ORIGINAL ARTICLE

Potential of *Hibiscus sabdariffa* L. Calyx (Rosella) extract as antibacterial agent in dental disease: Phytochemical and chemical components profiling

Dewi Fatma Suniarti, Trijani Suwandi[1], Salsabila Aqila Putri[2], Dikdik Kurnia[2]

Department of Oral Biology, Faculty of Dentistry, Universitas Indonesia, [1]Department of Periodontics, Faculty of Dentistry, Universitas Trisakti, West Jakarta, [2]Department of Chemistry, Faculty of Mathematics and Natural Sciences Universitas Padjadjaran, West Java, Indonesia

J. Adv. Pharm. Technol. Res.

ABSTRACT

Chemical characteristics of natural products are influenced by different external factors, varying according to the geographic origin. The ethanol extract of *Hibiscus sabdariffa* L calyx Indonesia has been studied *in vivo* and *in vitro* provide potential effect for dental field uses. Ethanol extract showed antibacterial to *Streptococcus sanguinis* as an inducer gingivitis, had an effect on the treatment of oral mucosa ulceration, and could inhibit the development of alveolar bone destruction. This study aims to determine the chemical groups and components of ethanol extract of *H. sabdariffa* L. calyces (Indonesia origin). Chemical group of ethanol extract *H. sabdariffa* L calyx Indonesia was analysis through phytochemical screening, whereas chemical components were detected through gas chromatography–mass spectrometry analysis. Saponins, tannins, phenolic, flavonoids, triterpenoids and glycosides, and 17 chemical components were identified in the ethanol extract of *H. sabdariffa* L calyx Indonesia. Among the chemical components, fatty acids group showed the most dominant. For standardization and develop of oral drug preparation, a better chemical components and phytochemical profiling are essential because the extract quality of herbs has various quality.

Key words: Antibacterial, chemical components, *Hibiscus sabdariffa L,* phytochemicals

INTRODUCTION

Hibiscus sabdariffa L. is a medicinal plant which is grown in tropical and subtropical countries such as Saudi Arabia, India, Thailand, Malaysia, and Indonesia.[1,2] It has the phytochemical components of phenolics, alkaloids, terpenoids, and natural pigments.[3] The leaves, fruit, seeds, stem, and roots of *H. sabdariffa* L have some pharmacological effects.[4] Conventionally, *H. sabdariffa* L can be used as a therapy for hypertension, inflammation, and liver disorders.[5] In pharmacological, *H. sabdariffa* L has antibacterial activity,[6] antioxidant agent in seed, calyx, leaf, and steam,[7] and it also has anticancer, anti-obesity, and antidiabetic activities.[8] In Indonesia, there are four superior varieties of *H. sabdariffa* L herbal plants, namely Roselindo 1 (red roselle), Roselindo 2 (Jamaica/purple squid roselle), Roselindo 3 (green roselle), and Roselindo 4 (purple roselle).[9]

The ethanol extract of *H. sabdariffa* L calyx has potential utilization in dentistry and has been investigated *in vitro* and *in vivo* through its pharmacological effects. The extract

Address for correspondence:
Prof. Dikdik Kurnia,
Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, West Java, Indonesia.
E-mail: dikdik.kurnia@unpad.ac.id

Submitted: 10-Mar-2022 **Revised:** 30-Apr-2022
Accepted: 10-May-2022 **Published:** 05-Jul-2022

Access this article online	
Quick Response Code:	Website: www.japtr.org
	DOI: 10.4103/japtr.japtr_64_22

How to cite this article: Suniarti DF, Suwandi T, Putri SA, Kurnia D. Potential of *Hibiscus sabdariffa* L. Calyx (Rosella) extract as antibacterial agent in dental disease: Phytochemical and chemical components profiling. J Adv Pharm Technol Res 2022;13:202-6.

of *H. sabdariffa* L calyx has the activity in biofilm to reduce *S. sanguinis* with the viability after treatment of 10%–34%.[10] The *H. sabdariffa* L calyx also has antibacterial activity against oral bacteria, such as *Fusobacterium nucleatum, Prevotella intermedia,* and *Porphyromonas gingivalis,* with the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values are 7.2–28.8 and 14.4–57.6 mg/mL, respectively,[11] then against *S. mutans, S. sanguinis, Capnocytophaga gingivalis,* and *S. aureus* with the MIC and MBC values of 5–20 and 25–33 mg/mL, respectively.[12] Furthermore, *in vivo* studied reported that in the concentration of 10%, ethanol extract of *H. sabdariffa* L calyx can inhibit alveolar bone damage, which is an early symptom of periodontitis.[13]

In the development of medicinal plants to standardized herbal medicines, the identity of the extracts must be well known. The chemical structures will be the basic compounds in the development of industrial agents because the phytochemical properties isolated from the plants are considered safe and effective with rational usage.[14] This identity is required for further research, as well as oral preparation with dosage forms appropriate for oral use so that they can be used for clinical trials. This article will present the identification results in the form of phytochemicals and chemical components of the ethanol extract of *H. sabdariffa* L calix whose plants are grown in the Institute for Medicinal and Aromatic Plants (BALLITRO), Bogor, Indonesia.

MATERIALS AND METHODS

Hibiscus sabdariffa L calyx extraction and phytochemical analysis

H. sabdariffa L calyx (Rosella) samples were obtained from Balai Tanaman Obat dan Aromatik (Balittro) Bogor, West Java, Indonesia. Petals were extract by maceration with 70% ethanol in 1:3 ratio in 30 days and then filtered. The extract was evaporated using rotary evaporator (Rotavapor® R-200 Buchi, Switzerland) in low pressures and temperature <50°C. The ethanol extract of *H. sabdariffa* L calyx was diluted in 1% dimethyl sulfoxide. Furthermore, the results of the extraction were analyzed into the phytochemical screening for the main component of secondary metabolites.

Gas chromatography–mass spectrometry analysis

Gas chromatography–mass spectrometry (GC-MS) analysis of *H. sabdariffa* L calyx extract was carried out using 680 PerkinElmer Clarus (PerkinElmer, Inc. USA) with the fused silica column and the capillary column (30 m in length ×250 μm in diameter × 0.25 μm in thickness). The carrier gas used is pure helium (99.99%). The ionization energy method was used for the detection of GC-MS spectrum with the high ionization energy of 70eV, 0.2 s for time scan, and 40–600 m/z for the range of fragments. Rasio split of injection is 10:1 with the quantity of 1 μL at the constant temperature of 250°C.[15]

RESULTS

The results of phytochemical screening and the chemical component are shown in Tables 1 and 2, respectively. Phytochemical analysis was carried out on the ethanol extract of *H. sabdariffa* L calyx. Then, the ethanol extract was analyzed for its chemical components using the GC-MS method. The results showed that the ethanol extract of *H. sabdariffa* L calyx contained 17 types of chemical components originating from several different groups.

DISCUSSION

Extract ethanol of *H. sabdariffa* L calyx showed the presence of saponin, tannis, phenols, flavonoids, triterpenoids, and glycoside. In the previous study, some medicinal plants have been reported as a potential treatment for dental disease. *Sapindus rarak* seeds extract contained saponin which has antibiofilm activity.[16] *Areca catechu* also has antibacterial activity against dental bacteria such as *Lactobacillus casei*.[17] *Syzygium aromaticum* is wealthy of phenolic compounds which has antibiofilm activity with the best inhibitory effect on *Staphylococcus*.[18] While *Citrus limon* has a broad spectrum of biological activity such as antibacterial. It has been investigated to have antimicrobial activity against *E. coli, Staphylococcus epidermidis, Streptococcus agalactiae,* and *Candida albicans*.[19] *Centella asiatica* L also has antibiofilm activity with one of the primary constituents is triterpenoids.[20,21] Then, *Digitalis purpurea* contains glycosides which are potential to be antioxidant.[22] However, based on the data from Table 1, *H. sabdariffa* L contain the higher phytochemicals than those plants. The abundant phytochemicals are saponins and triterpenoids.

The phytochemicals which present in *H. sabdariffa* L have a lot of benefits in dentistry, some of which provide antibacterial activity where this activity is indispensable in the treatment of dental disease. It was reported that saponins can provide antibacterial activity by causing the penetration of proteins and enzymes in bacterial cell.[23] Tannins is a

Table 1: Phytochemical of Indonesia extract ethanol *Hibiscus sabdariffa* L. Calyx

Scientific	Semi quantitative	Comparison
Saponin	++++	*Sapindus rarak*
Tannins	+++	*Areca catechin*
Phenols	+++	Cloves (*Syzygium aromaticum*)
Flavonoids	+++	Lemon oranges zest (*Citrus limon*)
Triterpenoids	++++	Leaves of *Centella asiatica* L.
Glycoside	++	Leaves of *Digitalis purpurea*

+: Presence, Number of +: No. of replications

Table 2: Chemical component of Indonesia ethanol extract *Hibiscus sabdariffa* L. Calyx

Type and sample code	RT	Quality	Chemical component	Content (%)
Solution of ethanol extract of *Hibiscus sabdariffa* L. Calix	6.287	91	Methane, Sulfonylbis-	16.65
	6.473	38	Methyl-D3-hydrazine	2.67
	7.094	64	*S*-Methyl methanethiosulfonate	1.91
	7.576	22	(2*E*)-5-Methyl, [2,3-D2] hexa-2,4-dieonic acid	12.80
	8.135	64	Propanoic acid	12.96
	8.735	27	Propane, 2-chloro-	2.26
	9.293	50	2-Chlormethylcyclopro pancarbonic acid methyl ester	8.35
	10.197	38	1-Propanol, 2,3-dichloro-	1.24
	11.789	78	2-Furancarboxaldehyde, 5-(Hydroxymethyl)-	3.70
	12.541	59	Furancarboxaldehyde, 5-(Hydroxymethyl)-	3.33
	21.022	78	4-*tert*-Butyl-1,3-oxazolidine-2-thione	2.33
	27.373	45	Phenol, 2-amino	7.38
	27.718	43	Benzothiazole, 2-methyl-	1.74
	28.014	98	*n*-Hexadecanoic acid	5.31
	28.497	35	Gamma-hexadecalactone	4.46
	29.172	96	(9*E*)-9-Octadeconoic acid	9.66
	30.710	72	1,2-Benzenedicarbobylic acid, diisooctyl ester	1.28

RT: Retention time

group of polyphenols which can increase the platelets aggregation to form temporary platelets plugs in injured blood vessels by depositing blood proteins. Therefore, in dentistry, tannins act as agents of astringents and gingival regeneration.[24] The phenolic group has been investigated to have antibiofilm activity against *P. aeruginosa*.[25] Flavonoid can be an inhibitor of GTF enzyme produced by *S. mutans* and is known to act as virulence factor in the pathogenesis dental caries.[26] Besides, the hydroxyl group of flavonoids also can interact to bacterial wall proteins such as ATPase and cause inactivation of enzymes and transport proteins.[27] Pentacyclic triterpenoids were reported to inhibit the biofilm formation of *S. mutans* and *Actinomyces viscosus*.[28] The group of glycosides also has antimicrobial activity to some bacteria which contribute to dental caries, such as *S. mutans*, *S. mitis*, *S. salivarius*, *S. aureus*, and *Lactobacillus acidophilus*.[29]

Furthermore, the analysis of the active ingredients in the *H. sabdariffa* L calyx was used to indicate the existence of chemical compounds which could provide medicinal properties. The results of chemical component analysis by GC-MS are shown in Table 2. The ethanol extract of *H. sabdariffa* L calix was dominated by fatty acid of 40.73%, namely (2*E*)-5-methyl, [2,3-D2] hexa-2,4-dienoic acid, propanoic acid, *n*-hexadecanoic acid, and (9*E*)-9-octadecenoic acid, and followed by fatty acid methyl ester of 8.35% (2-chlormethylcyclopro pancarbonic acid methyl ester). Then, the extract contained the group of furans 7.03%, namely 2-furancarboxaldehyde, 5-(hydroxymethyl)-and furancarboxaldehyde, 5-(hydroxymethyl)-, organosulfur 16.65%, hydrazine 2.67%, alkyl halide 2.26%, alcohol 1.24%, aminophenol 7.38%, benzothiazoles of 1.74%, lactone 4.46%, and dicarboxylic acid ester 1.28%.

Based on a previous study by Shen *et al*.,[30] calyces *H. sabdariffa* L essential oil extract from China showed that the most of the active compounds were 50.564% fatty acids which were similar to this study. Fatty acids are a group of volatile constituents in secondary metabolites.[31] Fatty acids are known to have an inflammatory response which can be used for periodontal disease of the teeth.[32] It was reported that ibuprofen, which is propanoic acid, has been shown to be useful in a clinical model oral analgesics in dental pain.[33] While hexadecanoic acid was known to act as anionic surfactants in providing antibacterial activity.[34] Beside it, the other active ingredients also have some benefits in medicinal uses, such as the group of organosulfur which have the potential to be antioxidant, antibacterial, antiviral, and anti-cancer,[35] alkyl halides can be used for anesthesia and antibiotic (clindamycin),[36] benzothiazoles are known to provide antimicrobial, anti-inflammatory, and antioxidant activities,[37] furans have antibacterial, antifungal, antiviral, anti-inflammatory, cardiovascular activity, and analgesic,[38] lactones are widely used for cardiovascular disease, anti-cancer, and anti-inflammatory.[39] While further research on the use of fatty acids and other active ingredient groups from the extract of *H. sabdariffa* L calyx for dental disease is still relatively rare. Based on this research, it can be concluded that the chemical compounds of *H. sabdariffa* L have the potential to be developed in the field of dentistry.

CONCLUSION

Screening of phytochemicals and chemical compounds is important to get the best quality of the herbal extract. *H. sabdariffa* L calyx extract has phytochemical constituents and some active ingredients which are potential to be used in dentistry. The GC-MS analysis showed the extract of *H.*

sabdariffa L has 17 chemical compounds and is dominated by fatty acids with the presentage of 40.73%. This study showed that the chemical compounds of *H. sabdariffa* L calyx extract are potential to be anti-inflammatory and antibacterial agent in dental disease.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Juhari NH, Bredie WLP, Toldam-Andersen TB, Petersen MA. Characterization of Roselle calyx from different geographical origins. Food Res Int 2018;112:378-89.
2. Juhari NH, Petersen MA. Physicochemical properties and oxidative storage stability of milled roselle (*Hibiscus sabdariffa* L.) seeds. Molecules 2018;23:E385.
3. Jabeur I, Pereira E, Caleja C, Calhelha RC, Soković M, Catarino L, *et al*. Exploring the chemical and bioactive properties of *Hibiscus sabdariffa* L. calyces from Guinea-Bissau (West Africa). Food Funct 2019;10:2234-43.
4. Al-Snafi AE. Medicinal plants possessed beneficial therapeutic effects in Alzheimer's disease and memory deficits. GSC Biol Pharm Sci 2021;17:008-33.
5. Shadhan RM, Bohari SP. Effects of *Hibiscus sabdariffa* Linn. fruit extracts on α-glucosidase enzyme, glucose diffusion and wound healing activities. Asian Pac J Trop Biomed 2017;7:466-72.
6. Afolabi OC, Ogunsola FT, Coker AO. Susceptibility of cariogenic *Streptococcus mutans* to extracts of *Garcinia kola*, *Hibiscus sabdariffa*, and *Solanum americanum*. West Afr J Med 2008;27:230-3.
7. Mohd-Esa N, Hern FS, Ismail A, Yee CL. Antioxidant activity in different parts of roselle (*Hibiscus sabdariffa* L.) extracts and potential exploitation of the seeds. Food Chem 2010;122:1055-60.
8. Da-Costa-Rocha I, Bonnlaender B, Sievers H, Pischel I, Heinrich M. *Hibiscus sabdariffa* L. – A phytochemical and pharmacological review. Food Chem 2014;165:424-43.
9. Nurnasari E, Anggraeni TD, Nurindah N. Seed Oil Profile of Four Varieties of Herbal Roselle (Hisbiscus sabdariffa var. sabdariffa) Indonesia. Bul Tanaman Tembakau Serat Minyak Industri 2019;11:8-15.
10. Suwandi T, Suniarti DF, Prayitno SW. Effect of ethanol extract of *Hibiscus sabdariffa* L. calyx on *Streptococcus sanguinis* viability *in vitro* biofilm based on crystal violet. J Med Plants Res 2013;7: 2476-82.
11. Sulistyani H, Fujita M, Miyakawa H, Nakazawa F. Effect of roselle calyx extract on *in vitro* viability and biofilm formation ability of oral pathogenic bacteria. Asian Pac J Trop Med 2016;9:119-24.
12. Baena-Santillán ES, Piloni-Martini J, Santos-López EM, Gómez-Aldapa CA, Rangel-Vargas E, Castro-Rosas J. Comparison of the antimicrobial activity of *Hibiscus sabdariffa* calyx extracts, six commercial types of mouthwashes, and chlorhexidine on oral pathogenic bacteria, and the effect of *Hibiscus sabdariffa* extracts and chlorhexidine on permeability of the bacterial membrane. J Med Food 2021;24:67-76.
13. Idrus E, Tsary DA, Setiadi DS, Calfina NE, Halim VA, Suniarti DF, *et al*. Inhibition of alveolar bone destruction by roselle extract (*Hibiscus sabdariffa* L.). J Int Dent Med Res 2020;13:830-5.
14. Milutinovici RA, Chioran D, Buzatu R, Macasoi I, Razvan S, Chioibas R, *et al*. Vegetal compounds as sources of prophylactic and therapeutic agents in dentistry. Plants (Basel) 2021;10:2148.
15. Konappa N, Udayashankar AC, Krishnamurthy S, Pradeep CK, Chowdappa S, Jogaiah S. GC-MS analysis of phytoconstituents from *Amomum nilgiricum* and molecular docking interactions of bioactive serverogenin acetate with target proteins. Sci Rep 2020;10:16438.
16. Pratiwi SU, Hamzah H. Inhibition and degradation activity of (*Sapindus rarak* seeds) ethanol extract against polymicrobial biofilm. Res J Pharm Technol 2020;13:5425-30.
17. Djohari M, Fernando A, Safitri A. Aktivitas Daya Hambat Ekstrak Etanol Biji Pinang (*Areca catechu* L.) terhadap Isolat Bakteri Gigi. J Ilmu Kefarmasian Indonesia 2020;18:81-7.
18. Abdul Majeed S. Investigation of anti-biofilm effect of *Syzygium aromaticum* plant extracts against oral pathogens. Int J Sci Res 2018;7:201-206.
19. Hindi N, Chabuck Z. Antimicrobial activity of different aqueous lemon extracts. J Appl Pharm Sci 2013;3:74-8.
20. Gohil KJ, Patel JA, Gajjar AK. Pharmacological review on *Centella asiatica*: A potential herbal cure-all. Indian J Pharm Sci 2010;72:546-56.
21. Rafey A, Amin A, Kamran M, Haroon U, Farooq K, Foubert K, *et al*. Analysis of plant origin antibiotics against oral bacterial infections using *in vitro* and *in silico* techniques and characterization of active constituents. Antibiotics 2021;10:1504.
22. Al-Snafi AE. Phytochemical constituents and medicinal properties of *Digitalis lanata* and *Digitalis purpurea* – A review. Indo Am J Pharm Sci 2017;4:225-34.
23. Purba H, Simanjuntak HA, Situmorang R. Phytochemical screening of bunga rosella (*Hibiscus sabdariffa* L) and antimicrobial activity test. J Pendidikan Kimia 2020;12:70-8.
24. Fauzi A, Lamma S, Ruslin M. Total tannin levels analysis of brown algae (*Sargassum* sp. and *Padina* sp.) to prevent blood loss in surgery. J Dentomaxillofac Sci 2018;3:37-40.
25. Jagani S, Chelikani R, Kim DS. Effects of phenol and natural phenolic compounds on biofilm formation by *Pseudomonas aeruginosa*. Biofouling 2009;25:321-4.
26. Achmad MH, Ramadhany S, Suryajaya FE. *Streptococcus* colonial growth of dental plaque inhibition using flavonoid extract of ants nest (*Myrmecodia pendans*): An *in vitro* study. Pesqui Bras Odontopediatria Clín Integr 2019;19:1-9.
27. Erlianda D, Rizal MF, Budiardjo SB. Antibacterial effect of flavonoids from propolis produced by trigona on atpase activity of *Streptococcus mutans*. Int J Appl Pharm 2017;9:6-9.
28. Raja AF, Ali F, Khan IA, Shawl AS, Arora DS. Acetyl-11-keto-β-boswellic acid (AKBA); targeting oral cavity pathogens. BMC Res Notes 2011;4: 1-8.
29. Jyothi KS, Seshagiri M. *In-vitro* activity of saponins of *Bauhinia purpurea*, *Madhuca longifolia*, *Celastrus paniculatus* and *Semecarpus anacardium* on selected oral pathogens. J Dent (Tehran) 2012;9:216-23.
30. Shen CY, Zhang TT, Zhang WL, Jiang JG. Anti-inflammatory activities of essential oil isolated from the calyx of *Hibiscus sabdariffa* L. Food Funct 2016;7:4451-9.
31. Radulovic N, Dekic M, Stojanovic-Radic Z, Palic R. Chemical composition and antimicrobial activity of the essential oils of *Geranium columbinum* L. and *G. lucidum* L.(*Geraniaceae*). Turk J Chem 2011;35:499-512.
32. Iwasaki M, Manz MC, Moynihan P, Yoshihara A, Muramatsu K, Watanabe R, *et al*. Relationship between saturated fatty acids and periodontal disease. J Dent Res 2011;90:861-7.
33. Cajaraville JP. Ibuprofen arginate for rapid-onset pain relief in daily practice: A review of its use in different pain conditions. J Pain Res 2021;14:117-26.
34. Fadzir UA, Mokhtar KI, Mustafa BE, Darnis DS. Evaluation of bioactive compounds on different extracts of *Linum usitatissimum* and its antimicrobial properties against selected oral pathogens.

Makara J Health Res 2018;22:121-127.

35. Lee DY, Li H, Lim HJ, Lee HJ, Jeon R, Ryu JH. Anti-inflammatory activity of sulfur-containing compounds from garlic. J Med Food 2012;15:992-9.
36. Gál B, Bucher C, Burns NZ. Chiral alkyl halides: Underexplored motifs in medicine. Mar Drugs 2016;14:E206.
37. Sharma PC, Sinhmar A, Sharma A, Rajak H, Pathak DP. Medicinal significance of benzothiazole scaffold: An insight view. J Enzyme Inhib Med Chem 2013;28:240-66.
38. Alizadeh M, Jalal M, Hamed K, Saber A, Kheirouri S, Pourteymour Fard Tabrizi F, *et al*. Recent updates on anti-inflammatory and antimicrobial effects of furan natural derivatives. J Inflamm Res 2020;13:451-63.
39. Chadwick M, Trewin H, Gawthrop F, Wagstaff C. Sesquiterpenoids lactones: Benefits to plants and people. Int J Mol Sci 2013;14:12780-805.