ABSTRACT

ANTIFUNGAL POTENCY of *Stevia rebaudiana Bertoni* LEAVES EXTRACT TOWARDS *Candida albicans*

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ABSTRACT

Introduction: *Candida* spesies are eukaryotic opportunistic pathogens that reside on the mucosa oral cavity, gastrointestinal tract, vagina and sometimes skin. An infection caused by *Candida* is termed candidiasis or candidosis. Oral candidiasis is a common opportunistic infection of the oral cavity caused by overgrowth of *Candida* spesies, the most common being *Candida albicans*. Risk factor for oral candidiasis are local factors, systemic factors and the physiological states. The most commonly used classes of antifungal drug treatment to *Candida* infections are the triazole, imidazole, polyenes and echinocandins. However, the management of *Candida* infections faces many problems, such as toxicity, resistance of *Candida* to antifungal drugs, relapse to *Candida* infection and the high cost of antifungal drugs. **Discussion:** Many investigators are exploiting alternative therapeutic strategies to overcome problems treating candidiasis. *Stevia rebaudiana Bertoni* leaves extracts has been known to have antimicrobial activity to treat candidiasis. **Aim:** This review article is to explain antifungal potency of *Stevia rebaudiana Bertoni* leaves extracts toward *Candida albicans*. **Conclusion:** *Stevia rebaudiana Bertoni* leaves extracts can use as cheap, natural and alternative agent to control candidiasis.

Keywords: antifungal, candidiasis, *Candida albicans*, *Stevia rebaudiana Bertoni*

INTRODUCTION

The most common fungal infection among human population is candidiasis, the etiology of which is mostly *Candida albicans*[1]. *Candida albicans* are normal microflora of an individual's mucosa oral cavity, gastrointestinal tract, vagina and sometimes the skin[2-5]. However, as a result of a discrupted balance of the normal flora or a compromised immune system, *Candida albicans* spesies can become pathogenic and

cause infection such as candidiasis[3, 5]. Oral candidiasis is a common opportunistic infection of the oral cavity caused by an overgrowth of *Candida* species, the most common being *Candida albicans*. The risk factor of oral candidiasis are local factors, systemic factors and the physiological states[4, 6].

A significant increased in the prevalence of infections caused by *Candida* species has been observed in the past two decades[1]. The most commonly used classes of antifungal agents to treat *Candida* infection are the azole, polyenes and echinocandins[6]. However, the management of *Candida* infections faces many problem, such as toxicity, resistance of *Candida* to commonly used antifungal drugs, relapse of *Candida* infection, the high cost of the antifungal drugs and retain many side effect[5-7].

To elude these problems, investigators are exploiting alternative therapeutic strategies, such as the use of natural products[8]. The screening of plant extract has been of great interest to scientists in the search for new drugs for greater effective treatment of several diseases[9]. Plant extracts and phytochemicals with known antimicrobial properties can be of great significance in therapeutic treatments[10].

One of the potent members of the Asteraceae family is *Stevia rebaudiana Bertoni*, which is used traditionally for the source of natural sweetener and found to contain over 100 phytochemicals[11-13]. Leaves extract of *Stevia rebaudiana Bertoni* has many medical applications, such as an antifungal[11, 13-15]. The objective of this article is to review the antifungal potency of *Stevia rebaudiana Bertoni* leaves extracts toward *Candida albicans*.

LITERATURE REVIEW

Candidiasis

Candidiasis or candidosis is the most common fungal infection among human population, the etiology of which is mostly *Candida albicans* (50-60% of cases).Other important pathogens include *Candida glabrata*(15-20%), *Candida parapsilopsis* (10-20%), *Candida tropicalis* (6-12%), *Candida pseudotropicalis* (<5%), *Candida lusitaniae*(<5%), *Candida dubliniensis*(<5%), and *Candida stellatoidea* (<5%)[1, 16, 17]. Most *Candida* infection only affect the mucosa lining, but the rare systemic, manifestations may have a fatal course[3]. In the oral cavity, lesion of *Candida albicans* can be seen as various forms, such as acute pseudomembranous candidiasis (*thrush*), acute atrophic candidiasis (antibiotic sore mouth), chronic atrophic candidiasis (denture stomatitis), chronic hyperplastic candidiasis (candidal leukoplakia), angular cheilitis (perleche, angular stomatitis), median rhomboid glossitis and linear gingival erythema. Angular cheilitis has been associated with intra oral *Candida* infection of the pseudomembranous or erythematous types, cutaneous *Staphylococcus* or *Streptococcus* infection, isolated nutritional deficiencies, particularly with vitamin B_{12} or iron and mouth breathing. Median rhomboid glossitis has been associated with smoking and the use of inhaled corticosteroids. Linear gingival erythema has been associated with signify disease progression in HIV and also been reported in healthy children[17].

The local risk factor of oral candidasis include xerostomia, used of steroid asthma inhaler, unhygienic denture, smoking and high carbohydrate diet. The systemic risk factor of oral candidasis include medication (antibiotics, corticosteroid or immunosuppressant, chemotherapy agents), uncontrolled diabetes, immune defects (HIV disease, leukaemias or malignancies),

deficiency Fe and vitamin $B_{12.}$ The physiological states factor of oral candidasis include pregnancy, infancy and old age[4, 6].

Candida albicans (habitat, transmission and characteristic)

Candida albicans is indigenous to the oral cavity, gastrointestinal tract, vagina and sometimes the skin as the normal microflora in approximately 50% of the population[3, 4]. The areas of the mouth most heavily colonized by *Candida* are the posterior dorsal tongue, palate and buccal mucosa[3, 16]. As a result of a disrupted balance of the normal flora or a compromised immune system, *Candida* spesies can become pathogenic[3]. *Candida albicans* infection is usually endogenous, although cross infection may occur from mother to baby, and among infant siblings[4].

Characteristics *Candida albicans* typically grown with three distinct morphologies- yeast, pseudohyphae and true hyphae. Unicellular yeast cells grow by budding. The spherical to oval budding yeast cells 3-5 x 5-10 µm in size. These yeast-phase cells are also called blastospores, but should not be confused with bacterial spores. In pseudohyphae cells, the buds elongate and fail to separate from the mother cell, producing filaments of elongated buds but retaining constrictions at the septal junction (elongated filamentous cells joined end to end), especially at lower incubation temperatures and on nutritionally poor media. True hyphae consist of chains of tube-like cells with no constrictions at the septal junction[3, 4].

Antifungal Drugs of Candidiasis

Candidiasis treatment usually uses antifungal drugs that work in two ways either fungistatic or fungicidal activity. Antifungal drugs acts against fungal cell membrane by binding to and disrupting them or blocking ergosterol formation[5, 6]. The most commonly used antifungal drugs belong to the group of polyenes and azoles. Polyenes such as nystatin and amphotericin B are the mostly used drugs in the treatment of oral

candidiasis[18]. Nystatin is not absorbed from the mucous membranes or through intact skin; taken orally, it is poorly absorbed from the gastrointestinal tract. Nystatin for oral candidiasis is available in the form of pastilles and an aqueous suspension as a rinse and expectorated, while for treating denture stomatitis usually used nystatin cream[5].

Azoles are divided into the imidazoles (clotrimazole, miconazole, ketoconazole) and triazoles (fluconazole, itraconazole, posaconazole, voriconazole)[17]. Imidazole useful in dentistry. Oropharyngeal candidiasis can treated using clotrimazole oral, lozenges or troches, while denture stomatitis can used clotrimazole cream[5].

Ketoconazole is indicated in the treatment and management of mucocutaneous and oropharngeal candidiasis. Ketoconazole has many drug interactions. The most serious adverse reaction associated with ketoconazole is hepatotoxicity[5]. Fluconazole is indicated for treatment of oropharyngeal and esophageal candidiasis and serious systemic candida infections. Fluconazole is now indicated to treat vaginal candidiasis and is used prophylactically against candidiasis in immunocompromised patients or for treatment of candida infection that do not respon to other agents[5, 17].

The administration of antifungal drugs is usually sufficient to treat candidiasis. However some fungal species such as *C. glabrata* and *C. krusei* are less susceptible to azole drugs while *C. albicans* are often resistant to azole drugs[19]. It is known that fungal resistance to antifungal drugs increases. In addition, antifungal drugs also have side effects that cannot be avoided by patients such as gastro-intestinal disturbances (nausea, vomiting, diarrhea, dyspepsia), rash, taste disturbance and renal toxicity[5, 6]. Echinocandins antifungals include drugs such as anidulafungin, caspofungin and micafungin, are used in the management of invasive candidiasis and administered by intravenous infusion[6].

Botanical Description, Nutritional Aspects, Biochemical Aspects and Health Benefits of *Stevia rebaudiana Bertoni*

Stevia rebaudiana Bertoni was discovered by Dr.Moises Santiago Bertoni in 1888 at Paraguay. Initially called *Eupatorium rebaudianum,* its name change to *Stevia rebaudiana Bertoni* in 1905 after a Paraguayan chemist Dr.Rebaudi[20]. Stevia is a genus of about 200 spesies of herbs and shrubs in the sunflower family (*Asteraceae*), native to the Amambay region in the north east of Paraguay. Today its cultivation has spread to other regions of the world, including Canada and some part of Asia and Europe [21]. Stevia plant grows up to 65 cm to as tall as 180 cm. It is a short day plant and flowering from January to March in the southern hemisphere. The flowers are white in color with a pale purple throat[22]. The suitable natural climate is semihumid subtropical with temperature extremes from 21^0C to 43^0C and average 24^0C[23]. The leaves are sessile, 3-4 cm long, elongate lanceolate or spatulate shaped with blunt tipped lamina, serrate margin from the middle to the tip and entire below [24] (Figure 1).

Figure 1. Herbaccous shrub plant of *Stevia rebaudiana Bertoni* [25]

Stevia rebaudiana Bertoni is a natural sweet herb native. Sweetness of 1 g of stevia in 100 ml water was equivalent to a sucrose solution containing 20 g of sucrose (sugar) [26]. In 2006, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) announced accepted daily intake (ADI) of stevioside of up to 5.0 mg/kg body weight[27, 28].

The leaves of Stevia naturally contain a complex mixture of eight sweet diterpene glycosides, including stevioside (300-fold sweeter tasting than sucrose), rebaudiosides A (250-to 450-fold sweetener). rebaudiosides B, rebaudiosides C, rebaudiosides D, rebaudiosides E, steviobioside, dulcoside A, isosteviol and dihydroisosteviol. The steviol glycosides are the compound responsible for the sweet taste and currently in use as a sweetener with non caloric in a number of industrial foods[29]. The dry extract from the leaves of stevia contains flavonoids, alkaloids, water soluble chlorophylls and xanthopyhlls, hydroxycinnamic acids (caffeine, chlorogenic, etc), neutral water-soluble oligosaccharides, free sugars, amino acids, lipids, essential oils and trace elements[29-32]. Among the 230 spesies in the genus Stevia, only the spesies *rebaudiana* and *phlebophylla* produce steviol glycosides[24, 31]**.** Medicinal value of Stevia leaves extract, due to the most important bioactive compound including alkaloids, flavonoids, tannins and phenolic compounds with antioxidant, antimicrobial and anti-inflammatory effects[24, 33].

Likes many plant glycosides, stevia has also shown activity as an antifungal[11, 13-15]. As toxicological studies has shown that stevioside does not have mutagenic, teratogenic or carcinogenic effects and no allergic reaction have been observed when it is used as a sweetener[34]. It can be safely used in herbal medicines.

Effectiveness antifungal potency of *Stevia rebaudiana Bertoni* leaves extract toward *Candida albicans*

Study conducted by Subramaniam R (2011) has been reported the antifungal activity of the 100% pure solution from Stevia leaves extract against *Candida albicans* that is responsible for oral candidiasis especially in increased number among diabetics patient. Stevia leaves extract showed statistically significant antifungal activity against *Candida albicans* in comparison to the control with the mean zone of inhibition was 15.25

mm. The minimal inhibitory concentration for *Candida albicans* was 12,5%[14].

Study by Shiddique AB et al (2014) was carried out to evaluate the phytochemical screening and the antimicrobial activity of *Stevia rebaudiana Bertoni* leaves extracted using various solvents like n-hexane, dichloromethane, acetone and ethyl alcohol. Preliminary phytochemical screenings showed the most abundant compound in the stevia leaves extract were alkaloid and steroid followed by tannins, saponins and flavonoids. The antifungal potency after incubation at 37^0C for 24 hour showed the largest diameter zone inhibition against *Candida albicans* was 12,08 mm with minimum inhibition concentration (MIC) values was 100µg/disc[13].

Similar results of the antifungal activity of the *Stevia rebaudiana Bertoni* leaves extract against *Candida albicans* were obtained in a study conducted by Jayaraman S et al (2008). Four solvent extract of *Stevia rebaudiana Bertoni* leaves (ethyl acetate, acetone, chloroform and water) were investigated against *Candida albicans*. All solvent extract of *Stevia rebaudiana Bertoni* leaves from this study were active to inhibited the growth of *Candida albicans* after incubation 24, 48, 72 and 96 hour[11].

Study by Muanda FN et al (2011) has been demonstrated the antifungal activities of the essential oil (EO), water extract (WE) and methanol-water extract (MWE) from *Stevia rebaudiana Bertoni* leaves against *Candida albicans*. The EO was analyzed by gas chromatography/mass spectrometry. The WE and MWE compounds were identified by RP-HPLC analysis. The antifungal activities was examined by the disk-diffusion method. More than 33 components were identified as yield of essential oils from *Stevia rebaudiana Bertoni* leaves extract, such as carvacrol, caryophyliene oxide, spathulenol, cardinal, α-pinene, ibuprofen, isopinocarveol, caryophylle, pinene-3 one, dispers yellow and limonene.

The EO, WE and MWE from *Stevia rebaudiana Bertoni* leaves presented an inhibitory activity against *Candida albicans*. MWE from *Stevia rebaudiana Bertoni* leaves have the most effective antifungal activity against *Candida albicans* with the diameter of inhibition zone 17 mm, while inhibition zone of EO and WE were 15 mm and 11 mm,respectively. EO has demonstrated weak antifungal activity with minimum inhibitory concentration (MIC) values always >1000 µg/ml compared to WE (> 700 µg/ml) and MWE (> 500 µg/ml)[15].

DISCUSSION

The phytochemical properties extract of the leaf of *Stevia rebaudiana Bertoni* have potencially beneficial antifungal (*in vitro*) towards *Candida albicans*[13-15]. The Minimum Inhibitory Concentration (MIC) for *Candida albicans* have been scientifically proven 12,5% from Stevia leaves extract in the study conducted by Subramaniam R (2011) and the MIC values was 100µg/disc in the Study by Shiddique AB et al (2014). Study by Muanda FN et al (2011) has also been demonstrated the antifungal activities of the essential oil (EO), water extract (WE) and methanol-water extract (MWE) from *Stevia rebaudiana Bertoni* leaves against *Candida albicans* with the MIC values of MWE, WE and EO was > 500 µg/ml, > 700 µg/ml and > 1000 µg/ml respectively. The Minimum Inhibitory Concentration (MIC) was reported as the lowest concentration of the extracts capable of inhibiting the growth of microrganisms tested. The MIC of Stevia leaves extract for *Candida albicans* was calculated as the lowest concentration of the Stevia leaves extract inhibiting the visible growth of for *Candida albicans*[14].

Study by Jayaraman S et al (2008) has been demonstrated all solvent extract of *Stevia rebaudiana Bertoni* leaves (ethyl acetate, acetone, chloroform and water) were active to inhibited the growth of *Candida albicans* after incubation 24, 48, 72 and 96 hour due to the greater stability of the active principles in the solvent over a longer period of time[11, 33].

Preliminary phytochemical screenings by Shiddique AB et al (2014) showed the most abundant compound in the stevia leaves extract using various solvents like n-hexane, dichloromethane, acetone and ethyl alcohol were alkaloid and steroid followed by tannins, saponins and flavonoids[13].

Medicinal value of Stevia leaves extract, due to the most important bioactive compound including alkaloids, flavonoids, tannins, essential oils and phenolic compounds with antioxidant, antimicrobial and anti-inflammatory effects[24, 33]. Carvacrol, caryophyllene,caryophyllene oxide, spathulenol, cardinal, limonene, isopinocarveol, α-pinene and ibuprofen were identified as major compounds essential oil from Stevia leaves extract with antioxidant, antimicrobial and antifungal activities[15].

Agati et al (2012) reported the antifungal effect which could attribute to the high content of flavonoid that involved in inhibition of nucleic acid biosynthesis and other metabolic processes[35]. Aboh et al (2014) reported that the lipophilic nature of flavonoid as antifungal can also interfere fungal cell membrane and damage the function of membrane and cell walls[36, 37].

Pyla et al (2010) reported the phenols mechanism against microbes may be conjugated with interactions that inactivate microbial adhesions, cell envelope transport proteins and non spesifik interactions with carbohydrates[38]. The mechanism of antifungal action of phenols occurs through its ability to denature protein bonds in cell membrane so the cell will lysis and subsequently phenols bind with sulfhydrile groups of fungal protein to change the shape of cell membraneproteins[36, 37]. Moreover Srivastava et al proposed that the inhibition impact was depending on changes of the intercellular synthesis of enzymes[39].

The mechanism of antifungal action of saponins occurs through its ability to damage the fungal cell membrane and inhibit the formation of yeast[37]. The mechanism of tannins as antifungal occurs through the hydrolysis of

esther bonds between gallic acid which affects the biosynthesis of cell walls and cell membrane. Changes in cell membrane permeability can cause a decrease in cell volume[36, 37].

Components presented in small amounts in essential oils such as carvacrol from Stevia leaves extract could play an important role in antimicrobial activity due to the possible synergistic action with other components. Carvacrol together with thymol can enter between the fatty acyl chains making up membrane lipid bilayers, thus altering the fluidity and permeability of cell membranes[8, 40]. Some authors indicate that this action on fungi particularly *Candida albicans*, affects the regulation and function of important membrane-bound enzyms that catalyze the synthesis of a number of major cell wall polysaccharide components, such as B-glucans, chitin and mannan, thus disturbing cell growth and envelope morphogenesis[8, 41].

CONCLUSIONS

Stevia rebaudiana Bertoni leaves extract has a great potential and an ideal candidate for future research into their uses for antifungal drugs towards *Candida albicans.* The antifungal property from *Stevia rebaudiana Bertoni* leaves extract can lead to cheap and readily available natural drugs for the control of candidiasis. The most important bioactive compounds (phytochemical properties) which potentially antifungal of Stevia leaves extract are alkaloids, flavonoids, tannins, saponin, phenolic compounds, and essential oils. However, *in vivo* studies need to be conducted to ascertain the safety and acceptability of these herbal product.

REFERENCES

1. Pfaller M, Diekema D. Epidemiology of invasive candidiasis: a persistent public health problem. Clinical microbiology reviews. 2007;20(1):133-63.
2. Shao L, Sheng C, Zhang W. Recent advances in the study of antifungal lead compounds with new chemical scaffolds. Yao xue xue bao= Acta pharmaceutica Sinica. 2007;42(11):1129-36.

3. Kim J, Sudbery P. Candida albicans, a major human fungal pathogen. The Journal of Microbiology. 2011;49(2):171.
4. Samaranayake L. Essential Microbiology for Dentistry 5th ed. Edinburgh: Elsevier Health Sciences; 2018.
5. Haveles EB. Applied Pharmacology for the Dental Hygienist 8th ed: Elsevier Health Sciences; 2020.
6. Wray D. Essential Dental Therapeutics. UK: John Wiley & Sons Ltd; 2018.
7. Martins N, Ferreira IC, Barros L, Silva S, Henriques M. Candidiasis: predisposing factors, prevention, diagnosis and alternative treatment. Mycopathologia. 2014;177(5-6):223-40.
8. Mandras N, Nostro A, Roana J, Scalas D, Banche G, Ghisetti V, et al. Liquid and vapour-phase antifungal activities of essential oils against Candida albicans and non-albicans Candida. BMC complementary and alternative medicine. 2016;16(1):330.
9. Dimayuga RE, Garcia SK. Antimicrobial screening of medicinal plants from Baja California Sur, Mexico. Journal of ethnopharmacology. 1991;31(2):181-92.
10. Rojas JJ, Ochoa VJ, Ocampo SA, Muñoz JF. Screening for antimicrobial activity of ten medicinal plants used in Colombian folkloric medicine: A possible alternative in the treatment of non-nosocomial infections. BMC complementary and alternative medicine. 2006;6(1):1-6.
11. Jayaraman S, Manoharan MS, Illanchezian S. In-vitro antimicrobial and antitumor activities of Stevia rebaudiana (Asteraceae) leaf extracts. Tropical Journal of Pharmaceutical Research. 2008;7(4):1143-9.
12. Bhimba BV, Meenupriya J, Joel EL, Naveena DE, Kumar S, Thangaraj M. Antibacterial activity and characterization of secondary metabolites isolated from mangrove plant Avicennia officinalis. Asian Pacific Journal of Tropical Medicine. 2010;3(7):544-6.
13. Siddique AB, Rahman SMM, Hossain MA, Rashid MA. Phytochemical screening and comparative antimicrobial potential of different extracts of Stevia rebaudiana Bertoni leaves. Asian Pacific Journal of Tropical Disease. 2014;4(4):275-80.
14. Subramaniam R, Mittal S, Hiregoudar M, Ratan G, Sakeenabi B, Prashant G, et al. Antimicrobial activity of Stevioside on periodontal pathogens and Candida Albicans-An invitro study. Journal of Indian Association of Public Health Dentistry. 2011;9(5):325.
15. Muanda FN, Soulimani R, Diop B, Dicko A. Study on chemical composition and biological activities of essential oil and extracts from Stevia rebaudiana Bertoni leaves. LWT-Food Science and Technology. 2011;44(9):1865-72.
16. Vazquez JA, Sobel JD. Mucosal candidiasis. Infectious disease clinics of North America. 2002;16(4):793-820, v.
17. Sharon V, Fazel N. Oral candidiasis and angular cheilitis. Dermatologic therapy. 2010;23(3):230-42.
18. Pappas PG, Kauffman CA, Andes D, Benjamin Jr DK, Calandra TF, Edwards Jr JE, et al. Clinical practice guidelines for the management of candidiasis: 2009 update by the Infectious Diseases Society of America. Clinical infectious diseases. 2009:503-35.
19. Niimi M, Firth NA, Cannon RD. Antifungal drug resistance of oral fungi. Odontology. 2010;98(1):15-25.
20. Carakostas M, Curry L, Boileau A, Brusick D. Overview: the history, technical function and safety of rebaudioside A, a naturally occurring steviol glycoside, for use in food and beverages. Food and Chemical Toxicology. 2008;46(7):S1-S10.
21. Hossain MA, Siddique A, Rahman SM, Hossain M. Chemical composition of the essential oils of Stevia rebaudiana Bertoni leaves. Asian J Tradit Med. 2010;5(2):56-61.
22. Singh S, Rao G. Stevia: The herbal sugar of 21 st century. Sugar tech. 2005;7(1):17-24.
23. Huxley AJ, Griffiths M. Dictionary of gardening: Stockton Press; 1992.

24. Lemus-Mondaca R, Vega-Gálvez A, Zura-Bravo L, Ah-Hen K. Stevia rebaudiana Bertoni, source of a high-potency natural sweetener: A comprehensive review on the biochemical, nutritional and functional aspects. Food chemistry. 2012;132(3):1121-32.
25. Šic Žlabur J, Voća S, Dobričević N, Ježek D, Bosiljkov T, Brnčić M. Stevia rebaudiana Bertoni-A review of nutritional and biochemical properties of natural sweetener. Agriculturae Conspectus Scientificus. 2013;78(1):25-30.
26. Cardello H, Da Silva M, Damasio M. Measurement of the relative sweetness of stevia extract, aspartame and cyclamate/saccharin blend as compared to sucrose at different concentrations. Plant Foods for Human Nutrition. 1999;54(2):119-29.
27. Organization WH. Evaluation of certain food additives and contaminants: thirty-third report of the joint FAO/WHO Expert Committee on food additives. Evaluation of certain food additives and contaminants: thirty-third report of the joint FAO/WHO expert committee on food additives1989.
28. Tarka S, Roberts A. Stevia: It's Not Just About Calories. Open Obes J. 2010;2:101-9.
29. Chatsudthipong V, Muanprasat C. Stevioside and related compounds: therapeutic benefits beyond sweetness. Pharmacology & therapeutics. 2009;121(1):41-54.
30. Komissarenko N, Derkach A, Kovalyov I, Bublik N. Diterpene glycosides and phenylpropanoids of Stevia rebaudiana Bertoni. Rast Res. 1994;1(2):53-64.
31. Brandle J, Telmer P. Steviol glycoside biosynthesis. Phytochemistry. 2007;68(14):1855-63.
32. Goyal S, Samsher GR, Goyal R. Stevia (Stevia rebaudiana) a bio-sweetener: a review. Int J Food Sci Nutr. 2010;61(1):1-10.
33. Edeoga HO, Okwu D, Mbaebie B. Phytochemical constituents of some Nigerian medicinal plants. African journal of biotechnology. 2005;4(7):685-8.
34. Pól J, Hohnová B, Hyötyläinen T. Characterisation of Stevia rebaudiana by comprehensive two-dimensional liquid chromatography time-of-flight mass spectrometry. Journal of Chromatography A. 2007;1150(1-2):85-92.
35. Agati G, Azzarello E, Pollastri S, Tattini M. Flavonoids as antioxidants in plants: location and functional significance. Plant science. 2012;196:67-76.
36. Aboh M, Olayinka B, Adeshina G, Oladosu P. Antifungal activities of phyto compounds from Mitracarpus villosus (Sw.) DC from Abuja, Nigeria. J Microbiol Res. 2014;4(2):86-91.
37. Negri M, Salci TP, Shinobu-Mesquita CS, Capoci IR, Svidzinski TI, Kioshima ES. Early state research on antifungal natural products. Molecules. 2014;19(3):2925-56.
38. Pyla R, Kim T-J, Silva JL, Jung Y-S. Enhanced antimicrobial activity of starch-based film impregnated with thermally processed tannic acid, a strong antioxidant. International journal of food microbiology. 2010;137(2-3):154-60.
39. Srivastava B, Singh P, Shukla R, Dubey NK. A novel combination of the essential oils of Cinnamomum camphora and Alpinia galanga in checking aflatoxin B 1 production by a toxigenic strain of Aspergillus flavus. World Journal of Microbiology and Biotechnology. 2008;24(5):693-7.
40. Ahmad A, Khan A, Akhtar F, Yousuf S, Xess I, Khan L, et al. Fungicidal activity of thymol and carvacrol by disrupting ergosterol biosynthesis and membrane integrity against Candida. European journal of clinical microbiology & infectious diseases. 2011;30(1):41-50.
41. Braga P, Dal Sasso M, Culici M, Alfieri M. Eugenol and thymol, alone or in combination, induce morphological alterations in the envelope of Candida albicans. Fitoterapia. 2007;78(6):396-400.