Malays. Appl. Biol. (2018) 47(2): 53–56

PHYTOCHEMICAL AND ANTIMICROBIAL ACTIVITIES OF DIFFERENT SOLVENT EXTRACTS OF *Hibiscus sabdariffa*

NUR ROYHAILA MOHAMAD[1], SITI SALWA ABD GANI[1,2*], ROSWANIRA ABDUL WAHAB[3] and USWATUN HASANAH ZAIDAN[4]

[1]Halal Product Research Institute (HPRI), Universiti Putra Malaysia
UPM, Putra Infoport, 43400 Serdang, Selangor, Malaysia
[2]Department of Agriculture Technology, Faculty of Agriculture, Universiti Putra Malaysia
UPM, 43400 Serdang, Selangor, Malaysia
[3]Department of Chemistry, Faculty of Science, Universiti Teknologi Malaysia,
81310 UTM Johor Bahru, Malaysia
[4]Department of Biochemistry, Faculty of Biotechnology & Biomolecular Sciences,
Universiti Putra Malaysia, UPM, 43400 Serdang, Selangor, Malaysia
[*]E-mail: ssalwaag@upm.edu.my or ssalwa.abdgani@gmail.com

Accepted 13 April 2018, Published online 25 May 2018

ABSTRACT

The use of plant extracts and phytochemicals with known antimicrobial properties is becoming commonplace worldwide and gaining great significance for therapeutic uses. Thus, the purpose of the present study is to investigate the effects of numerous extraction solvents (hexane, ethyl acetate, ethanol) on the phytochemical constituents and antimicrobial activities of *Hibiscus sabdariffa*. Phytochemical screening for saponins, tannins, flavonoids, phenolic and alkaloid compounds revealed that the ethanol and ethyl acetate extracts of *Hibiscus sabdariffa* contained all the aforementioned phytochemical constituents, for the exception of tannins and alkaloids that were absent in the ethyl acetate extract. Interestingly, the hexane extract did not afford such constituents. The ethanol extract exhibited stronger inhibitory activity against *Bacillus subtilis* (B29), *Staphylococcus aureus* (S276), *Staphylococcus epidermis* (S273), *Pseudomonas aeruginosa* (ATCC 15442) and *Escherichia coli* (E266) than ethyl acetate extract, but the difference was less evident for the hexane extract of *Hibiscus sabdariffa* which inhibited only *B. subtilis*, as determined by the paper disc method. Base on the findings, it can be construed that the ethanol and ethyl acetate extracts of HS have prospective applications as antimicrobial agent as well as one of the sources of therapeutically useful products.

Key words: *Hibiscus sabdariffa*, phytochemical, antimicrobial activity

INTRODUCTION

Medicinal plants are age long remedies for human diseases because they contain beneficial phyto-chemical components that are of exceptional therapeutic value (Adegoke *et al.,* 2009). Such plants have found significant application in modern medicine as valuable raw material and rich sources of ecologically developed secondary metabolites for syntheses of important drugs (Audu *et al.,* 2007) as remedies for different ailments. Revival of the scientific community interest in bioactive plant extracts showed antimicrobial activity is largely contributed by rising instances of microbial resistance towards current antibiotics and drugs (Nagendra *et al.,* 2010). Extensive and perhaps, irresponsible prescriptions of antibiotics by medical practitioners inadvertently lead to selected groups of clinical isolates showing multidrug resistance. To make matters worse, development of synthetic antibiotics is rather slow and only a few new types of antibiotics are launched each year (Prabhakaran *et al.,* 2016). In view of these circumstances, concerted efforts into the identification of novel plant extracts that exhibit exceptional antimicrobial properties are therefore, necessary as well as timely.

Herein, the study was focused on the plant *Hibiscus sabdariffa*, notorious in English as roselle or red sorrel, and in Arabic as karkadeh, a tropical plant native to India and Malaysia, but extensively

* To whom correspondence should be addressed.

grown elsewhere in Central and West Africa and Southeast Asia. It is an annual, erect, bushy, herbaceous sub-shrub that grows up to 8 ft in height and, the flower is usually characterized by a red calyx with five large sepals (Rodriguez-Medina, 2009). *Hibiscus sabdariffa* has been globally used for various medicinal applications, for instance, to treat hypertension, pyrexia, liver damage and leukemia in China (Tseng *et al.,* 2000). Muhammad and Shakib (1995) reported that the consumption of *Hibiscus sabdariffa* can prevent cancer, lowering blood pressure and improve the digestive system in humans. Likewise, the calyces extract of *Hibiscus sabdariffa* has been found effective for treating patients with kidney stones due to the inherent uricosuric effect of compounds present in this plant (Prasongwatana *et al.,* 2008). In addition to having the abovementioned activities, extracts of HS have been associated with good antioxidant property. Such property protects the human body against the damaging effects of low density lipoprotein (LDL)-oxidation, presumably contributed by the inherent *in vivo* hypolipidemic effects of these extracts. Extracts of HS are also used as food preservatives (Hirunpanich *et al.,* 2006).

In this study, we report the extraction of *Hibiscus sabdariffa* calyces using three different solvents, *viz*. hexane (non-polar), acetyl acetate (moderately polar) and ethanol (polar) on screening its phytochemical constituents and each extract was then assessed using *in-vitro* antagonistic activity against five well-known strains of pathogenic bacteria, namely *B. subtilis*, *S. aureus*, *S. epidermis*, *P. aeruginosa* and *E. coli.*

MATERIALS AND METHODS

Preparation of plant extracts

An amount of 1000 g of calyces of *Hibiscus sabdariffa* was air-dried for 1 week at room temperature and then ground using an electric grinder to a mesh size of 1 mm. A 10 g sample of HS powder was transferred into a beaker containing 100 mL of solvent (hexane, ethyl acetate, ethanol) and soaked for 24 h, filtered and the supernatant was vacuum evaporated. Each extract of HS was lyophilized and stored at 4°C until further analysis.

Phytochemical screening

Alkaloid, saponins, tannins, phenolic and flavonoid of the extract of *Hibiscus sabdariffa* was screened according to the method described by Sofowora (1993).

Antimicrobial activities

Paper discs measuring 6 mm in diameter previously soaked in an antibiotic solution was placed onto plates containing growing cultures of bacteria *viz*. *S. aureus*, *S. epidermis*, *P. aeruginosa* and *E. coli*. Standards, streptomycin and nystatin were used to test for anti-microbial activity against bacteria nystatin as well as yeast and fungi. Each culture was standardized to 0.5 McFarland standards containing approximately 10^8 bacterial cells. The plates were inverted and incubated between 30–37°C for durations of 18–24 h, 24–48 h or until sufficient growth has occurred. After incubation, each plate was observed and measured for diameters of inhibition zones. Each zone was measured to the nearest whole mm, using sliding calipers or a ruler, held on the back of the inverted petri dish. The evaluation of inhibition can be set out into three categories; very active (above 11 mm), medium activity (active) (between 6–11 mm), while non-active (6 mm). This evaluation was based on the diameter of zones of inhibition (Silalahi *et al.,* 2014).

RESULTS AND DISCUSSION

Phytochemical screening

Phytochemical screening on extracts from new plants is of paramount importance to identify potential new sources of commercially therapeutic compounds. Such step is crucial in order to make the best and prudent use of available natural wealth (Ram, 2001). Screening on the phytochemical composition of *Hibiscus sabdariffa* obtained using different types of solvents constitutes vary according to the polarity of solvents used during the extraction. For this experiment, the extracts of HS obtained from extractions using different solvents; hexane, acetyl acetate and ethanol were analysed and observed for bioactive compounds *viz*. alkaloids, saponins, tannins, phenolic and flavonoids. The presence and absence of each compound is depicted as presence (+) and absence (–), (Table 1). Ethanol and ethyl acetate extracts of *Hibiscus sabdariffa* were also showed presence of flavonoids. These findings were similar to the flavonoids being a common class of bioactive compounds extractable using ethyl acetate and ethanol (Zafar & Mujeeb,

Table 1. Phytochemical constituent of hexane, ethyl acetate, and ethanol extracts of calyces of *H. sabdariffa*

Metabolites	*Hexane*	*Ethyl acetate*	*Ethanol*
Saponins	–	+	+
Tannins	–	–	+
Phenolic	–	+	+
Flavanoid	–	+	+
Alkaloid	–	–	+

Note: (+), Presence and (-), Absence of bioactive compounds.

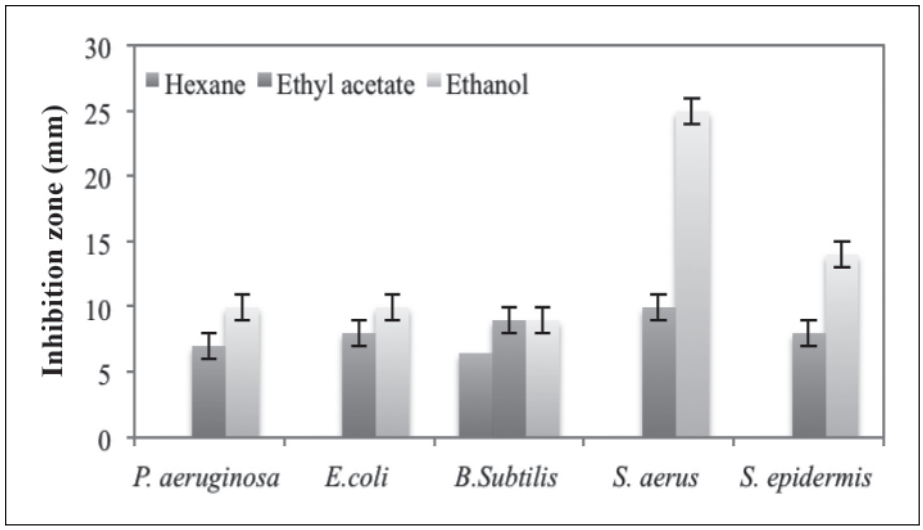

Fig. 1. Antimicrobial activity of hexane, ethyl acetate, and ethanol extracts of *Hibiscus sabdariffa* against five selected pathogenic bacteria.

2002). It is a class of compounds prominent for their anti-viral, anti-inflammatory, antioxidant activity, cytotoxic and, is also used for treating hypertension, diabetes, rheumatic fever etc. Thus, indicate that the calyces of *Hibiscus sabdariffa* is a potential source of flavonoids that be useful to treat such diseases.

Likewise, phenolic compounds were detected in *Hibiscus sabdariffa* extracts of ethyl acetate and ethanol. This class of compound is well-documented for their excellent antioxidant properties (Shirwaikar *et al.,* 2003). Saponins and tannins were also present in the ethanolic extract of *Hibiscus sabdariffa*. Tannins have been found beneficial as a potent anti-oxidant as well as preventing unwanted bacterial proliferation, in which the compound blocks microbial metabolism of certain key enzymes (Tom *et al.,* 2013). Indeed, herbs having tannins as their predominant component are often astringent in nature and used for treating intestinal disorders such as diarrhea and dysentery (Dharmananda*,* 2003). Alkaloids were also found in the ethanol extract of *Hibiscus sabdariffa*. However, the tests indicated this compound was isolated in higher quantities in the ethanol extract than that in the ethyl acetate and hexane extract. This was probably due to ethanol being more hydrophilic as compared to ethyl acetate and hexane. Notably, ethanolic extract of *Hibiscus sabdariffa* qualitatively demonstrated presence of more groups of compounds, and to a lesser extent, in the ethyl acetate and absence in hexane extracts.

Antimicrobial Activities

The antimicrobial activity of hexane, ethyl acetate and ethanol extracts of *Hibiscus sabdariffa* was tested against pathogenic bacteria namely *B. subtilis*, *S. aureus*, *S. epidermis*, *P. aeruginosa* and *E. coli*. The ethanol extract of *Hibiscus sabdariffa* exhibited the strongest anti-microbial activities against all the tested microorganisms (Figure 1) and afforded the largest inhibition zone on *S. aureus* (25 mm), followed by *S. epidermis* (14 mm), *P. aeruginosa* and *E. coli* (10 mm), with the lowest inhibition on *B. subtilis* (9 mm). The data implied that the ethanol extract contained higher quantities of compounds with antimicrobial activities. Although the mechanism of anti-microbial action in phytochemicals of *Hibiscus sabdariffa* is not fully understood, it has been suggested that polar extracts from this plant are high in phenolic compounds such as flavonoids and cyanidin (Lacombe *et al.,* 2013). Antimicrobial properties of phenolic compounds may be attributed to actions of these compounds which deprives these bacteria of iron, which is an essential element for assembly of certain enzymes (Guo *et al.,* 2007). The largest inhibition zone in the ethyl acetate extracts were observed on *S. aureus* (10 mm) followed by *B. subtilis* (9 mm), *S. epidermis* and *E. coli* (8 mm), and the lowest was for the plate that contained *P. aeruginosa* (7 mm) culture. Conversely, anti-microbial activity was observed for *B. subtilis* in the hexane extract of *Hibiscus sabdariffa* (6.5 mm).

CONCLUSION

Our results suggest that ethanol extracts of *Hibiscus sabdariffa* is a potential source of antimicrobial agents due to the presence of all phytochemical constituents and could be largely contributed for clinical evaluation and development of commercial drugs.

ACKNOWLEDGEMENTS

The authors would like to thank the Ministry of Higher Education of Malaysia (MyBrain15) for fund and Universiti Putra Malaysia (UPM) for the use of their lab facilities.

REFERENCES

Adegoke, A.A., Adebayo-tayo & Bukola, C. 2009. Antibacterial activity and phytochemical analysis of leaf extracts of *Lasienthera africanum*. *African Journal of Biotechnology*, **8(1)**: 77-80.

Audu, S.A., Ilyas, M. & Kaita, H.A. 2007. Phytochemical screening of the leaves of *Lophiralanceolata* (Ochanaceae). *Life Science Journal*, **4(4)**: 75-79.

Dharmananda, S. 2003. Gallnuts and the Uses of Tannins in Chinese Medicine, Institute for Traditional Medicine, Portland, Oregon.

Guo, M.L., Perez, C., Wei, Y.B., Rapoza, E., Su, G. & Bou-Abdallah, F. 2007. Iron-binding properties of plant phenolics and cranberry's bio-effects. *Dalton Trans*, **43**: 4951-4961.

Hirunpanich, V., Utaipat, A., Molales, N.P., Bunyapraphtsala, N., Sato, H. & Herunsale, A. 2006. Hypocholesterolemic and antioxidant effects of aqueous extracts from the dried calyx of *Hibiscus sabdariffa* L. in hypercholesterolemic rats. *Journal of Ethnopharmacology*, **103**: 252-260.

Lacombe, A., McGivney, C., Tadepalli, S., Sun, X. & Wu, V.C. 2013. The effect of American cranberry (*Vaccinium macrocarpon*) constituents on the growth inhibition, membrane integrity, and injury of *Escherichia coli* O157:H7 and *Listeria monocytogenes* in comparison to *Lactobacillus rhamnosus*. *Food Microbiology*, **34(2)**: 352-359.

Muhammad, T.B. & Shakib, A.B. 1995. Jus hibiscus: Bukan sekadar minuman biasa. Dewan Ekonomi. 12–14pp.

Nagendra, K.K., Rangaiah, G.S., Varaprasad, B. & Sirisha, C. 2010. Bactericidal activities of different medicinal plants extract against ocular pathogen viz *Corynebacterium macginleyi*. *Drug Invention Today*, **29(1)**: 5-7.

Prasongwatana, V., Woottisin, S., Sriboonlue, P. & Kukongviriyapan, V. 2008. Uricosuric effect of roselle (*Hibiscus sabdariffa*) in normal and renal-stone former subject. *Journal of Ethnopharmacology*, **117(3)**: 491-495.

Prabhakaran, D., Rajeshkanna, A. & Senthamilsevi, M.M. 2016. Antimicrobial activity of *Hibiscus sabdariffa* (flowers). *Indo American Journal of Pharmaceutical Research*, **6(5)**: 5494-5498.

Ram, R.L. 2001. Preliminary phytochemical analysis of medicinal plants of South Chotanagpur used against dysentery. *Advances in Plant Sciences,* **14**: 525-530.

Rodriguez-Medina, I.C., Beltran-Debon, R., Molina, V.M., Alonso-Villaverde, C., Joven, J. & Menendez, J.A. 2009. Direct characterization of aqueous extract of *Hibiscus sabdariffa* using HPLC with diode array detection coupled to ESI and ion trap MS. *Journal of Separation Science*, **32(20)**: 3441-3448.

Sofowora, A. 1993. Phytochemical screening of medicinal plants and traditional medicine in Africa, 2nd Edition. Spectrum Books Ltd Nigeria. 150-156pp.

Shirwaikar, A., Malini, S. & Kumari, S.C. 2003. Protective effect of *Pongamia pinnata* flowers against cisplatin and gentamicin induced nephrotoxicity in rats. *Indian Journal Experimental Biology*, **1**: 58-62.

Silalahi, J., Permata, Y.M. & Putra, E.D.L. 2014. Antibacterial activity of hydrolyzed virgin coconut oil. *Asian Journal of Pharmaceutical Clinical Research*, **7(2)**: 90-94.

Tom, V., Rodolfo, J.H., James, E.S. & Qing-Li, W. 2013. *ACS Symposium Series*, **1127(14)**: 209-230.

Tseng, T., Kao, T., Chu, C., Chou, F., Lin, W. & Wang, C. 2000. Induction of apoptosis by hibiscus protocatechuic acid in human leukemia cells via reduction of retinoblastoma (RB) phosphorylation and Bcl-2 expression. *Biochemical Pharmacology*, **60**: 307-315.

Zafar, R. & Mujeeb, M. 2002. Rotenoid and rutin in callus culture of *Tephrosia purpurea* (L) Pers. *Indian Journal Pharmaceutical Science,* **64(3)**: 217-221.