ORIGINAL ARTICLE
Volume 15 Issue 1 2020
DOI: 10.21315/aos2020.15.1.427
ARTICLE INFO
Submitted: 20/02/2020
Accepted: 29/06/2020
Online: 15/07/2020

Antibacterial Property of *Synsepalum dulcificum* Leaves Aqueous Extract against Oral Pathogens and its Chemical Compounds

Hanim Afzan Ibrahim[a,b], Nur Karyatee Kassim[a,b], Noor Azlin Azraini Che Soh[b], Zulkhairi Othman[c], Tuan Nadrah Naim Tuan Ismail[a*]

[a]*School of Dental Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia*

[b]*Department of Chemical Pathology, School of Medical Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia*

[c]*School of Health Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia*

[*]Corresponding author: tnadrah@usm.my

To cite this article: Ibrahim HA, Kassim NK, Che Soh NAA, Othman Z, Tuan Ismail TNN (2020). Antibacterial property of *Synsepalum dulcificum* leaves aqueous extract against oral pathogens and its chemical compounds. *Arch Orofac Sci*, **15**(1): 55–64. https://doi.org/10.21315/aos2020.15.1.427

To link to this article: https://doi.org/10.21315/aos2020.15.1.427

ABSTRACT

Synsepalum dulcificum (*S. dulcificum*) commonly known as "miracle fruit" because its berries have the capability to modify sour taste to the sweet taste when eaten. Beside the berries, *S. dulcificum* leaves were also known to possess biological properties such as antioxidant, antimutagenic and antidiabetic activities. However, the study of its antimicrobial activity against oral pathogen is still lacking. Thus, this study aimed to evaluate the antibacterial activity of its leaves against cariogenic bacteria and to analyse its phytochemical compounds. The samples of *S. dulcificum* leaves were collected in Kelantan, the east coast region of Peninsular Malaysia and extracted with distilled water using a Soxhlet technique. The antibacterial activity of the *S. dulcificum* leaves aqueous extract against *Streptococcus mutans* (*S. mutans*), *Streptococcus sobrinus* (*S. sobrinus*) and *Lactobacillus salivarius* (*L. salivarius*) was evaluated using the broth microdilution assay. The identification of the phytochemical compounds was performed using gas chromatography-mass spectrometry (GC-MS). The antibacterial study showed the minimum inhibitory concentration of *S. dulcificum* leaves aqueous extract against *S. mutans* and *S. sobrinus* were 16 mg/mL and 8 mg/mL, respectively. Interestingly, there was no inhibitory effect of *S. dulcificum* leaves aqueous extract against *L. salivarius*. A total of 42 chemical compounds were identified and major identified bioactive compounds groups were heterocyclic and phenolic compounds. Our results suggested *S. dulcificum* leaves aqueous extract has antimicrobial properties against *S. mutans* and *S. sobrinus*, but no inhibitory activity against oral normal flora, with the presence of bioactive compounds has potential in oral care products application.

Keywords: *Synsepalum dulcificum leaves, antimicrobial activity, oral pathogens, gas chromatography mass spectrometry, broth microdilution assay*

INTRODUCTION

Synsepalum dulcificum (*S. dulcificum*) is a shrub native to tropical West Africa. It is called miracle fruit due to its berry's ability to cause sour foods to taste sweet. It was reported that the glycoprotein (miraculin) is the active compound that works by altering the sweet receptor (Koizumi *et al.*, 2011). There were few studies of chemical compounds of its berries that obtained from regions such as Malaysia (He *et al.*, 2016) and China (Du *et al.*, 2014). The results revealed that all samples were rich in phenolic contents and had antioxidant properties.

However, the benefit of *S. dulcificum* is not limited to its berries only. In Malaysia, its leaves were traditionally used to treat toothache by chewing the leaves. Previous studies of *S. dulcificum* leaves had revealed the presence of antioxidant, antimutagenic, antidiabetic and antimicrobial properties in this plant. Study of methanol extract of its leaves grown from Nigeria showed that the extract was rich in phenolic contents and had antioxidant activity (Obafemi *et al.*, 2017b) as well as has potential as antidiabetic agent (Obafemi *et al.*, 2017a). On the other hand, *S. dulcificum* leaves aqueous extract collected in Taiwan showed that the extract was also rich in phenolic contents and exhibited antimutagenic and anti-oxidative damage activities (Chen *et al.*, 2015). However, there were various types of phenolic compounds identified in both studies. Methanol extract of *S. dulcificum* leaves collected in Nigeria has that gallic acid, chlorogenic acid, caffeic acid, ellagic acid, catechin, epicatechin, quercetin, quercitrin, isoquercitrin, rutin and kaempferol as the identified phenolic compounds (Obafemi *et al.*, 2017a). While study of water extract of *S. dulcificum* leaves collected in Taiwan has p-hydroxybenzoic acid, vanillic acid, syringic acid, trans-p-coumaric acid and veratric acid as the identified phenolic compounds (Chen *et al.,* 2015). These results showed that the method of extraction and cultivation area could be the factors that contributed to the discrepancies.

A previous study of methanol and ethanol extracts of *S. dulcificum* leaves found that both extracts possessed antibacterial activity against facultative anaerobic bacterium *Listeria monocytogenes* (Wasoh *et al.*, 2017). The study of antibacterial activity of *S. dulcificum* leaves is still lacking. To date, no other antibacterial study of *S. dulcificum* leaves has been investigated except study by Wasoh *et al.* (2017). Thus, our focused in the current study was to evaluate the antibacterial activity of *S. dulcificum* leaves against oral pathogens which are *Streptococcus mutans* (*S. mutans*) and *Streptococcus sobrinus* (*S. sobrinus*). Its inhibitory effect against oral normal flora which is *Lactobacillus salivarius* (*L. salivarius*) was also evaluated. *S. mutans* is a facultative anaerobic bacterium that is associated with the development of dental caries. The acid producing *S. mutans* dissolved tooth structure in the presence of carbohydrate (Guo *et al.*, 2013). The second harmful oral pathogen that related to the development of tooth cavities is *S. sobrinus* (Damle, 2018). On the other hand, *L. salivarius* is a probiotic that has beneficial effects in the oral cavity by inhibiting cariogenic streptococci and candida (Meurman, 2005). Thus, the best antibacterial agent should have antibacterial activity against oral pathogens but not against oral probiotic.

An antimicrobial agent such as chlorhexidine has been reported to prevent dental carries effectively. However, a recent study showed that long term use of chlorhexidine in oral care product is potentially causing multidrug-resistant (Saleem *et al.*, 2016). The use of the natural product can contribute as the alternative to the synthetic chemical substance for caries prevention. Hence, in the present study, the antibacterial property of *S. dulcificum* leaves aqueous extract was evaluated. Water extract is preferred since alcohol is well-known to cause dryness to the oral mucosa. The use

of water is needed, especially for oral care products (McCullough and Farah, 2008). Its phytochemical compounds were also analysed since the region of plantation might contribute to the phytochemical discrepancy. The previous studies of methanol extract of *S. dulcificum* leaves collected from two countries showed that the chemical compounds were different (Chen *et al.*, 2010; Obafemi *et al.*, 2017b). In the present study, the chemical compounds of *S. dulcificum* leaves was analysed by gas chromatography-mass spectrometry (GC-MS) because this technique is highly sensitive, fast and no standards are needed since the identification of the chemical compounds was carried out by comparing the mass spectra with the libraries (Hübschmann, 2015).

MATERIALS AND METHODS

Sample of *S. dulcificum*

S. dulcificum was collected from Kota Bharu, Kelantan. The plant was confirmed by botanist, Dr. Mohd Firdaus Ismail from Institute of Bioscience, University Putra Malaysia and the voucher specimen was deposited at the Herbarium of Institute of Bioscience, University Putra Malaysia. The voucher specimen number was SK 3263/17.

Preparation of *S. dulcificum* Leaves Aqueous Extract

S. dulcificum leaves aqueous extract was prepared using Soxhlet extraction method. The *S. dulcificum* leaves were harvested and cleaned first with tap water and rinsed twice in sterile distilled water (Seong *et al.*, 2018). The leaves were then dried at 37°C in the oven and ground into powder. The *S. dulcificum* leaves were extracted with distilled water using a Soxhlet apparatus, and the resulting water extract was freeze-dried to obtain crude water extract (Ranasinghe *et al.*, 2012). The dried extract was kept at −20°C.

Antimicrobial Activity

In the current study, *S. mutans* (ATCC 25175) and *S. sobrinus* (ATCC33478) were obtained from the American Type Culture Collection, USA and purchased from Oxoid Ltd., UK. *L. salivarius* (clinical isolate) was obtained from the Craniofacial Laboratory, School of Dental Sciences, Universiti Sains Malaysia, Kelantan, Malaysia. The Mueller Hinton Blood Agar (MHBA) and Brain Heart Infusion (BHI) medium were purchased from Oxoid Ltd., UK.

Preparation of the Bacterial Suspension

S. mutans, S. sobrinus and *L. salivarius* were cultured on MHBA and were incubated at 37°C for 48 h under an anaerobic condition. After incubation, the colonies were suspended in 1 mL of peptone water and were standardised to 0.5 McFarland (1×10^8 CFU/mL) by using a nephelometer (Balto *et al.*, 2017).

Determination of Minimum Inhibitory Concentrations (MIC)

The MIC of *S. dulcificum* leaves extract against tested oral bacteria was determined by broth microdilution method with some modifications (Balto *et al.*, 2017). A crude extract of dry *S. dulcificum* leaves was dissolved in distilled water at a concentration of 32 mg/mL. Two-fold serial dilutions with BHI medium in a sterile 96-well microtiter plates were performed. Final concentrations of *S. dulcificum* leaves extract ranged from 0.25 mg/mL to 32 mg/mL. Each plate had a set of controls: a column with the chlorhexidine (0.12%) as the positive control, a column with all solutions with the exception of the *S. dulcificum* leaves extract as a negative control and a column with all solutions with the exception of the bacterial suspension as a sterility control. Each well, except for the sterility control, was inoculated with 20 µl of bacterial suspension (1×10^6 CFU/mL). The plates containing tested oral

bacteria were incubated at 37°C for 48 h under anaerobic condition. After incubation, each well was added with 10 µl of resazurin (Sigma Aldrich, US) (0.01%) indicator solution and was incubated for 2 h at 37°C under anaerobic condition. After two hours, the lowest concentration at which colour change to pink occurred was taken as the MIC value. This experiment was done in triplicate independently. Resazurin is a blue non-fluorescent and non-toxic dye that becomes pink and fluorescent when reduced to resorufin by oxidoreductases within viable cells (Adan *et al.*, 2016).

GC-MS Conditions and Parameters

The chemical compounds of *S. dulcificum* leaves aqueous extract was analysed by GC-MS system operating on electron impact ionisation (EI) mode fixed at 70eV on Hewlett Packard 6890 Gas Chromatograph coupled to 5973N Mass Selective Detector (Agilent Technologies, USA) equipped with fused silica capillary column, HP-5 30 m length × 0.25 mm internal diameter × 0.25 µm film thickness. Helium was used as carrier gas with flow rate 1.0 mL/min. The initial temperature of the column was programmed from 50°C (held for 2 min) and then was heated to 280°C (held for 10 min) with a 20°C/min rate. The injection and interface temperatures were set at 250°C and 280°C, respectively. The sample (1 µl) injected in splitless mode and analysed in MS full scan mode; range *m/z* 40-650. Acquisition of data was performed using Chemsation software. The sample was derivatised by addition of MSTFA (N-methyl-N-trimethylsilyl-trifluoroacetamide) prior to the analysis to make the compounds more volatile and thus amenable for GC analysis.

Identification of Chemical Compounds

The *S. dulcificum* compounds were identified by comparing their mass spectra with National Institute of Standards and Technology (NIST02) and Wiley275 libraries (≥ 80% matching). The percentage compound was calculated from the summation of the peak areas of *S. dulcificum* compounds.

RESULTS

The antimicrobial activity of *S. dulcificum* leaves aqueous extract is shown in Table 1. *S. dulcificum* leaves aqueous extract showed inhibitory activity against *S. mutans* and *S. sobrinus* with MIC value of 16 mg/mL and 8 mg/mL; respectively. Interestingly, there was no inhibitory activity of aqueous extract of *S. dulcificum* leaves against *L. salivarius* at a concentration at least 32 mg/mL. The lowest concentration at which colour change to pink occurred was taken as the MIC value.

Table 1: Antibacterial activity of *Synsepalum dulcificum* leaves aqueous extract against oral pathogens

Microorganism	Inhibitory effect				Positive control (0.12% CHX*)	Negative control (Brain Heart Infusion)
	32 mg/mL of *S. dulcificum* leaves aqueous extract	16 mg/mL of *S. dulcificum* leaves aqueous extract	8 mg/mL of *S. dulcificum* leaves aqueous extract	4 mg/mL of *S. dulcificum* leaves aqueous extract		
Streptococcus mutans	+	+	-	-	+	-
Streptococcus sobrinus	+	+	+	-	+	-
Lactobacillus salivarius	-	-	-	-	+	-

The result of GC-MS analysis of water extract of *S. dulcificum* leaves is shown in Table 2. A total of 42 chemical compounds were identified and the major identified compounds were N-furfuryl pyrrole (5.45%), 2-Furancarboxaldehyde, 5-(hydroxymethyl)- (5.28%), 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (4.31%) and 2,3-Dihydro-5-hydroxy-6-methyl-4H-pyran-4-one (4.22%).

The chemical compounds were categorised into few groups, which were heterocyclic compounds, phenolic compounds, terpenoids and steroid. The heterocyclic compounds were the main identified group and contributed to the 15.98%. The main identified heterocyclic compounds include N-furfuryl pyrrole (5.45%), 2-Furancarboxaldehyde and 5-(hydroxymethyl)- (5.28%). The phenolic compounds were the second main identified group and contribute to 8.15%. The main identified phenolic compounds were 4-vinyl-phenol (2.31%) and 2-Methoxy-4-vinylphenol (1.83%).

Table 2: Chemical compounds of *Synsepalum dulcificum* leaves aqueous extract

No	Retention time (min)	% of total	Compounds name	% Library matching
			Heterocyclic compounds	
1.	4.700	0.75%	Furfural	96
2.	5.450	1.14%	2-Furanmethanol	99
3.	5.891	0.13%	5-Methylene-2(5H)14-furanone	91
4.	6.521	0.83%	2(3H)-Furanone, dihydro-	91
5.	6.409	0.11%	Ethanone,1-(2-furanyl)-	87
6.	6.850	0.12%	2(3H)-Furanone, 5-methyl-	83
7.	6.899	0.16%	2,5-Furandione, 3-methyl-	90
8.	6.948	0.23%	2,5-Dimethyl-3(2H)Furanone	80
9.	7.158	1.27%	5-methyl furfural	96
10.	7.326	0.51%	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	91
11.	9.147	5.45%	N-furfuryl pyrrole	90
12.	9.693	5.28%	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	83
			Phenolic	
13.	7.396	0.53%	Phenol	94
14.	9.399	2.31%	4-vinyl-phenol	87
15.	10.008	1.83%	2-Methoxy-4-vinylphenol	98
16.	10.225	1.57%	Phenol,2,6-dimethoxy-	97
17.	10.764	0.89%	Phenol,2-methoxy-4-(1-propenyl)-	95
18.	11.975	1.02%	Phenol,2,6-dimethoxy-4-(2-propenyl)p	91
			Terpenoids	
19.	16.086	0.07%	Squalene	91
20.	20.441	0.27%	3-beta-o-cinnamoyl-lupeol	86
21.	21.022	0.09%	Norolean-12-ene	91
22.	21.575	0.35%	Lup-20(29)-en-3-ol, acetate, (3.beta.)	94

(*Continued on next page*)

Table 2 (*Continued*)

No	Retention time (min)	% of total	Compounds name	% Library matching
			Steroids	
23.	19.482	0.12%	Chondrillasterol	95
			Others	
24.	2.817	3.70%	Acetic acid	90
25.	5.695	0.23%	Acetol acetate	90
26.	5.982	0.56%	1,3-Cyclopentenedione	95
27.	6.311	0.61%	1-Hydroxy-3-penten-2-one	86
28.	6.675	0.81%	2-Hydroxy-2cyclopenten-1-one	91
29.	7.487	0.48%	Pyrazine	86
30.	7.718	0.27%	Cycloheptatriene	90
31.	7.788	0.98%	Pyrimidine	86
32.	7.886	0.53%	2-Cyclopenten-1-one, 2-hydroxy-3-methyl-	94
33.	8.418	4.22%	2,3-Dihydro-5-hydroxy-6-methyl-4H-pyran-4-one	90
34.	8.524	4.31%	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	80
35.	8.573	0.94%	Cyclopentene	81
36.	11.450	2.16%	Megastigmatrienone	96
37.	11.821	1.97%	Dihydro-coniferyl alcohol	90
38.	12.165	0.51%	Myristic acid	96
39.	15.581	0.05%	9,17-Octadecadienal	95
40.	15.911	0.02%	9-Octadecenamide	87
41.	17.808	0.08%	Vitamin E	99

The other groups that were identified in the present study were terpenoids and steroid. Terpenoids and steroid were identified in a small percentage which contributes to the 0.78% and 0.12%, respectively. The main identified terpenoids were lu-20(29)-en-3-ol,acetate, 3(beta) (0.35%), 3.beta.-O-cinnamoyl-lupeol (0.27%).

DISCUSSION

Our study showed that water extract of *S. dulcificum* leaves had antibacterial activity against *S. mutans* and *S. sobrinus*. However, no inhibitory activity of water extract of *S. dulcificum* leaves against *L. salivarius*. The antibacterial activity of *S. dulcificum* leaves against oral pathogens was studied here for the first time. A previous study found that both ethanol and methanol extracts of *S. dulcificum* leaves had antibacterial activities against *Listeria monocytogenes* (Wasoh *et al.*, 2017). In the present study, water extract was used as an alternative to solvent extract as it is a universal solvent used for natural product extraction and has been widely used among traditional healers to extract various medicinal plants (Balakrishnan *et al.*, 2014). Furthermore, water-based is the best formulation for oral mucosa compared to alcohol based because of its potential increase in cancer risk (Lachenmeier, 2012). Water is also cost-effective and more favourable when compared to other solvents since it is non-toxic and can be easily absorbed. Likewise, some bio-active compounds such as phenolic compounds are water soluble (Saltveit, 2017).

In the present study, the chemical compounds were categorised into few groups and heterocyclic compounds were the main identified group. This was the first time the heterocyclic compounds were found as major compounds. Method of chemical compounds analysis could be the factor that contributed to this discrepancy. Since heterocyclic compounds were volatile compounds, they were amenable to the GC-MS analysis. The main identified compounds include N-furfuryl pyrrole (5.45%), 2-Furancarboxaldehyde, 5-(hydroxymethyl)- (5.28%), 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (4.31%) and 2,3-Dihydro-5-hydroxy-6-methyl-4H-pyran-4-one (4.22%) were found for the first time from this plant. A previous study showed 2-furancarboxaldehyde, 5-(hydroxymethyl) has antimicrobial activity against both gram positive and negative bacterial as well as fungi (Mohammed, 2013).

The phenolic compounds were the second main identified group and contributed to 8.15%. Similar finding was observed in *S. dulcificum* leaves that obtained from Nigeria (Obafemi *et al.*, 2017b). However, study by Obafemi *et al.* (2017b) found that none of the identified phenolic compounds were the same. This study used methanol as a solvent and the extract was analysed by high-performance liquid chromatography. The method of extraction (Azmir *et al.*, 2013) and analysis technique (Koparde *et al.*, 2017) could be the factors that influenced the chemical compounds in the *S. dulcificum* leaves. In the current study, the main identified phenolic compounds were 4-vinyl-phenol (2.31%) and 2-methoxy-4-vinylphenol (1.83%). Phenolic compounds were documented to possess antibacterial effect by disrupting the bacterial lipid-protein interface due to its ability to acts as nonionic surface-active agent (Greenberg *et al.*, 2008).

The other groups identified in the present study were terpenoids and steroid. Terpenoids and steroid were identified in a small percentage which contributed to the 0.78% and 0.12%, respectively. The main identified terpenoids were lu-20(29)-en-3-ol,acetate, 3(beta) (0.35%), 3.beta.-O-cinnamoyl-lupeol (0.27%). The previous study of GC-MS analysis of methanol extract of *S. dulcificum* leaves obtained in Taiwan showed that none of the terpenoids were similar (Ragasa *et al.*, 2015). Terpenoids are well-known to possess antibacterial activity (Ludwiczuk *et al.*, 2017). The antibacterial mode of action of terpenoids could be due to their ability to inhibit oxygen uptake and oxidative phosphorylation which are crucial processes for microbial survival (Griffin *et al.*, 1999).

Although steroid was identified in small percentage, this compound was not identified in methanol extract of *S. dulcificum* leaves obtained in Nigeria (Obafemi *et al.*, 2017b). On the other hand, methanol extract of *S. dulcificum* leaves collected in Taiwan showed the presence of steroids such as β-sitosterol and stigmasterol (Chen *et al.*, 2010). This finding showed that the cultivation area might influence the chemical compounds of the *S. dulcificum*. The only steroid identified in the present study was chondrillasterol (0.12%). A recent study of chondrillasterol showed that this compound has the antibacterial property against *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* (Mozirandi *et al.*, 2019).

CONCLUSION

The aqueous extract of *S. dulcificum* leaves against *S. mutans* and *S. sobrinus*, the cariogenic bacteria revealed the promising result, suggesting its potential application in the oral care product. Further cytotoxicity test of its aqueous extract on gingival human cell and preclinical testing is needed to promote application of crude extract of *S. dulcificum* in the oral health care industry.

ACKNOWLEDGEMENTS

The authors wish to thank Mr. Mohamad Ezany Yusoff from Craniofacial Science Laboratory, School of Dental Sciences, Universiti Sains Malaysia for his technical assistance in antimicrobial testing. The authors would like to thank Universiti Sains Malaysia for providing financial support under Short Term Grant, (304/PPSG/61313196).

REFERENCES

Adan A, Kiraz Y, Baran Y (2016). Cell proliferation and cytotoxicity assays. *Curr Pharm Biotechnol*, **17**(14): 1213–1221. https://doi.org/10.2174/1389201017666160808160513

Azmir J, Zaidul ISM, Rahman MM, Sharif KM, Mohamed A, Sahena F *et al.* (2013). Techniques for extraction of bioactive compounds from plant materials: A review. *J Food Eng*, **117**(4): 426–436. https://doi.org/10.1016/j.jfoodeng.2013.01.014

Balakrishnan B, Paramasivam S, Arulkumar A (2014). Evaluation of the lemongrass plant (*Cymbopogon citratus*) extracted in different solvents for antioxidant and antibacterial activity against human pathogens. *Asian Pac J Trop Dis*, **4**(Suppl 1): S134–S139. https://doi.org/10.1016/S2222-1808(14)60428-X

Balto H, Al-Sanie I, Al-Beshri S, Aldrees A (2017). Effectiveness of *Salvadora persica* extracts against common oral pathogens. *Saudi Dent J*, **29**(1): 1–6. https://doi.org/10.1016/j.sdentj.2016.11.001

Chen CY, Wang YD, Wang HM (2010). Chemical constituents from the leaves of *Synsepalum dulcificum*. *Chem Nat Compd*, **46**(3): 495. https://doi.org/10.1007/s10600-010-9658-6

Chen TY, Kang ZC, Yen MT, Huang MH, Wang BS (2015). Inhibitory effect of aqueous extracts from Miracle Fruit leaves on mutation and oxidative damage. *Food Chem*, **169**: 411–416. https://doi.org/10.1016/j.foodchem.2014.08.022

Damle SG (2018). Competence and transformation of oral *Streptococcus sobrinus* in dental caries. *Contemp Clin Dent*, **9**(Suppl 2): S195–S196. https://doi.org/10.4103/ccd.ccd_689_18

Du L, Shen Y, Zhang X, Prinyawiwatkul W, Xu Z (2014). Antioxidant-rich phytochemicals in miracle berry (*Synsepalum dulcificum*) and antioxidant activity of its extracts. *Food Chem*, **153**: 279–284. https://doi.org/10.1016/j.foodchem.2013.12.072

Greenberg M, Dodds M, Tian M (2008). Naturally occurring phenolic antibacterial compounds show effectiveness against oral bacteria by a quantitative structure–Activity relationship study. *J Agric Food Chem*, **56**(23): 11151–11156. https://doi.org/10.1021/jf8020859

Griffin SG, Wyllie SG, Markham JL, Leach DN (1999). The role of structure and molecular properties of terpenoids in determining their antimicrobial activity. *Flavour Frag J*, **14**(5): 322–332. https://doi.org/10.1002/(SICI)1099-1026(199909/10)14:5<322::AID-FFJ837>3.0.CO;2-4

Guo L, Hu W, He X, Lux R, McLean J, Shi W (2013). Investigating acid production by *Streptococcus mutans* with a surface-displayed pH-sensitive green fluorescent protein. *PLOS ONE*, **8**(2): e57182. https://doi.org/10.1371/journal.pone.0057182

He Z, Tan JS, Abbasiliasi S, Lai OM, Tam YJ, Ariff AB (2016). Phytochemicals, nutritionals and antioxidant properties of miracle fruit *Synsepalum dulcificum*. *Ind Crops Prod*, **86**: 87–94. https://doi.org/10.1016/j.indcrop.2016.03.032

Hübschmann HJ (2015). *Handbook of GC-MS: Fundamentals and Applications*, 3rd edn. New York: John Wiley & Sons.

Koizumi A, Tsuchiya A, Nakajima KI, Ito K, Terada T, Shimizu-Ibuka A *et al.* (2011). Human sweet taste receptor mediates acid-induced sweetness of miraculin. *Proc Natl Acad Sci U S A*, **108**(40): 16819–16824. https://doi.org/10.1073/pnas.1016644108

Koparde AA, Magdum CS, Doijad RC (2017). Phyto active compounds from herbal plants extracts: Its extraction, isolation and characterization. *World J Pharm Res*, **6**(8): 1186–1205.

Lachenmeier DW (2012). Alcohol-containing mouthwash and oral cancer–Can epidemiology prove the absence of risk? *Ann Agric Environ Med*, **19**(3): 609–613.

Ludwiczuk A, Skalicka-Woźniak K, Georgiev MI (2017). Terpenoids. In: Badal S, Delgoda R (eds.), *Pharmacognosy*: *Fundamentals, Applications and Strategies*. Amsterdam: Elsevier Inc., pp. 233–266. https://doi.org/10.1016/C2014-0-01794-7

McCullough MJ, Farah CS (2008). The role of alcohol in oral carcinogenesis with particular reference to alcohol-containing mouthwashes. *Aust Dent J*, **53**(4): 302–305. https://doi.org/10.1111/j.1834-7819.2008.00070.x

Meurman JH (2005). Probiotics: Do they have a role in oral medicine and dentistry? *Eur J Oral Sci*, **113**(3): 188–196. https://doi.org/10.1111/j.1600-0722.2005.00191.x

Mohammed KS (2013). Antibacterial activity of *Allium Sativum* (Garlic) and identification of active compound by GC-MS analysis. *Int J Pharm Bio Sci*, **4**(4): 1071–1076.

Mozirandi W, Tagwireyi D, Mukanganyama S (2019). Evaluation of antimicrobial activity of chondrillasterol isolated from Vernonia adoensis (Asteraceae). *BMC Complement Altern Med*, **19**(1): 249. https://doi.org/10.1186/s12906-019-2657-7

Obafemi TO, Akinmoladun AC, Olaleye MT, Agboade SO, Onasanya AA (2017a). Antidiabetic potential of methanolic and flavonoid-rich leaf extracts of *Synsepalum dulcificum* in type 2 diabetic rats. *J Ayurveda Integr Med*, **8**(4): 238–246. https://doi.org/10.1016/j.jaim.2017.01.008

Obafemi TO, Akinmoladun AC, Olaleye MT, Onasanya A, Komolafe KC, Falode JA *et al.* (2017b). High performance liquid chromatography (HPLC) fingerprinting, mineral composition and *in vitro* antioxidant activity of methanol leaf extract of *Synsepalum dulcificum* (Sapotaceae). *J Appl Pharm Sci*, **7**(11): 110–118. https://doi.org/10.7324/JAPS.2017.71117

Ragasa CY, Cornelio K, Bauca T, Chua S, Shen CC (2015). Chemical constituents of the leaves, stems, and fruits of *Synsepalum dulcificum*. *Chem Nat Compd*, **51**(3): 588–589. https://doi.org/10.1007/s10600-015-1356-y

Ranasinghe P, Perera S, Gunatilake M, Abeywardene E, Gunapala N, Premakumara S *et al.* (2012). Effects of *Cinnamomum zeylanicum* (Ceylon cinnamon) on blood glucose and lipids in a diabetic and healthy rat model. *Pharmacognosy Res*, **4**(2): 73–79. https://doi.org/10.4103/0974-8490.94719

Saleem HG, Seers CA, Sabri AN, Reynolds EC (2016). Dental plaque bacteria with reduced susceptibility to chlorhexidine are multidrug resistant. *BMC Microbiol*, **16**: 214. https://doi.org/10.1186/s12866-016-0833-1

Saltveit ME (2017). Synthesis and metabolism of phenolic compounds. In: Yahia EM (ed.), *Fruit and Vegetable Phytochemicals: Chemistry and Human Health*, 2nd edn. New York: John Wiley & Sons. https://doi.org/10.1002/9781119158042.ch5

Seong J, Oyong GG, Cabrera EC (2018). *Synsepalum dulcificum* extracts exhibit cytotoxic activity on human colorectal cancer cells and upregulate *c-fos* and *c-jun* early apoptotic gene expression. *Asian Pac J Trop Biomed*, **8**(3): 173–178. https://doi.org/10.4103/2221-1691.227999

Wasoh H, Tajuddin S, Halim M, Mohd-Hairul AR, Sobri MZM, Lajis AFB, Yusof MT *et al.* (2017). Antibacterial activity of *Synsepalum dulcificum* leaf extract against *Listeria monocytogenes* and its comparison with *Strobilanthes crispus* and *Morus alba*. *J Bio-Sci*, **25**: 73–75.