Chemical profile and scavenging activity of four extracts from *Hibiscus sabdariffa* L. calyx

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Research note

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Abstract

Objective

Hibiscus sabdariffa L., also known as roselle and karkadeh, is commonly used in traditional medicine and has many applications on food, pharmaceutical and cosmetic industries. More than 100 cultivars of *H. sabdariffa* are found. Despite the fact that Sudan is considered as one of the principal producer of *H. sabdariffa* there has been limited systematic study on the efficiency of different solvent extraction on its bioactive molecules. Therefore, this study was designed to investigate the chemical constituents of *H. sabdariffa* calyx using acetone, ethanol (EtOH), 50% EtOH and water as extraction solvents and their impact on the DPPH free radical scavenging activity.

Results

Results showed that the total polyphenol, flavonoids and vitamin C contents of the dried calyces were significantly ($P < 0.05$) affected by the solvent extraction used. HPLC analysis using 16 standards revealed the presence of 11–13 compounds in the four extracts with the 50% EtOH extract recovered the highest concentrations of identified compounds. Moreover, the 50% EtOH extract revealed highest DPPH free radical scavenging activity (IC_{50} 1.544 ± 0.3 µg/mL). Based on these results, maceration the calyx on 50% EtOH was therefore suggested for optimal extraction of bioactive compounds with high antiradical activity from *H. sabdariffa* calyx.

Introduction

Hibiscus sabdariffa L. (Family Malvaceae), with common names roselle and karkadeh, is believed to be domesticated by the people of western Sudan sometime before 4000 BC [1]. The calyx is widely used in Sudan to prepare cold or hot beverage, in addition to, a traditional fermented food known as Furundu is prepared from the seed [2]. *H. sabdariffa* is considered as important economic crop and a source of income for small farmers in western Sudan [3]. It is also well-thought-out as famine food because of its resistance to hard conditions of drought [4]. In traditional medicine, the calyces are mixed with fruits of *Adansonia digitata* and *Tamarindus indica* to cure malaria [5]. The calyx is also used to treat hypertension, flu, haemorrhoids [6], headache [7], fever, snake bite, scorpion sting [7; 8], as hypotensive and antispasmodic and for relaxation of uterine muscle [9]. A detailed review carried out by Da-Costa-Rocha [15] and Riaz and Chopra [16] summarized the chemical constituents and pharmacological properties of *H. sabdariffa*.

Biological activities of plants are associated with their phytochemical constituents [17], therefore it is important to determine the appropriate extraction method and extractive solvents used concerning a targeted biological activity. Despite the fact that Sudan is considered as one of the principal producer of *H. sabdariffa* and its different application on food, pharmaceutical and cosmetic industries there has

been limited systematic study on the efficiency of different solvent extraction on bioactive molecules. In this context this study was designed to investigate the chemical constituents of *H. sabdariffa* calyx using acetone, ethanol and water as extraction solvents and their impact on antiradical property.

Materials And Methods

Plant materials

Dried calyces of *H. sabdariffa* was purchase from the local market in Khartoum in June/2018.
Preparation of extracts

The dried calyces were cleaned from debris and were pulverized and subjected to extraction using four solvents; acetone, ethanol (EtOH), 50% ethanol/water (50% EtOH) and water. 10 g of sample was accurately measured and macerated in 50 mL of appropriate solvent for one hour. All extracts were filtered through Whatman No.1 filter paper. Then, acetone and EtOH extracts were concentrated using a rotary evaporator under reduced pressure while aqueous extracts were freeze-dried.

Determination of total polyphenol content

The total polyphenolic content was determined by using the method described by Wolfe et al. [18]. The total phenolic contents were expressed as gallic acid equivalents (mg/g on a dry weight basis).

Determination of total flavonoid content

The total flavonoid content was determined by adopting the method described by Ordonez et al. [19]. Total flavonoids content was expressed as quercetin (mg/g on a dry weight basis).

Determination of vitamin C content

The content of vitamin C was evaluated spectrophotometrically according to method PN-A-04019 [20]. Results are presented as mg vitamin C/100 g extract on a dry weight basis.

HPLC analysis

HPLC analysis was carried out using an Agilent 1260 series. The separation was carried out using Eclipse Plus C18 column (4.6 mm x 250 mm i.d., 5 µm). The mobile phase consisted of water (A) and 0.02% tri-floro-acetic acid in acetonitrile (B) at a flow rate 1 ml/min. The mobile phase was programmed consecutively in a linear gradient as follows: 0 min (80% A); 0–5 min (80% A); 5–8 min (40% A); 8–12 min (50% A); 12–14 min (80% A) and 14–16 min (80% A). The multi-wavelength detector was monitored at 280 nm. The injection volume was 10 µL for each of the sample solutions. The column temperature was maintained at 35 °C. Compounds were identified and quantified (µg/g extracts) with comparison standards retention times and UV–Vis spectra.

Determination of antiradical activity

The antioxidant DPPH free radical scavenging activity was determined using the method described by Brand-Williams [21]. The IC_{50} value was calculated from the linear regression of plots of concentration of the test sample against the mean percentage of the antioxidant activity.

Statistical analysis

All experiments were performed in triplicate and the obtained results were expressed as the mean ± standard deviation. One-way ANOVA was performed for determining significant differences between the four extracts and their antiradical activity.

Results

Extraction yield

Calyx of *H. sabdariffa* was macerated in four solvents; acetone, EtOH, 50% EtOH and water to evaluate the effect of type of solvent on the chemical composition and consequently the antiradical activity of the plant. Results of extraction yield (percentage of mass of extract/mass of dry matter) are depicted in Table 1. The four extracts yield were ranked in a descending order as follows: EtOH (77.2%) > 50% EtOH (58.4%) > water (6.6%) > acetone (3.1%).

Table 1
Effect of solvent type on yield, vitamin C content and antiradical activity from *Hibiscus sabdariffa* L. calyx.

Extraction solvent	Yield (%)	Vitamin C[1] (mg/100 g)	DPPH antiradical activity[1] IC_{50} (µg/mL)
Acetone	3.1	420 ± 9.7[a]	16.754 ± 0.7[a]
EtOH	77.2	230 ± 7.5[b]	2.481 ± 0.3[c]
50% EtOH	58.4	80 ± 0.7[d]	1.544 ± 0.3[d]
Water	6.6	100 ± 1.2[c]	11.616 ± 1.1[b]
Vitamin C*			1.500 ± 0.1[d]

* Positive control; [1], Results were expressed as the mean of triplicates ± standard deviation; Different superscript letters in the same column indicate significant difference ($p < 0.05$).

Total polyphenol content

The total polyphenol content values were expressed as mg gallic acid equivalent (GAE)/g. Results are presented in Fig. 1. Both EtOH (39.07 ± 0.08 mg GAE/g) and 50% EtOH (38.64 ± 0.33 mg GAE/g) extracts

revealed significant ($p \leq 0.05$) highest polyphenolic content followed by the water (31.83 ± 0.62 mg GAE/g) and acetone extracts (29.67 ± 0.22 mg GAE/g) respectively.

Total flavonoid content

The total flavonoids content values were expressed as mg quercetin equivalent (QE)/g. Results are presented in Fig. 1. The total flavonoid contents were significantly ($p \leq 0.05$) different in the four studied extracts. The highest content was obtained from the water extract (31.80 ± 0.83 mg QE/g) followed by the 50% EtOH (26.38 ± 0.55 mg QE/g) and acetone (19.11 ± 0.32 mg QE/g) extracts. EtOH extract (9.05 ± 0.85 mg QE/g) displayed the least flavonoid content.

Phenolic profile

The four extracts of *H. sabdariffa* calyx was analyzed using HPLC to determine the effect of the extraction solvent on the composition and concentration of extracted metabolites (Table 2). From the 16 standard compounds used, 13 were detected in the water and 50% EtOH extracts while 11 compounds were identified in the acetone and EtOH extracts. Generally, the 50% EtOH extract recovered the highest concentrations of identified compounds (10 compounds) while the acetone extract gave the least amount of all detected compounds. The four extracts were dominated by chlorogenic acid (hydroxycinnamic acid) and naringenin (flavonoid) with highest concentrations obtained from the 50% EtOH (14549.69 and 12574.15 µg/g respectively) followed by the EtOH (10895.07 and 8062.12 µg/g respectively) and water (10288.41 and 3326.99 µg/g respectively) extracts respectively. Rutin (flavonoid) was also present in considerable amount with highest concentration in the EtOH (1265.24 µg/g) followed by the 50% EtOH (1224.82 µg/g) and water (1004.92 µg/g) extracts respectively. Gallic acid (phenolic acid) present in highest concentration in the EtOH extract (9306.03 µg/g) followed by the water (7656.05 µg/g) and 50% EtOH (5884.05 µg/g) extracts respectively. Caffeine was found to be present in the 50% EtOH (171.05 µg/g) and water (138.11 µg/g) extracts and absent or in low amount in the water and acetone extracts.

Table 2
Chemical profile of *Hibiscus sabdariffa* L. calyx extracted by different solvent types.

	Concentration (µg/g)			
	Acetone	EtOH	50% EtOH	Water
Phenolic acids/ derivative				
Gallic acid	36,02	9306,03	5884,05	7656,05
Ellagic acid	0,00	188,02	209,27	107,01
Syringic acid	0,00	0,00	0,00	0,00
Propyl Gallate	1,23	0,00	411,85	73,95
Phenolic aldehydes				
Vanillin	0,00	61,42	109,77	0,00
Hydroxycinnamic acids				
Chlorogenic acid	81,09	10895,07	14549,69	10288,41
Caffeic acid	2,06	296,29	329,00	263,37
Coumaric acid	0,00	66,57	108,37	29,49
Ferulic acid	0,00	0,00	0,00	0,00
Cinnamic acid	0,29	17,08	63,65	22,61
Flavonoids				
Catechin	12,26	0,00	0,00	2826,53
Rutin	5,41	1265,24	1224,82	1004,92
Naringenin	28,53	8062,12	12574,15	3326,99
4`.7-DihydroxyisoFlavone	1,40	98,35	281,22	59,60
Querectin	2,14	231,05	245,95	103,83
Alkaloid				
Caffeine	2,23	0,00	171,05	138,11

Vitamin C content

Vitamin C content in the four extracts of *H. sabdariffa* calyx was also determined and results are presented in Table 1. The highest content in vitamin C was obtained from the acetone (420 ±

9.7 mg/100 g) and EtOH (230 ± 7.5 mg/100 g) extracts respectively while the water (100 ± 1.2 mg/100 g) and 50% EtOH (80 ± 0.7 mg/100 g) extracts had the least content.

DPPH free radical scavenging activity

The antioxidant DPPH free radical scavenging activity was determined for the four extracts of *H. sabdariffa* calyx and IC_{50} values are depicted in Table 1. The 50% EtOH extract revealed highest antiradical activity with IC_{50} value (1.544 ± 0.3 µg/mL) comparable to that obtained from the positive control (1.5 ± 0.1 µg/mL). The EtOH extract (2.481 ± 0.3 µg/mL) exerted also considerable antiradical activity. The water and acetone respectively exhibited the least antiradical activity with IC_{50} values of 11.616 ± 1.1 and 16.754 ± 0.7 µg/mL.

Discussion

Active metabolites usually exist in low concentration in plants [22, 23]. Therefore, it is necessary to select the appropriate solvent to be able to obtain extracts with high yield and best properties for the targeted biological activity. Extraction yield was used as an indicator of the effects of the extraction conditions. In this study the four solvents used to extract metabolites from *H. sabdariffa* calyx varied in their extraction yield with best amount obtained from the EtOH (77.2%) and 50% EtOH (58.4%) solvents respectively. This results were in agreement with finding of Grigonisa et al. [24] who reported that the highest extract yields were obtained with polar alcohol-based solvents.

The results of the present study demonstrated that the total content of polyphenol, flavonoid and Vitamin C in calyces of *H. sabdariffa* significantly ($p \leq 0.05$) influenced by type of solvent used for extraction. EtOH and 50% EtOH extracted highest content of polyphenols while the water and acetone extracts showed the highest flavonoid and vitamin C contents respectively. Previous study by Koffi et al [25] showed that alcohol (ethanol and methanol) was better in extracting phenols than acetone. However, it would also be important to consider that the high contents of phenolics in alcoholic extracts are more likely associated with biomolecules including proteins, carbohydrates (glycosides), terpenes, chlorophyll, lipids and inorganic compounds, which could be also extracted by these solvents and consequently interfere in determination of total polyphenol by Folin-Ciocalteu reagent [26].

Total flavonoid content in the present study were best extracted by the water extract and was in contradiction to the results of Koffi et al [25] who showed that flavonoids in *H. sabdariffa* calyx were better extracted from the acetone extract. This difference in results could be attributed to nature of flavonoid molecules present in *H. sabdariffa* sample from Sudan which could be affected by many biotic and abiotic factors such as differences of cultivars, soil of cultivation, climatic conditions and storage condition [27, 28].

Furthermore, the chemical profiling of the four extracts revealed that out of the 15 phenolic standards used 12 compounds were present in *H. sabdariffa* with chlorogenic acid, naringenin rutin and gallic acid at noteworthy concentrations in most extracts. Generally, the highest amount of the identified compounds

was obtained from the 50% EtOH extract. Previous studies [15, 16] reported the presence of most of these compounds in *H. sabdariffa* calyx, but to the best of our knowledge this is the first reports on comparing the effect of the four used solvents on the recovery of these compounds.

Result of the antioxidant DPPH free radical scavenging activity showed that 50% EtOH extract followed by EtOH extract exhibited the best anti-DPPH radical activity while acetone extract revealed the least activity. Previous study showed that the alcoholic (methanol) extract gave the highest DPPH value in comparison with ethyl acetate and acetone [28, 29]. As noted in the phenolic profile of these two extracts, many compounds are well known for their antiradical activity such as rutin, qurecetin [30], gallic acid [31], ellagic acid [32], chlorogenic acid, coumaric acid [33], caffeic acid [34] vanillin and catechin [35]. Although the water extract contained also considerable amount of these compounds, its antiradical activity was lower than that observed in the 50% EtOH and EtOH extracts. This might be to antagonistic effect and steric hindrances of other components which are also present in the crude extract [36]. Furthermore, the antiradical activity of the acetone extract was probably associated to its high content in vitamin C. Soobrattee et al. [37] reported that although the antiradical potential of vitamin C was found to be weaker than that of quercetin and similar to that of trolox, it can still contribute to the antiradical activity of berries and fruits.

From the Pearson's correlation coefficient of total polyphenols, flavonoids, vitamin C with antioxidant activity, it was suggested that the antioxidant DPPH free radical scavenging activity of *H. sabdariffa* calyx was mainly associated to polyphenolic content ($R^2 = 0.9836$) rather than the total flavonoid content ($R^2 = 0.0625$) or vitamin C ($R^2 = 0.3962$).

Conclusion

The present study provide a better insight into the influence of type of solvent extraction on the chemical composition of metabolites from *H. sabdariffa* calyx and their amount. The obtained results suggested that 50% EtOH could extract more phenolic compounds with highest concentration which was reflected on highest DPPH free radical scavenging activity.

Limitation

Types of anthocyanins and organic acids should be determined.

Other antioxidant assays should be performed.

Abbreviations

EtOH: ethanol; HPLC: High-performance liquid chromatography; GAE: gallic acid equivalent; QE: quercetin equivalent. DPPH: 2,2-diphenyl-1-picrylhydrazyl.

Declarations

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Authors’ contributions

SY conceptualized the project, provided technical support and prepared the manuscript; ZH, provided technical support.

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Availability of data and materials

The data can be requested from the corresponding author.

Ethics approval and consent to participate

No human or animal studies were used in this work.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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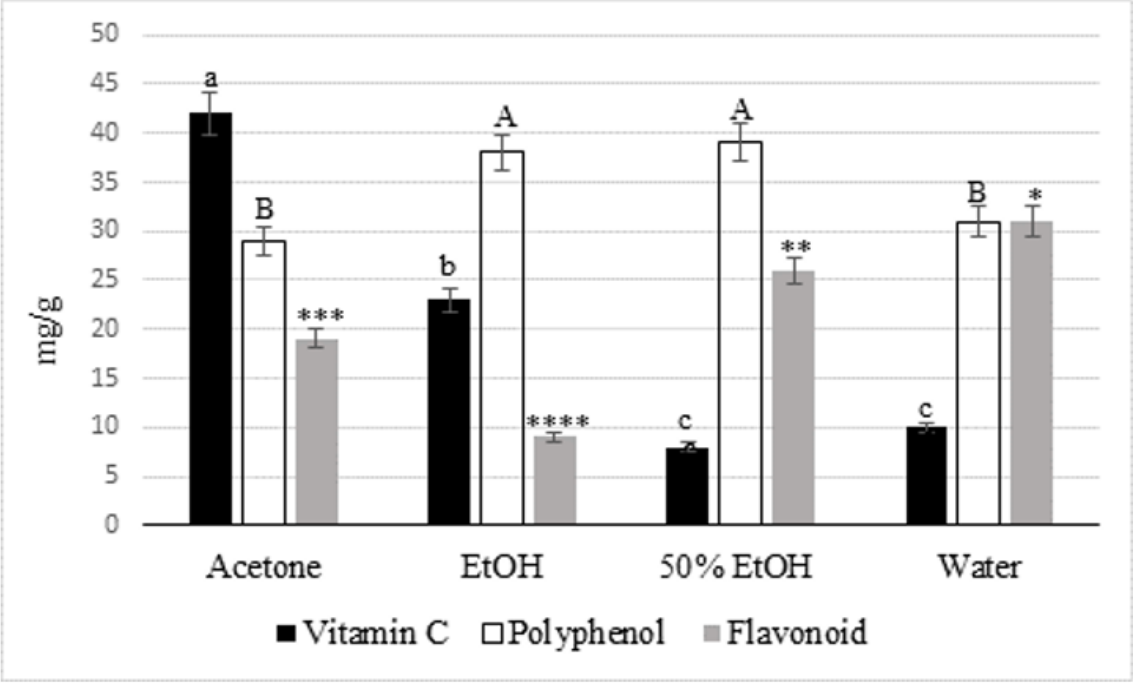
Figures



Different superscript letters or signs of the same bar indicate significant difference ($p < 0.05$).

Figure 1

Total vitamin C, polyphenol and flavonoid contents of Hibiscus sabdariffa L. calyx extracted by different solvent types.