some other β-D-glucans. International Journal of Biological Macromolecules 72: 125-130.

Gond, S. K., Kharwar, R. N. and White Jr., J. F. 2014. Will fungi be the new source of the blockbuster drug taxol? Fungal Biology Review 28: 77-84.

Kagimura, F. Y., da Cunha, M. A. A., Theis, T. V., Malfatti, C. R. M., Dekker, R. F., Barbosa, A. M., Teixeira, S. D. and Salome, K. 2015. Carboxymethylation of (1→6)-β-glucan (lasiodiplodan): Preparation, characterization and antioxidant evaluation. Carbohydrate Polymers 127: 390-399.

Khanzada, M. A., Lodhi, A. M. and Shahzad, S. 2004. Mango dieback and gummosis in Sindh, Pakistan caused by *Lasiodiplodiatheobromae*. Plant Management Network-2 March 2004.

Kumar, P. S. and Singh, L. 2009. *Lasiodiplodiatheobromae* is a mycoparasite of a powdery mildew pathogen. Mycobiology 37(4): 308-309.

Leung, P. H., Zhao, S., Ho, K. P. and Wu, J. Y. 2009. Chemical properties and antioxidant activity of exopolysaccharides from mycelial culture of *Cordycepssinensis* fungus Cs-HK1. Food Chemistry 114: 1251-1256.

Li, P., Luo, H., Meng, J., Sun, W., Sun, W., Wang, X., Lu, S., Peng, Y. and Zhou, L. 2014. Effects of oligosaccharides from endophytic *Fusarium oxysporum*Dzf17 on activities of defense-related enzymes in Dioscoreazingiberensis suspension cells and seedling cultures. Electronic Journal of Biotechnology 17: 156-161.

Matsuura, H., Nakamori, K., Omer, E. A., Hatakeyama, C., Yoshihara, T. and Ichikara, A. 1998. Three lasiodiplodins from *Lasiodiplodiatheobromaeifo* 31059 In honour of Professor GH Neil Towers 75th birthday. Phytochemistry 49(2): 579-584.

Mello, M. B., Machado, C. S., Ribeiro, D. I., Aissa, A. F., Burim, R. V., Alves da Cunha, M. A., Barcelos, G. R. M., Antunes, I. M. G. and Bianchi, M. L. P. 2016. Protective effects of the exopolysaccharideLasiodiplodin against DNA damage and inflammation induced by doxorubicin in rats: Cytogenetic and gene expression assays. Toxicology. [In Press]. *http://dx.doi.org/10.1016/j. tox.2016.05.010*

Mulcahy, N. 2013. Strong case for weekly paclitaxel in breast cancer. Medscape Medical News, June 03, 2013.

Mycobank. 2016. Botryodiplodiatheobromae. Retrieved on September 19, 2016 from Mycobank Website: *http://www.mycobank.org/Biolomics.aspx?Table=Mycobank&Page=200&ViewMode=Basic*

Navaratnam, P.,Arasaratnam, V., Mahendran, S. and Balasubramaniam, K. 1996. Formulation of medium and recycling of biomass for glucoamylaseproduction by *Botryodiplodiatheobromae*. Process Biochemistry 31(1): 77-80.

Nghia, N. A., Chi, V. T. Q. and Dong, N. X. 2012. Molecular analysis of *Botryodiplodiatheobromae* isolates from rubber in Vietnam using rDNA, its sequencing and ISSR markers. Retrieved on July 28, 2016 from IRRDB Website: *http://clinic.rubberboard.org.in/PDF/ircpdf/BT-05%20Nguyen%20Anh.pdf*

Norhayati, M., Erneeza, M. H. and Kamaruzaman, S. 2016. Morphological, pathogenic and molecular characterization of *Lasiodiplodiatheobromae:* A causal pathogen of black rot disease on Kenaf seeds in Malaysia. International Journal of Agriculture and Biology 18: 80-85

Nwufo, M. I. and Fajola, A. O. 1988. Production of amylolytic enzymes in associated with corm rots of *Colocasiaesculenta*. ActaMicrobiologica Hungarica 35(4): 371-377.

Ogundero, V. W. 1987. Crown rot of fungi of Nigerian bananas cv. Robusta and the effects of benomyl on their exo-enzymes. Journal of Basic Microbiology 27(1): 43-47.

Oliveira, K. S. M., Di Bastiani, M., Cordeiro, L. M. C., Costa, M. F., Toleado, K. A., Iacomini, M., Babosa, A. M., Dekker, R. and Nascimento, V. M. G. 2015. (1→6)- and (1→3) (1→6)-β-glucans from Lasiodiplodiatheobromae MMBJ: Structural characterization and pro-inflammatory activity. Carbohydrate Polymers 133: 539-546.

Pandi, M., Kumaran, R. S., Choi, Y-K., Kim, H. J. and Muthumary, J. 2011. Isolation and detection of taxol, an anticancer drug produced from *Lasiodiplodiatheobromae*, an endophytic fungus of the medicinal plant Moringacitrifolia. African Journal of Biotechnology 10(8): 1428-1435.

Pandi, M., Manikandan, R. and Muthumary, J. 2010. Anticancer activity of fungal taxol derived from *Botryodiplodiatheobromae* Pat., an endophytic fungus against 7,12 dimethyl benz(a)anthracene (DMBA)-induced mammary gland carcinogenesis in Sprague Dawley rats. Biomedicine and Pharmacotherapy 64: 48-53.

Pandi, M., Rajapriya, P., Suresh, G., Ravichandran, N., Manikandan, R., Thiagarajan, R. and Muthumary, J. 2011. A fungal taxol from *Botryodiplodiatheobromae* Pat. Attenuates 7,12 dimethyl benz(a)anthracene (DMBA)-induced biochemical changes during mammary gland carcinogenesis. Biomedicine and Preventive Nutrition 1: 95-102.

Purwandari, U. 2000. Aflatoxin in gathotan in relation to fungal distribution. Melbourne: Royal Melbourne Institute of Technology University, Master Thesis.

Purwandari, U., TristianaG. R. andHidayati, D. 2014. Gluten-free noodle made from gathotan flour: antioxidant activity and effect of consumption on blood glucose level. International Food Research Journal 21(4): 1629-1634.

Queiroz, E. A. I. F., Fortes, Z. B., da Cunha, M. A. A., Barbosa, A. M., Khaper, N. and Dekker, R. F. H. 2015. Antiproliferative and pro-apoptotic effects of three fungal exocellular β-glucans in MCF-7 breast cancer cells is mediated by oxidative stress, AMP-activated protein kinase (AMPK) and the Forkhead transcription factor, $FOXO_3$a. The International Journal of Biochemistry and Cell Biology 67: 14-24.

Ray, R.C. 2004. Extracellular amylase(s) production by fungus *Botryodiplodiatheobromae* and *Rhizopusoryzae* grown on cassava starch residue. Journal of Environmental Biology 25(4): 489-495.

Roopa, G., Madkusudkan, M. C., Sunil, K. C. R., Lisa, N., Calvin, R., Poornima, R., Zeinab, N., Kini, K. R., Prakash, H. S. and Geetha, N. 2016. Identification of taxol-producing endophytic fungi isolated from *Salaciaoblonga* through genomic mining approach. Journal of Genetic Engineering and Biotechnology 13: 119-127.

Salami, A. O. and Akintokun, A. K., 2008. Post-harvest enzymatic activities of healthy and infected cassava (*Manihotesculenta* Crantz) tubers. Emirate Journal of Agriculture 21(1): 1-17.

Saleem, A. and Ebrahim, M. K. H. 2014. Production of amylase by fungi isolated from legume seeds collected in Almadinah Almunawwarah, Saudi Arabia. Journal of Taibah University for Science 8:90-97.

Schulz, B., Boyle, C., Draeger, S., Rommert, A-K. and Krohn, K. 2002. Endophytic fungi: a source of novel biologically active secondary metabolites. Mycological Research 106(9): 996-1004.

Sheppard, D. C. and Howell, P. L. 2016. Biofilm exopolysaccharides of pathogenic fungi: Lessons from bacteria. Journal of Biological Chemistry 291: 12529-12537.

Sunitha, V. H., Ramesha, A., Savitha, J. and Srinivas, C. 2012. Amylase production by endophytic fungi *Cylindrocephalum* sp. isolated from medicinal plant Alpiniacalcarata (Haw.) Roscoe. Brazilian Journal of Microbiology 43(3): 1213-1221.

Twumasi, P., Ohene-Mensah, G. and Moses, E. 2014. The rot fungus *Botryodiplodiatheobromae* strains cross infect cocoa, mango, banana and yam with significant tissue damage and economic loss. African Agricultural Research 9(6): 613-619.

Umezurik, G. M. 1971. The purification and properties of extracellular β-glucosidase from *Botryodiplodiatheobromae* Pat. Biochimicaet Biophysica Acta (BBA)-Enzymology 227(2): 419-428.

Vasconcelos, A. D., Monteiro, N. K., Dekker, R. F. H., Barbosa, A. M., Carbonero, E. R., Silveira, J. I. M., Sassaki, G. L., da Silva, R. and da Silva, M. dL. C. 2008. Three exopolysaccharide of the β-(1 → 6)-D-glucan type and β-(1 → 3)-D-glucan produced by strains of *Botryospheriarhodina* isolated from rotting tropical fruit. Carbohydrate Research 343: 2481-2485.

Venkatachalam, R., Kamalraj, S. and Muthumary, J. 2008. Taxol from *Botryodiplodiatheobromae* (BT115)-An endophytic fungi of Taxusbaccata. Abstract III3-P-006. Journal of Biotechnology 136S: S187-S197.

Xie, H-H, Wei, J-G., Liu, F., Pan, X-H. and Yang, X-B. 2014. First report of mulberry rot caused by *Botryodiplodiatheobromae* in China. APS Journals 98(11): 1581.3

Zhao, W., Bai, J., McCollum, G., Baldwin, E. and Drake, H. L. 205. High incidence of preharvest colonization of Huanglobing-symptomatic Citrus sinensis fruit by *Lasiodiplodiatheobromae (Diplodianatalensis)* and exarcebation of postharvest fruit decay by that fungus. Applied and Environmental Microbiology 81(1): 364-372.

Zwawiak, J. and Zaprutko, L. 2014. A brief history of taxol. Journal of Medical Science 1(83): 47-52.