Available online at www.elixirpublishers.com (Elixir International Journal)

Pharmacy

Elixir Pharmacy 45 (2012) 7660-7663

ISSN: 2229-712X

# Evaluation of total flavonoid, and total Phenolic contents of dried calyx preparations of Bissap *(Hibiscus Sabdariffa)*

Elom S. Achoribo[1], Daniel Achel[1], Abass Gibrilla[2], Adaboro M.R[1] and Linda Palm[2]

[1]Applied Radiation Biology Centre, Radiological and Medical Sciences Research Institute, Ghana Atomic Energy Commission, P.O. Box LG 80, Legon-Accra.

[2]Nuclear Chemistry and Environmental Research Centre, National Nuclear Research Institute, Ghana Atomic Energy Commission, P.O. Box LG 80, Legon-Accra.

**ARTICLE INFO**

**Article history:**

Received: 21 February 2012;

Received in revised form:

17 March 2012;

Accepted: 28 March 2012;

**Keywords**

*Hibiscus sabdariffa*,

Anti-cancer property,

Phenolic.

**ABSTRACT**

The dried calyces of *Hibiscus sabdariffa* L., have gained importance as local soft drink and medical herb in Ghana. There are speculations it has anti-cancer property, and that the decoction is better than the infusion. In view of these the Total Phenolic and Flavonoid content of both preparations was investigated. The decoction method gave the highest % yield of crude extract (76%) and of total flavonoid content (5887.5µg QE/g) while the infusion method gave the highest TPC value (46.123mg GAE/g). Also higher values of TPC and TFC were obtained from the S. samples compare to the B. samples. All the TFC values obtained from the decoction method were higher than the infusion values. Out of the 8 samples, TPC values of 3 (Kaneshie.S, Madina.S, and Nima.B) of the decoction method were lower than that of infusion. As flavonoids play vital role in scavenging free radicals in organisms, the decoction process can be recommended. Since the differences in the decoction and infusion's data are not big, both preparations can be used.



## Introduction

Excess of free radicals that naturally occur in mammalian body through oxidative process is known to be involved in many human diseases such as Alzheimer, ageing process, cataracts, acute liver toxicity, cardiovascular diseases, arteriosclerosis, nephritis, diabetes mellitus, rheumatism, inflammatory process and DNA damage that can lead to carcinogenesis (Favier, 2003; Kassab *et al*., 2003; Atawodi, 2005). The level of these species produced by mitochondrial respiration, phagocytosis, redox cycles or radiation is maintained by neutralizing excess free radical species by nutritional trappers (vitamins C, E, carotenoids, polyphenols) or destruction by various enzyme systems (superoxide dismutases, glutathione peroxidases). Unfortunately, oxidative stress can result from a disruption of the balance between the systems generating free radicals and systems permitting their elimination leading to excess of free radicals that are highly reactive oxygen species; superoxide ($O^{\bullet}_2$), hydroxyl ($OH^{\bullet}$), peroxyl ($ROO^{\bullet}$), peroxinitrite ($^{\bullet}ONOO$) and nitric oxide ($NO^{\bullet}$) (Atta-Ur-Rahman and Choudhary, 2001). For the prevention and treatment of these diseases involving for the treatment of these diseases, whose mechanisms involve the process of oxidative stress, many antioxidant based drug formulations are used (Wong *et al*., 2006). Because of restrictions on synthetic antioxidants due to their carcinogenicity, interest has increased considerably in finding naturally occurring antioxidants for use in foods, cosmetics or medicine materials to replace the synthetics ones (Sasaki *et al*., 2002).

Indeed, phenolic compounds found in vegetables, fruits or medicinal plants are known for their antioxidant potential and their role in prevention of human diseases (Cai *et al*., 2004) and number of papers highlighted a positive correlation between the antioxidant activity and the total phenolic content (Tawaha *et al*., 2007). Same can be said of Flavonoids, known to be potent antioxidants and have aroused considerable interest recently because of their potential beneficial effects on human health in fighting diseases. The capacity of flavonoids to act as antioxidants depends upon their molecular structure. The position of hydroxyl groups and other features in the chemical structure of flavonoids are important for their antioxidant and free radical scavenging activities. Quercetin, the most abundant dietary flavonol, is a potent antioxidant because it has all the right structural features for free radical scavenging activity (Jay M Patel, 2008; Chen YT *et al*., 1990).

*Hibiscus sabdariffa* is an herbaceous plant, cultivated largely in tropical and subtropical areas of both hemispheres. This plant is used for its fibre; mainly for its calyx, which is of three types: green, red and dark red. The leaves are deeply three to five lobed, arranged alternately on the stems. The flowers are white to pale yellow with a dark red spot at the base of each petal, and have a stout fleshy calyx at the base, fleshy and bright red as the fruit matures. In Senegal, Mali, Burkina Faso, Ghana, Benin and Niger, and France it is called *Bissap*. The plant is considered to have antihypertensive properties and in folk medicine used in as a diuretic, food colourings, mild laxative, and treatment for cardiac and nerve diseases and cancer.

The dried calyces of *Hibiscus sabdariffa* L., have gained importance as local soft drink and medical herb in local regions in Ghana. And people claim that the decoction is better than the infusion. With regard to the claim on its anti-cancer property, it is of importance to determine the total phenolic and flavonoid content of both extractions.

## Materials and Methods:

Chemicals: Folin-Ciocalteu phenol reagent (FCR), 2,2-diphenyl-1-picrylhydrazyl (DPPH), Gallic acid, Quercetin, and Vitamin C were purchased from Sigma. $AlCl_3$, $Na_2CO_3$,

Tele:

E-mail addresses: seyrama@gmail.com

$CH_3COOK$, were from Merck (Darmstadt, Germany). Ethanol and methanol were from Jansen Chimica (Beerse, Belgium). All chemicals were of analytical grade.

Plant Material: The dried calyx of Hibiscus sabdariffa Linn were purchased from 5 different markets in Accra/Ghana: Madina, Dome, Kaneshie, Nima, Achimota. Two different types (fig 1) were obtained from Madina, Dome, and Kaneshie which make the total number of 8 samples.

Extraction Processes: Two method of extraction were performed:

*Infusion*: 20ml of water was brought just to a boil and then poured over 1g grounded dried leaves, and then covered and allowed to steep for 30mn.

*Decoction*: Instead of just steeping it in hot water, 1g of the grounded dried leaves was boiled with 20ml of water to soften the material and release its active constituents.


**Fig 1: Type of samples**

**Extraction yield**

Extraction yields were established for each extraction. Two (2) ml of each extract were evaporated to dryness to establish the amount of extractable solids (extraction yield). Each determination was performed in triplicate.

***Methods:***

**Phenolic Content**

The total phenolic contents (TPC) were measured by the Folin Ciocalteu method using Gallic acid as standard (Singleton, *et al*., 1999) with modifications. Briefly, 50µl each of the extracts was mixed with 3ml of distilled water ($dH_2O$) and 250µl of FCR. The mixtures were allowed to stand for 5 min, and then 750µl of 20% $Na_2CO_3$ was added. After incubation of the resulting reaction mixtures for 30 min at room temperature absorbance values were measured at 760nm using a UV-VIS Spectrophotometer (Shimadzu, 1201, Japan). All determinations were performed in triplicate. A calibration curve was prepared using serial dilutions of 0.2mg/ml, 0.4mg/ml, 0.6mg/ml, 0.8mg/ml and 1mg/ml from a stock solution of 1000mg/ml Gallic acid dissolved in water. 50µl each of these solutions were treated like the samples and a calibration linear regression equation: Absorbance (Y) = 1.2961 mg Gallic Acid (x), $r^2$ = 0.982, was developed to explain the model. Total phenolic content in each extract was expressed in Gallic Acid Equivalents (GAE) by using the following formula $C = c \times V / m$

Where;

C= total content of phenolic compound in mg/g plant extract, in GAE.

c = the concentration of Gallic acid established from the calibration curve in mg/g.

V= the volume of extract in ml.

m= the weight of plant extract in grams.

Flavonoid Content

The aluminum chloride colorimetric assay method as described by Zhishen *et al*, was employed to evaluate total flavonoid content (TFC) in the samples using Quercetin as standard. 500µl of extracts were mixed with 1500µl of 99.9% EtOH, 100µl of 1 M potassium acetate, 100µl of 10% aluminum chloride and 3000µl of distilled water. The resulting mixtures were incubated for 30 minutes at room temperature and corresponding absorbance measured at 415 nm. All determinations were carried out in triplicates. A standard calibration curve was constructed using Quercetin standard solutions of 75ug/ml, 100ug/ml, 150µg/ml, 175µg/ml and 200µg/ml. 500µl of each standard was treated in the same manner as the samples above. Flavonoid content was determined as micro gram (µg) Quecertin Equivalent (QE)/g using the calibration linear regression equation: Absorbance (Y) = 0.0064 µg Quercetin(x) ($r^2$ = 0.990) with the following formula (Chang et al., 2002): $TFC = (C * D.F * V) / W$

Where;

C– Concentration obtained from the standard curve

D.F - Dilution factor

V - Volume of stock Solution

W - Weight of plant used in the experiment.

**Results and Discussion:**

The extraction yield obtained from the Infusion and Decoction (expressed as w/w percentage) is showed in fig.2. This ranges from 55 to 67% for the infusion process and 57 to 76% for Decoction. The differences in the % extraction yield for both methods are not much. The gap is only seen in Madina.B sample (61- 76%).

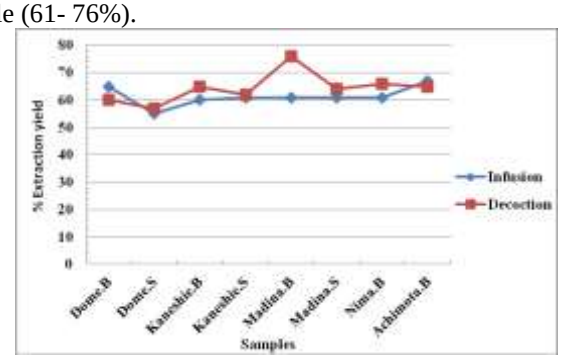


**Fig2: % Extraction Yield of the crude extracts from infusion and decoction**

Fig.3 as represented below presents the profile of the Total Phenolic Content (TPC) in the dried calyces of *Hibiscus sabdariffa* L. The highest TPC of 46.12mg GAE/g was observed from Infusion of Kaneshie.S with a corresponding Decoction TPC value of 43.56mg GAE/g, followed by Dome.S (42.48mg GAE/g), Madina.S (36mg GAE/g), Dome.B (29.67mg GAE/g), Nima.B (23.44mg GAE/g), Achimota.B (22.77mg GAE/g), Kaneshie.B (20mg GAE/g), and Madina.B (19.32mg GAE/g). Their corresponding Decoction TPC values are 43.02mg GAE/g, 32.92mg GAE/g, 33.18mg GAE/g, 21.36mg GAE/g, 23.86mg GAE/g, 31.64mg GAE/g, and 19.39mg GAE/g respectively.

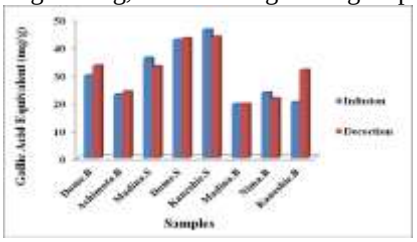


**Fig.3: Gallic Acid Equivalent (mg GAE/g) obtained from decoction and infusion.**

From the Decoction process, Kaneshie.S gives the highest TPC value of 43.56mg GAE/g followed by Dome.S (43.02mg GAE/g). But this pattern change from Dome.S to Dome.B instead to Madina.S as in Infusion. In all, there are no big differences in the infusion and decoction processes. It can be said that both preparations (Decoction, Infusion) of dried calyces of *Hibiscus sabdariffa* L. used by Ghanaian Communities does not affect much the total phenolic content.

Fig.4 shows clearly that the total phenolic content values depend on the nature of the dried calyces, because it is seen that the TPC values in the S. samples are higher than that in B. samples. This can be explained by the relatively small size of the S. samples. Also the harvesting, and processes they undergo may have increased the extraction of the phenolic compounds.

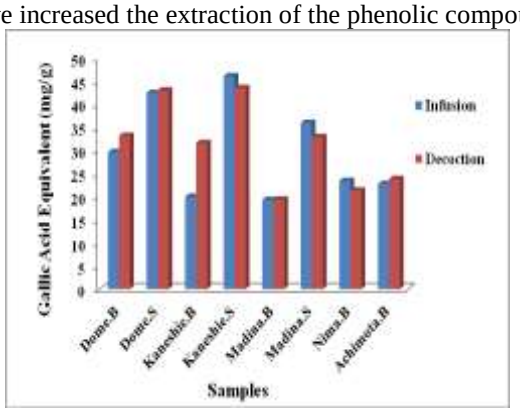


**Fig.4: Differences in the Gallic Acid Equivalent values between the S. and B. Samples**

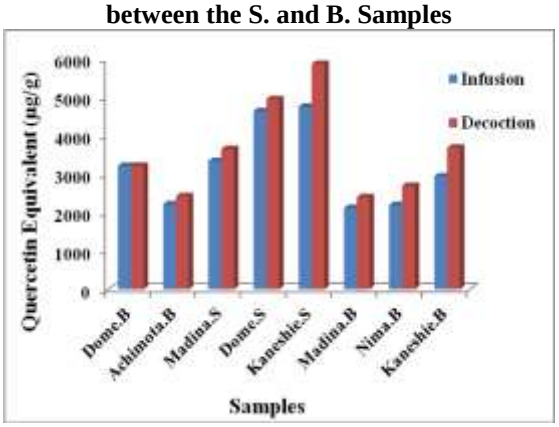


**Fig.5: Quercetin Equivalent (µg QE/g) obtained from decoction and infusion.**

The Total Flavonoid Content (TFC) in the dried leaves of *Hibiscus sabdariffa* L. is represented in Fig.5 which depicted higher TFC values for the Decoction method than the infusion. 5887.5µg Quecertin Equivalent(QE)/g) TFC value was obtained from Kaneshie.S sample, followed by Dome.S (4963.54µg QE/g), Kaneshie.B (3697.92µg QE/g), Madina.S (3661.46µg QE/g), Dome.B (3223.96µg QE/g), Nima.B (2702.08µg QE/g), Achimota.B (2431.25µg QE/g), and Madina.B (2406.25µg QE/g). Their corresponding infusion TFC values are 4772.92µg QE/g 4653.13µg QE/g, 2952.08µg QE/g, 3351.04µg QE/g, 3222.92µg QE/g, 2201.04µg QE/g, 2220.83µg QE/g, and 2121.88µg QE/g respectively. Contrary to the TPC values, the highest values were obtained from the Decoction, and the infusion values decrease according to the same pattern. The highest content of flavonoid observed can be attributed to the rich abundance of anthocyanidines in combination with other flavonoid, as documented by Chau, J. W., *et al* (2000). The decoction method gave the highest % yield of crude extract (76%) and of total flavonoid content (5887.5µg QE/g) while the infusion method gave the highest TPC value (46.123mg GAE/g).

The increase in TFC values of the S. and B. samples is portrayed in Fig.6.

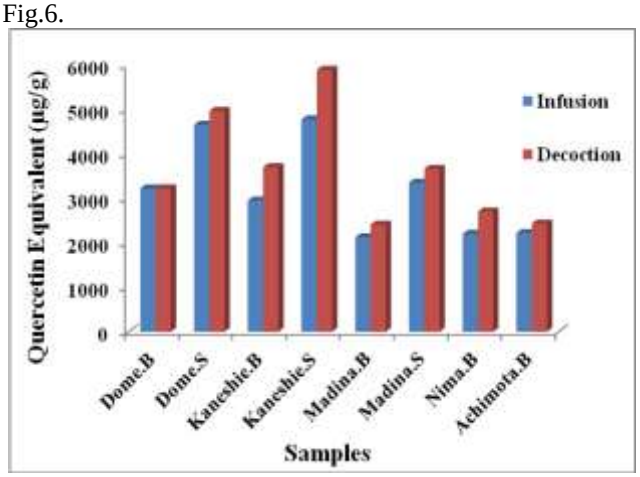


***Fig.6: Differences in the Quercetin Equivalent values between the S. and B. Samples***

It can be said from Figures 3, and 5 that the S. samples gave higher TPC and TFC values compare to the B. samples.

**Conclusion:**

The decoction method gave the highest % yield of crude extract (76%) and of total flavonoid content (5887.5µg QE/g) while the infusion method gave the highest TPC value (46.123mg GAE/g). Also higher values of TPC and TFC were obtained from the S. samples compare to the B. samples. All the TFC values obtained from the decoction method were higher than the infusion values. Out of the 8 samples worked on, 3 samples (Kaneshie.S, Madina.S, and Nima.B) TPC values of the decoction method were lower than that of infusion. As flavonoids play vital role in scavenging free radicals in organisms, the decoction process can be recommended. Since the differences in the decoction and infusion's data are not big, both preparations can be used.

**References:**

Atawodi, S.E., 2005. Antioxidant potential of African medicinal plants. Afr. J.Biotechnol., 4:128-133.

Atta-Ur-Rahman and M.I.Choudhary, 2001. Bioactive natural products as potential source of new pharmacophores: A theory of memory. Pure Applied Chem., 73: 555-5560

Cai, Y., Q. Luo, M. Sun and H.Corke, 2004. Antioxidant activity and phenolic compound of 112 traditional Chinese medicinal plants associated with anticancer. Life Sci., 74: 2157-2184.

Chau, J. W., Jin, M. W., Wea, L. L., Chia, Y. C., Fen, P. C. and Tsui, H. T. (2000). Protective effect of *Hibiscus* anthocyanins against tert-butyl hydroperoxide-induced hepatic toxicity in rats. *Food and Chemical Toxicology* 38: 411-416.

Chen YT et al. Free Radic Biol Med 1990; 9(1): 19-21.

Favier, A., 2003. Le stress oxidant: Interet conceptual et experimental dans la comprehension des mecanismes des maladies et potential therapeutique (Oxidative stress: Conceptual and experimental interest in the understanding of disease mechanisms and potential therapeutic). L'actualite chimique, 269: 108-270

Jay M Patel. Lethbridge Undergraduate Research Journal 2008; 3(2).

Kassab, A., S. Laradi, S.Ferchichi, A. Omezzine and B. Charfeddine et al., 2003. Oxidative parameters in type 2 diabetes mellitus. Immuno-Analyse Biol. Specialisee, 18: 79-85

Sasaki, Y.F., S. Kawaguchi, A. Kamaya, M.Ohshita and K.Kabasawa et al., 2002. The comet assay with 8 mouse organs: Results with 39 currently used food additives. Mutat. Res./Gen. Toxicol. Environ. Mutagenesis, 519: 103-119.

Singleton VL, Orthofer R, Lamuela-Raventós RM, Lester P. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. In *Methods in Enzymology* (ed). Academic Press: pp. 152-178.

Tawaha, K., F.Q.Alali, M.Gharaibeh, M.Mohammad and T. El-Elimat, 2007. Antioxidant activity and total phenolic content of selected Jordanian plant species. Food Chem., 104: 1372-1378.

Wong, C.C., H.B. Li, K.W. Cheng and F. Chen, 2006. A systematic survey of antioxidant activity of 30 Chinese medicinal plants using the ferric reducing antioxidant power assay. Food Chem., 97: 705-771.

Zhishen J, Mengcheng T, Jianming W. 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry* 64:555-559.