A Mini Review on the Antibacterial Activity of Roselle (Hibiscus sabdariffa L.) Phytochemicals

A Prasetyoputri*, S I Rahmawati, A Atikana, F N Izzati, Y Hapsari, E Septiana, Bustanussalam and M Y Putra

Research Centre for Biotechnology, Indonesian Institute of Sciences (LIPI). Jl. Raya Bogor KM 46, Cibinong, Indonesia 16911

E-mail: anggia.prasetyoputri@uq.net.au

Abstract. Roselle (Hibiscus sabdariffa L.) has been shown to have various bioactivities with therapeutic benefits. These bioactivities are owing to the different kinds of phytochemicals, which include anthocyanins, phenolic acids, and flavonoids. This mini review aims to summarize the reported antibacterial activity of roselle phytochemicals in the literature in the past decade (2011-2021). The results revealed that roselle extracts from various extraction methods were shown to have antibacterial activity against clinical isolates and food pathogens, including multidrug resistant bacteria. Furthermore, there is evidence that roselle extract showed potential synergy with antibiotics. Overall, phytochemicals in roselle have the potential as an antibacterial for different beneficial applications.

Keywords: Hibiscus sabdariffa L.; phytochemicals; antibacterial activity; bioactive compounds; synergy

1. Introduction
In light of antibiotic resistance and limited new antibiotics entering the clinic, various efforts are undertaken to find sources of new antimicrobials. As many plants are used as traditional medicine, plant-derived compounds are therefore a promising source of new antimicrobial molecules [1]. Several main phytochemicals that contribute to the antimicrobial activities of plant extracts include flavonoids, alkaloids, terpenes, polyphenols, and coumarins [1,2]. These phytochemicals exert varying mechanisms of action against bacteria, which include membrane disruption, complex formation with bacterial cell wall, or substrate deprivation [2].

One of medicinal plants that have been investigated for their antibacterial activity is Hibiscus sabdariffa L. (Hs). Distributed worldwide, Hs is also called roselle or karkade. Hs belongs to the Malvaceae family native to tropical Africa, though Hs also cultivated in several places such as in America and South-east Asia, including Indonesia. It is an annual herbaceous plant living in subtropical and tropical, dry, mountain climates. The calyces are harvested after around 3-4 months when the plants are mature [3]. The bright red colour of the fleshy calyces (Figure 1) is due to an abundance of anthocyanins [4]. The high concentrations of dietary fibre and polyphenol [5] and other nutrients such as high levels of vitamin C [3] led to Hs calyces widespread use as herbal medicine.
Furthermore, the popular consumption of Hs is owing to its nice taste, colourful appearance, as well as its multiple medicinal benefits and use for culinary purposes.

![Figure 1. Hs dried calyx](image)

Studies have shown that a wide range of therapeutic effects are exhibited by Hs, including antioxidant, antipyretic, anti-inflammatory, antibacterial, anticancer, immunomodulatory, antihypertensive, and hepatoprotective effects [4,6-9]. Furthermore, in insulin-resistant rats, both antihyperglycemia and antihyperlipidemia activities have been demonstrated, as well as evidence of reduced cardiac hypertrophy in hypertensive rats and patients [6]. Several the bioactivities as mentioned above are due to its rich source of high antioxidant polyphenols in its calyces [10]. Moreover, the dietary fibre from polysaccharide content in Hs was found to be useful in obesity management as the fibre can reduce body weight, adiposity, plasma total cholesterol and glucose [8].

2. Phytochemicals found in Hs

Hs is an attractive commodity for food and traditional medicine, therefore, phytochemicals that are present in Hs have been extensively studied [5,7,11]. The major constituents of Hs phytochemicals include anthocyanins, flavonoids, phenolics and organic acids [9,12]. These compounds are responsible for the many biological activities of Hs extracts.

Among many biological activities, Hs is known for its rich antioxidant properties, potentially owing to the diverse polyphenols that it produces, such as anthocyanins. High concentrations of anthocyanins can be found in Hs calyces [7]. These compounds are responsible for the calyces’ red colour, which varies with the changing of its pH [7,13]. Among different anthocyanins, delphinidin-3-sambubioside and cyanidin-3-sambubioside are the major anthocyanins present in extracts of Hs calyces, with cyanidin-3-glucoside and delphinidin-3-glucoside present in lesser amounts [7,13,14] (Figure 2). These two major anthocyanins are suggested to be the compounds contributing to the antihypertensive and anticholesterol activities of Hs extract [15]. Additionally, delphinidin-3-sambubioside has also demonstrated an apoptosis-inducing potential in cancer cells [12].

Polyphenols of the flavonol and flavanol type in simple or polymerised form have also been reported in Hs extracts, as well as a number of flavonoids [7]. Antioxidant, antihypertensive, antimicrobial, anti-inflammatory, anticancer and antidiabetic potentials have been demonstrated by the polyphenols and flavonoids from Hs [16]. Sabdaritrin, hibiscitrin (hibiscetin-3-glucoside), gossypitrin, gossypirin, quercetin, luteolin, chlorogenic acid, protocatechuic acid, pelargonidic acid, eugenol, and phytosterol (β-sitosterol and ergosterol) are some of the flavonoids that can be found in Hs extracts [7,11]. Moreover, chlorogenic acids, caffeic acids, quercetin compounds have also been found in Hs extract, with activities ranging from antioxidant, anti-inflammatory, antiparasitic and many more [9]. A number of bioactivities have been demonstrated by polyphenol compounds, for example, protocatechuic acid was shown to have anticancer activity due to its apoptosis-inducing potential [12].
Additionally, chemopreventive activity was associated with the flavonoids from aqueous Hs extract administration in Wistar rats [17].

In addition to phenolic acids, flavonoids, and anthocyanins, Hs extracts also contain a high percentage of organic acids, including hydroxycitric acid, hibiscus acid, malic, tartaric, and citric acids [9]. The composition of acid from different cultivars of Hs may vary as they are influenced by genetics, environment, ecology and harvest conditions [8]. A number of biological activities are attributed to these organic acids. For example, hibiscus acid was shown to have antihypertensive activity as it demonstrated to be a potent vasorelaxant in a rat aorta [18]. Moreover, hibiscus acid was also found to have antibacterial activity against multidrug resistant bacteria [19].

It is evident from the literature that Hs extracts and phytochemicals have a lot of potential with regards to their antioxidant, anticancer, and antihypertensive properties, as well as many other important biological activities [5,7,9,11,12]. However, their antibacterial activity has been investigated to a lesser extent. As plant-derived compounds are potential as sources for new antibacterial lead compounds [20], a summary of the antibacterial activities of Hs extracts and phytochemicals would be beneficial. Therefore, this mini review will attempt to summarise various studies on antibacterial activities of extracts derived from Hs in between 2011 and 2021. In addition to elaborating on the different pathogens that can be inhibited by bioactive compounds from Hs, this paper will also review the potential synergism of Hs extracts with antibiotics.

3. Antibacterial activity of Hs extract and phytochemicals

Antibacterial activity of Hs extracts has been reported against many bacterial strains, including multi-drug resistant strains (Table 1). A number of studies have also investigated the specific active compounds that may be responsible for the antibacterial activity of Hs extracts. Hs is known to primarily contain organic acids, flavonoids and phenolic acids, anthocyanins, and polysaccharides, as well as various volatile compounds [7].

Flavonoids, anthocyanins, terpenoids, and coumarins have been known to be active against various Gram-positive and Gram-negative bacteria [21], suggesting the presence of these compounds is the reason for antibacterial activity of Hs extracts. Indeed anthocyanins from various plants have been shown to exert antibacterial activity through disruption of bacterial cell wall and have activities against Escherichia coli and Salmonella [22]. Similarly, flavonoid compounds have been shown to have activity against pathogenic bacteria through various mechanisms, which include nucleic acid synthesis inhibition, biofilm inhibition, DNA gyrase inhibition, and inhibition of membrane function [23]. Interestingly, a study characterising 25 varieties of Mexican Hs found that a high total content of phenolic compounds that include flavonoids and anthocyanins does not always translate to good antibacterial activity [24]. This study suggested that certain phenolic composition within the plant extract and potential interaction with other minor compounds could contribute to the antibacterial activity of Hs extracts [24].

Phenolic rich extracts from Hs was found to contain various compounds, including anthocyanin, flavonoids, alcoholic compounds, triazine derivatives, and esters, which were suggested to contribute to their potent antibacterial activity [25]. Hs extracts also contain various organic acids, such as hibiscus acid [9] (Figure 3). Hibiscus acid was found to inhibit bacterial growth of E. coli, Salmonella typhimurium, Pseudomonas aeruginosa, Staphylococcus aureus, and Vibrio cholerae [19]. This mechanism was suggested to be a disruption in bacterial cell membrane permeability [19]. Additionally, the contribution of fatty acids from Hs calyces to its antibacterial activity has also been reported [26].
Figure 2. Chemical structure of anthocyanins found in Hs. Cyanidin-3-sambubioside (R1=OH; R2=H, R3=Sambubioside), delphnidin-3-sambubioside (R1=OH; R2=OH, R3=Sambubioside), cyanidin-3-glucoside (R1=OH; R2=H, R3=Glucose), and delphnidin-3-glucoside (R1=OH; R2=OH, R3=Glucose). Figure redrawn from [7].

Figure 3. Chemical structure of hibiscus acid.
| No. | Material preparations | Pathogen                                    | Method of testing | Antibacterial activity                                                                 | References |
|-----|----------------------|---------------------------------------------|-------------------|---------------------------------------------------------------------------------------|------------|
| 1.  | Aqueous calyx extract| *Salmonella typhi*                          | Well diffusion method | Concentrations of 150, 250, 500 mg/mL produced inhibition zones of 18, 25, and 27 mm, respectively | [27]       |
| 2.  | Methanol calyx extract| *E. coli O157:H7* isolates from food, veterinary, and clinical samples | Disc diffusion method | Extract concentrations of 2.5%, 5%, and 10% were found to inhibit *Escherichia coli O157:H7*, with the 10% concentration being the most effective. Overall mean zones of inhibition were 8.9, 10.75, and 12.66 mm for 2.5%, 5%, and 10% concentrations, respectively | [28]       |
| 3.  | Ethanol extract of 25 Mexican Hs varieties | *E. coli* (ATCC 25922), *Salmonella enteritidis* (ATCC 14028), *S. aureus* (ATCC 25923), and *Micrococcus luteus* (ATCC 9341) | Agar well diffusion method | All extracts exhibited antibacterial activity against the Gram-positive and Gram-negative strains tested. Greater antibacterial activity was observed against *S. aureus* and *M. luteus* compared to *E. coli* and *S. enteritidis*. Zone of inhibition range for *S. aureus*: 16-22 mm; *M. luteus*: 10-18 mm; *E. coli* and *S. enteritidis*: 10-16 mm. | [24]       |
| 4.  | Methanol extract of Hs twigs | A total of 27 strains of *E. coli*, *Enterobacter aerogenes*, *E. cloacae*, *Klebsiella pneumoniae*, *Providencia stuartii*, *P. aeruginosa* | A rapid p-lodonitrotetrazolium chloride (INT) colorimetric assay | Hs extract was found to inhibit 18/27 (66.6%) bacteria tested (six *E. coli* strains, six *E. aerogenes* strains, one *E. cloacae* strain, four *K. pneumoniae* strains, and one *P. stuartii* strain, but no *P. aeruginosa* strain) | [29]       |
| 5.  | Crude, phenolic rich ethanol extract | *S. aureus* DSM 1104, *Streptococcus pyogenes* ATCC 19615, *L. monocytogenes* LMG10470, *E. coli* LMG 8223, *K. pneumoniae* ATCC 43816 and *P. aeruginosa* LMG 8029 | Agar well diffusion assay, MIC assay, as well as SEM and TEM microscopy | Using 2000 µg/mL extract, the inhibition zones against *S. aureus*, *Streptococcus pyogenes*, *Listeria monocytogenes*, *E. coli*, *K. pneumoniae*, *P. aeruginosa* ranged between 32 – 48 mm. MICs of phenolic rich extract against the above bacteria were the highest at 250 µg/mL concentration with inhibition zones ranging between 22- 30 mm. SEM and TEM images displayed malformations in bacterial cells in the presence of crude phenolic rich extract. | [25]       |
| 6.  | Acetonic extract, hibiscus | *E. coli*, *Salmonella Typhimurium* | Gel diffusion with paper disc, MIC and | Testing with multidrug resistant *E. coli* and *Salmonella Typhimurium*, zones of inhibition of acetonic extract ranged between | [19]       |
| 6th International Conference on Biotechnology Engineering (ICBioE 2021) | IOP Conf. Series: Materials Science and Engineering | 1192 (2021) 012017 | IOP Publishing | doi:10.1088/1757-899X/1192/1/012017 |

| acid, and chromatographic fractions | MBC | 9.8 – 12.6 mm, while hibiscus acid was 10.5-16.0 mm. With the same pathogens as above, MICs of acetonic extract and hibiscus acid was 7 mg/mL and 4-7 mg/mL, respectively. Additionally, the MBCs of acetonic extract and hibiscus acid was 7-10 mg/mL and 5-7 mg/mL, respectively. |

| 7. Aqueous methanol extract of leaves and stem | S. aureus, P. aeruginosa | Disc diffusion assay | Concentrations of 20, 40, 80, 160 mg/mL extract were tested. Inhibition zones of aqueous methanolic leaves extracts against S. aureus ranged between 5-11 mm and 6-12 mm for P. aeruginosa. Aqueous methanolic stem extracts of Hs were only active against S. aureus at >80 mg/mL and against P. aeruginosa at 160 mg/mL. |

| 8. Ethanolic extract | E. coli ATCC 25922, L. monocytogenes ATCC 51774, S. aureus ATCC 6538, E. aerogenes ATCC 13048 and S. Typhimurium ATCC 14028 | Disc diffusion assay | Inhibition zone diameters for E. coli: 23.43 mm, S. aureus: 24.15 mm, S. Typhimurium: 23.85 mm, E. aerogenes: 8.49 mm, L. monocytogenes: 26.37 mm. |

| 9. Aqueous extract | S. aureus, P. aeruginosa, E. coli, K. pneumoniae | Disc diffusion assay | Dose-dependent inhibition was displayed. Extract concentrations of 25, 50, 100, and 200 mg/mL resulted in the following zones of inhibition, respectively: S. aureus (8.0, 12.0, 13.0, 13.5 mm), K. pneumoniae (10.5, 12.5, 14.0, 14.0 mm), P. aeruginosa (9.0, 11.0, 12.5, 17.0 mm), and E. coli (8.5, 9.0, 12.0, 13.5 mm). |

| 10. Infusions of dry calyces in hot water (infused for 7 mins at 75°C) and cold water (25°C, let to stand for 2h) | S. Typhimurium, P. aeruginosa, E. coli and S. aureus | Broth microdilution on 96-well plates to determine MIC and MBC | Cold tea was more effective than hot tea at inhibiting P. aeruginosa (MIC 62.5 mg/ml), S. Typhimurium, E. coli and S. aureus (MIC 125 mg/ml). With regards to MBC, cold hibiscus tea was also more effective than hot tea against S. Typhimurium and P. aeruginosa (MBC 125.0 mg/ml for both strains). |
Bacterial pathogens, especially with multidrug resistance, are of interest in developing new antibiotics or in the discovery of antimicrobials. Plant extracts, including those derived from Hs, have the potential as antibacterial as shown by the different bioactive compounds that can exert antibacterial activity. The different pathogens that have been tested for the inhibition potential of Hs extracts are discussed below.

3.1. Human pathogens
Many studies have investigated the antibacterial potential of Hs extracts in vitro against bacterial pathogens of clinical importance. Aqueous methanolic extract has been tested against various pathogens with varying results. For example, aqueous methanolic extracts of Hs leaves were found to be more active against S. aureus and P. aeruginosa compared to extracts obtained from the stem [30]. Antibacterial activity of the leaves extract was also found to be concentration dependent, where the highest activity was found at the highest concentration tested of 160 mg/mL with an inhibition zone of 12.33 mm [30]. Methanolic extract of Hs was also tested against E. coli O157:H7 from different sources, including clinical isolates, with extract concentrations of 2.5%, 5%, and 10%. Here, the result also showed a concentration-dependent inhibition with 10% extract displaying the largest overall zone of inhibition of 12.66 mm [28]. Another study found that methanolic extract of Hs twigs were active against a number of E. coli strains, as well as against E. cloacae, E. aerogenes, and K. pneumoniae but not against the P. aeruginosa [29].

Ethanolic extracts of Hs are similarly showing in vitro antibacterial activity against a range of pathogens. Ethanolic extracts rich in phenolics were determined to not only have antibacterial activity in vitro, but also antifungal activity [25]. At 2000 µg/mL in an agar diffusion assay, crude phenolic rich extract was able to inhibit the growth of Gram-positive (S. aureus, S. pyogenes, L. monocytogenes) and Gram-negative bacteria (E. coli, K. pneumonia, P. aeruginosa) with inhibition zones ranging between 32 – 48 mm [25]. Interestingly, microscopic analysis revealed that in the presence of 50 µg/mL Hs extracts, cell deformation and other malformations were observed [25], suggesting that antibacterial activity of Hs extract affected the bacterial cell wall and/or membrane.

Ethanolic extracts of Hs was also found to inhibit Bacillus subtilis in vitro to a similar extent as the water extracts [34]. Other bacterial pathogens that could be inhibited by Hs extracts in vitro included oral bacteria Streptococcus mutans, Streptococcus sanguinis, and Capnocytophaga gingivalis [35], as well as Fusobacterium nucleatum, Prevotella intermedia, and Porphyromonas gingivalis [36], clinical isolates of Acinetobacter baumannii [37] and Mycobacterium tuberculosis [38]. Moreover, studies have shown that Hs extracts can inhibit biofilm formation [36,39]. A Drosophila infection model was also recently used to assess Hs extract activity against S. aureus, providing evidence that Hs extract antibacterial activity can be assessed in vivo [40]. It is therefore evident that Hs has a broad spectrum of antibacterial activity against pathogenic bacteria.

3.2. Foodborne pathogens
In addition to elucidating the antibacterial properties of Hs extracts against human pathogens, studies have also been conducted on the potential of Hs extracts against foodborne pathogens. This is very useful as Hs calyces are also consumed and addition of Hs to food may provide additional benefit as food additive or preservative and prevent contamination of foodborne bacteria.

Against bacteria obtained from food or other foodborne pathogens, Hs extract has shown antibacterial activity to a varying degree. In addition to E. coli O157:H7 from food samples [28], Hs extracts also inhibited a number of pathogens isolated from dairy products in a lawn diffusion assay [41]. Inhibition of food pathogens E. coli, S. aureus, L. monocytogenes, E. aerogenes and S. Typhimurium were also observed in vitro by ethanolic extracts of Hs [31]. With the exception of E. aerogenes, in that study, Hs extracts displayed the best antibacterial activity against all tested bacteria compared to other spice plants tested, providing further evidence of its potential as a food preservative.

Hs extracts have also been shown to inhibit a number of enteric pathogens, including clinical isolates [42]. Using broth microdilution, crude Hs extract was able to inhibit growth of six ATCC
strains (MC 50-200 mg/ml) and 16 clinical isolates (MIC 25-200 mg/ml), with the lowest MIC was exhibited against clinical isolates of *Edwardsiella tarda* and *Yersinia pseudotuberculosis* [42].

In another study, ethanolic extract of Hs calyces was tested as preservative and was found to increase the duration of shelf-life of beef [43]. Here, the Hs phenolic extract was also determined to exert antibacterial activity against *E. coli*, *S. enterica* var *Typhimurium*, *S. aureus*, *L. monocytogenes*, and *B. cereus*. Moreover, the phenolic extract was separated into aqueous and organic phases, and a fraction from the organic phase that was rich in phenolic acids was found to have the lowest MIC and MBC against the tested bacteria compared to aqueous phase and other fractions containing anthocyanin [43].

The spectrum of Hs extract antibacterial activity was further demonstrated against seven food pathogens, namely *B. cereus* 10451, *S. aureus* 10786, *E. coli* GIM1.708, *S. enteritidis* 10982, *Vibrio parahaemolyticus* 17802, and *P. aeruginosa* (B) 10104 with both aqueous and ethanolic extracts displaying bacterial growth inhibition at 20% w/v [44]. MIC determination resulted in similar MICs between the aqueous and ethanolic extracts, with MICs of 0.625%-10% w/v for aqueous extracts and 2.5%-5% w/v for ethanolic extracts. Interestingly, measurement of cytoplasmic internal pH suggested that the plant extract affected the bacterial cell membrane [44], similar to what has been reported in other studies [25,35]. The broad-spectrum nature of Hs extracts against foodborne pathogens warrants further investigation of its use as food additive and preservatives.

4. Synergistic activity of Hs phytochemicals with antibiotics in vitro

In addition to finding sources of new antibacterial agents, efforts to alleviate antibiotic resistance issue include discovering molecules or agents that have synergistic activities to improve the activity of currently-available antibiotics, as an alternative strategy to tackle antibiotic resistance. Plant extracts are a potential source of compounds that may have synergistic activity with antibiotics against various pathogenic bacteria [1,45]. A number of studies have therefore attempted to elaborate on the potential synergistic activity of plant extracts against bacterial pathogens, including multi-drug resistant bacteria [30,46-50].

Synergy of Hs extract in inhibiting bacterial growth in vitro was demonstrated against *Helicobacter pylori* clinical isolates by agar dilution method using aqueous Hs extracts with clarithromycin and metronidazole [49]. Another study determined that Hs extract at concentrations of 3.13, 1.56, and 0.78 mg/mL displayed synergistic activity with ciprofloxacin at concentrations 1.25 and 0.625 µg/mL against *S. aureus*, but no synergistic activity was observed with higher concentrations of both ciprofloxacin and Hs extracts [51]. Against *M. tuberculosis*, Hs extracts were found to be a good combination to rifampicin in inhibiting growth of isoniazid/ethambutol resistant *M. tuberculosis*, but displayed antagonistic effects when combined with streptomycin, ethambutol and isoniazid [38].

The potential synergy of Hs extract with gentamicin has also been tested for wound healing [52]. In this study, methanol extract of Hs calyx was formulated as a cream in combination with gentamicin and tested on wounds on rats. Application of the cream containing a combination of 1% w/w extract and 1% w/w gentamicin showed reduced time required for wounds closure by five days compared to 1% w/w gentamicin alone [52]. Additionally, the cream formulation showed no significant changes in pH [52], demonstrating the potential of Hs extract as a formulation with antibiotics for topical applications.

Considering the different methods were used in determining synergy, caution must be taken to interpret synergy of Hs extracts with antibiotics. However, current results show potential of Hs extracts to improve the activity of antibiotics.

5. Conclusions

Hs extracts contain various phytochemicals that can inhibit growth of various Gram-positive and Gram-negative bacteria in vitro, including multidrug resistant strains. The suggested mode of action of antibacterial activity that has been reported seems to be the disruption of bacterial cell wall and/or membrane. The major components of bioactive compounds in Hs are primarily known and some of
those have been shown to contribute to its antibacterial activity. Due to its large numbers of bioactive compounds, further investigation into the Hs extracts as a source of antibacterial lead compounds need to be performed, especially as the majority of studies were performed in vitro. Identification of specific compounds that are responsible for the antibacterial activity in vitro can assist in potentially selecting candidates for further studies in vivo. Additionally, the potential of such candidate compounds can be investigated further by synthesizing analogues to elucidate structure-activity relationship correlating to the antibacterial activity. This in turn may provide potential lead compounds that can be developed as new antibiotics. Alternatively, since synergy with antibiotics has also been demonstrated in a number of studies, Hs extracts or its bioactive compounds may also be explored further to improve antibiotic efficacy and alleviate antibiotic resistance. Information of synergy opens up the opportunity of Hs-derived compounds as antibiotic potentiators, which could help revive antibiotics that are losing their efficacy and therefore helping to invigorate the antibiotic pipeline.

6. References

[1] Savoia D 2012 *Future Microbiol* 7 979-90  
[2] Cowan MM 1999 *Clin Microbiol Rev* 12 564  
[3] Ismail A, Ikram EHK and Nazri HSM 2008 *Food* 2 1-16  
[4] Sindi HA, Marshall LJ and Morgan MR 2014 *Food Chem* 164 23-9  
[5] Guardiola S and Mach N 2014 *Endocrinología y Nutrición (English Edition)* 61 274-95  
[6] Si L-Y-N, Ramalingam A, Ali SS, Aminuddin A, Ng P-Y, Latip J, Kamisah Y, Budin SB and Zainalabidin S 2019 *EXCLI Journal* 18 876-92  
[7] Da-Costa-Rocha I, Bonnlaender B, Sievers H, Pischel I and Heinrich M 2014 *Food Chem* 165 424-43  
[8] Moyano G, Sáyago-Ayerdi SG, Largo C, Caz V, Santamaria M and Tabernero M 2016 *J Funct Foods* 21 1-9  
[9] Izquierdo-Vega JA, Arteaga-Badillo DA, Sánchez-Gutiérrez M, Morales-González JA, Vargas-Mendoza N, Gómez-Aldapa CA, Castro-Rosas J, Delgado-Olivares L, Madrigal-Bujaidar E and Madrigal-Santillán E 2020 *Biomedicines* 8 100  
[10] Si LY-N, Ali SAM, Latip J, Fauzi NM, Budin SB and Zainalabidin S 2017 *Life Sci* 191 157-65  
[11] Bedi PS, Bekele M and Gure G 2020 *Int Res J Pure Appl Chem* 21 41-54  
[12] Laskar YB and Mazumder PB 2020 *Biomed Pharmacother* 127 110153  
[13] Grajeda-Iglesias C, Figueroa-Espinoza MC, Barouh N, Baréa B, Fernandes A, de Freitas V and Salas E 2016 *J Nat Prod* 79 1709-18  
[14] Herranz-López M, Fernández-Arroyo S, Pérez-Sanchez A, Barrajón-Catalán E, Beltrán-Debón R, Menéndez JA, Alonso-Villaverde C, Segura-Carretero A, Joven J and Micol V 2012 *Phytotherapy* 19 253-61  
[15] Hopkins AL, Lammm MG, Funk JL and Ritenbaugh C 2013 *Fitoterapia* 85 84-94  
[16] Riaz G and Chopra R 2018 *Biomed Pharmacother* 102 575-86  
[17] Gheller AC, Kerkhoff J, Vieira Júnior GM, de Campos KE and Sugui MM 2017 *Sci World J* 2017 9392532  
[18] Zheoat AM, Gray AI, Igoli JO, Ferro VA and Drummond RM 2019 *Fitoterapia* 134 5-13  
[19] Portillo-Torres LA, Bernardino-Nicanor A, Gómez-Aldapa CA, González-Montiel S, Rangel-Vargas E, Villagómez-Ibarra JR, González-Cruz L, Cortés-López H and Castro-Rosas J 2019 *Antibiotics* 8  
[20] Shin J, Prabhakaran V-S and Kim K-s 2018 *Microb Pathog* 116 209-14  
[21] Barbieri R, Coppo E, Marchese A, Daglia M, Sobarzo-Sánchez E, Nabavi SF and Nabavi SM 2017 *Microbiol Res* 196 44-68  
[22] Ma Y, Ding S, Fei Y, Liu G, Jang H and Fang J 2019 *Food Control* 106 106712  
[23] Adamczak A, Ożarowski M and Karpiński T 2020 *J Clin Med* 9
[24] Borrás-Linares I, Fernández-Arroyo S, Arráez-Roman D, Palmeros-Suárez PA, Del Val-Díaz R, Andrade-Gonzáles I, Fernández-Gutiérrez A, Gómez-Leyva JF and Segura-Carretero A 2015 *Ind Crops Prod* **69** 385-94

[25] Abdel-Shafi S, Al-Mohammadi A-R, Sitohy M, Mosa B, Ismaiel A, Enan G and Osman A 2019 *Molecules* **24** 4280

[26] Sultan FI, Khorsheed AC and Mahmod A-RK 2014 *IJRRAS* **19** 140-49

[27] Mohammad GJ 2020 *Int J Drug Deliv Technol* **10** 217-21

[28] Fullerton M, Khatiwada J, Johnson JU, Davis S and Williams LL 2011 *J Med Food* **14** 950-56

[29] Djeussi DE, Noumedem JAK, Seukep JA, Fankam AG, Voukeng IK, Tankeo SB, Nkuete AHL and Kuete V 2013 *BMC Complement Altern M* **13** 164

[30] Kumar S and Anna Sheba L 2019 *Asian J Pharm Clin Res* **12** 198-201

[31] Akarca G, Tomar O, Güney İ, Erdur S and Gök V 2019 *J Food Sci Technol* **56** 5253-61

[32] Sulaiman FA, Kazeem MO, Waheed AM, Temowo SO, Azeez IO, Enan G and Osman A 2020 *Molecules* **24** 4280

[33] Sultan FI, Khorsheed AC and Mahmod A-RK 2014 *IJRRAS* **19** 140-49

[34] Mohammad GJ 2020 *Int J Drug Deliv Technol* **10** 217-21

[35] Fullerton M, Khatiwada J, Johnson JU, Davis S and Williams LL 2011 *J Med Food* **14** 950-56

[36] Djeussi DE, Noumedem JAK, Seukep JA, Fankam AG, Voukeng IK, Tankeo SB, Nkuete AHL and Kuete V 2013 *BMC Complement Altern M* **13** 164

[37] Kumar S and Anna Sheba L 2019 *Asian J Pharm Clin Res* **12** 198-201

[38] Akarca G, Tomar O, Güney İ, Erdur S and Gök V 2019 *J Food Sci Technol* **56** 5253-61

[39] Sulaiman FA, Kazeem MO, Waheed AM, Temowo SO, Azeez IO, Enan G and Osman A 2020 *Molecules* **24** 4280

[40] Sulistyani H, Fujita M, Miyakawa H and Nakazawa F 2016 *Asian Pac J Trop Med* **9** 119-24

[41] Abdallah EM 2016 *J Acute Dis* **5** 512-16

[42] Fauziyah PN, Sukandar EY and Ayuningtyas DK 2017 *Sci Pharm* **85**

[43] Sartini S, Djide MN and Nainu F In *Correlation Phenolic Concentration to Antioxidant and Antibacterial Activities of Several Ethanolic extracts from Indonesia*, Journal of Physics: Conference Series, 2019;10; IOP Publishing: 2019; p 072009.

[44] Farooqui A, Khan A, Borghetto I, Kazmi SU, Rubino S and Paglietti B 2015 *PloS one* **10** e0118431-e31

[45] Maia NL, de Barros M, de Oliveira LL, Cardoso SA, dos Santos MH, Pieri FA, Ramalho TC, da Cunha EFF and Moreira MAS 2018 *Front Microbiol* **9**

[46] Gonelimali FD, Lin J, Miao W, Xuan J, Charles F, Chen M and Hatab SR 2018 *Front Microbiol* **9**

[47] Stefanovič OD, Synergistic Activity of Antibiotics and Bioactive Plant Extracts: A Study Against Gram-Positive and Gram-Negative Bacteria. In *Bacterial Pathogenesis and Antibacterial Control*, Kırmusaoğlu, S., Ed. IntechOpen: United Kingdom, 2018, DOI: 10.5772/intechopen.72026.

[48] Faroqui A, Khan A, Borghetto I, Kazmi SU, Rubino S and Paglietti B 2015 *PloS one* **10** e0118431-e31

[49] Maia NL, de Barros M, de Oliveira LL, Cardoso SA, dos Santos MH, Pieri FA, Ramalho TC, da Cunha EFF and Moreira MAS 2018 *Front Microbiol* **9**

[50] Haroun MF and Al-Kayali RS 2016 *Iran J Basic Med Sci* **19** 1193-200

[51] Ezenwanyi NE 2016 *Br J Appl Sci Technol* **15** 1-8

[52] Builders PF, Kabele-Toge B, Builders M, Chindo BA, Anwunobi PA and Isimi YC 2013 *Indian J Pharm Sci* **75** 45-52