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

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**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. It has the phytochemical components of phenolics, alkaloids, terpenoids, and natural pigments. The leaves, fruit, seeds, stem, and roots of *H. sabdariffa* L have some pharmacological effects. Conventionally, *H. sabdariffa* L can be used as a therapy for hypertension, inflammation, and liver disorders. In pharmacological, *H. sabdariffa* L has antibacterial activity, antioxidant agent in seed, calyx, leaf, and steam, and it also has anticancer, anti-obesity, and anti-diabetic activities. 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).

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...
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 studies 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 calyx 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 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, tannins, 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

| Scientific | Semi quantitative | Comparison |
|------------|------------------|------------|
| Saponin    | +++++            | Sapindus rarak |
| Tannins    | +++              | Areca catechu |
| 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
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 antibiotic 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 calyx was dominated by fatty acid of 40.73%, namely (2E)-5-methyl, [2,3-D2]-hexa-2,4-dienoic acid, propanoic acid, n-hexadecanoic acid, and (9E)-9-octadeconoic acid, and followed by fatty acid methyl ester of 8.35% (2-chloromethylcyclopropane carboxylic 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.
Hibiscus 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.

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Conflicts of interest
There are no conflicts of interest.

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