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RESEARCH ARTICLE

SYNERGISTIC AND ANTIMICROBIAL EFFECTS OF FRESH AND DRIED EXTRACTS OF RED AND GREEN VARIETIES OF *HIBISCUS SABDARIFFA* CALYXES ON *SALMONELLA TYPHI* AND *ESCHERICHIA COLI*

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ABSTRACT

Synergistic and antimicrobial effects of fresh and dried extracts of red and green varieties of *Hibiscus sabdariffa*calyxes on two enteric pathogens were investigated. Isolates of enteric pathogens namely: *Salmonella typhi* and *Escherichia coli* were obtained from Benue State University Teaching Hospital, Makurdi. Test organisms were subjected to confirmatory tests using standard methods of analysis. Samples of fresh and dried red and white calyxes of *Hibiscus sabdariffa* were purchased from Modern Market, Makurdi. Dried calyxes of the two varieties were washed, air dried at room temperature to a constant weight. The dried calyxes were converted to a powdered form using a blender. The powdered form was subjected to ethanolic extraction. Fresh calyxes of the two varieties were cleaned air dried and meshed using ethanol, it was allowed to evaporate in a water bath for 92 hours until a gummy concentrate was obtained. Phytochemical screening was carried out on the fresh and dried extracts of *H. sabdariffa* calyxes. Size of inoculum developed from isolates was calibrated using Mac Farland standard 1. Agar well diffusion method was used for susceptibility testing of the test organisms with ethanolic extracts of the dried red and green calyxes. Results of phytochemical screening of dried calyxes revealed that flavonoids was absent in the green variety but present in the red variety. Steroids, terpenoides and phlobatanins were absent in both varieties. Alkaloids, tanins and phenols were present in all varieties, Also phytochemical screening of (fresh) red and green calyxes extracts showed the presence of alkaloids, tannins and phenols as the bioactive components, saponins was absent in the fresh varieties of green and red. Although, flavonoid was also present in the (fresh) red variety but absent in the (fresh) green variety. *Salmonella typhi* recorded a zero activity with extract of dried green variety. The (fresh) green variety had zero activity against *E.coli* and inhibited *Salmonella typhi* at concentration of 40mg/ml. However, when a synergy was established between the two extracts (red to white) at ratio 25:25, diameter of zone of inhibition (9mm) was recorded with *Salmonellatyphi* as oppose to zero activity with extract of the green calyx alone. There was significant difference ($p<0.05$) in the effectiveness of the two varieties on test organisms. Statistical analysis also revealed that there was significant difference ($p<0.05$) in effectiveness between (fresh) red and (fresh) green varieties. Combining (dried) red and green ethanolic extracts of *Hibiscus sabdariffa* at different ratio revealed that there was no significant difference ($p>0.05$) between concentration and ratio of red to green variety on the growth of the tested organisms. Therefore, a combination of these two extracts in equal ratio can be recommended in the treatment of enteric diseases.

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INTRODUCTION

The genus Hibiscus includes more than 300 species (Ines *et al.,* 2014), and it is believed to have originated from East Africa (Llondu and Lloh, 2007). *Hibiscus sabdariffa* also known as *Roselle* in English and*Zoborodo* in Hausa is a multipurpose plant (Adebayo-Tayo and Samuel, 2000).Two major varietiesare: *Hibiscus sabdariffa* var. *altissimawester* and *Hibiscus sabdariffa* var. *sabdariffa* (Morton, 1987). The green type is generally common in Southern Guinea Savanna of Nigeria, while the red type is more prevalent in Northern Guinea and Southern Savanna (Doughari *et al.,* 2011). The fleshly red calyxes are the most popular, because it extracts are used to make non-alcoholic drink called *zobo* in Hausa (Yang *et al.,* 2005).Long before mankind discovered the existence of microbes, the idea that plants have some healing potential and they contain what we characterize as antimicrobial principle was well accepted (Doughari *et al.,* 2011). Plants extracts has medicinal properties and bioactive compounds such as flavonoids, tannins, steroids, phlobatannins that can be used singly or synergistically to treat infectious diseases because of their antimicrobial activities (Onyeagba *et al.,* 2004).

MATERIALS AND METHODS

Sample Collection

Samples of fresh and dried calyxes of *Hibiscus sabdariffa* (green and red varieties) were purchased from Makurdi Modern market, Benue state. Samples were packaged separately in clean polythene bags and were clearly labeled. Samples were transported to the laboratory, Department of Biological Sciences, University of Agriculture Makurdi for analyses.

Identification of Plant Samples

Identification of the plant samples was carried out by a plant scientist in the Department of Crop Breeding, Faculty of Agronomy, University of Agriculture, Makurdi.

Preparation of Calyxes for Extraction

Dried calyxes of *Hibiscus sabdariffa* (green and red varieties) were washed with distilled water and air dried at room temperature to a constant weight. The dried calyxes were converted into powdered form using a laboratory blender. The powdered samples obtained were collected in sterile cellophane bags. Samples were kept in a cool dry place for further analysis. Fresh calyxes also were cleaned using distilled water and were allowed to air dry before extraction

Method of Extraction for Dried calyxes

Each powdered samples (100g) was measured and poured into separate jars. 95% ethanol (500ml) was poured into the jars up to 2cm height above the sample surface. The plastic jars were closed properly with aluminium foil. Extraction was carried out for five days with occasional stirring and shaking. The plant extracts were filtered with a piece of clean cotton material and then through Whatman filter paper no 1. After filtration, the residues were taken for re-extraction in the jars using 250ml, 95% ethanol for two days. The jars were shaken and stirred severally during the process and the extracts were filtered. The filtrate was evaporated to obtain a gummy concentrate using a water bath (model-TE-7 Tempette) at 38^0C for 92 hours (Salie *et al.,* 1996).

Method of Extraction for Fresh Calyxes

About 100g of fresh cleaned calyxes of green and red varieties of *Hibiscussabdariffa*was measures in a beaker, it was meshed and mixed with ethanol. The meshed samples of fresh calyxes of green and red varieties were kept for 72 hours, with occasional stirring at intervals of 4 hours, it was then filtered and the residue was soaked for 2 days with 250ml of 95% ethanol. The extract of the fresh calyxes was kept in a water bath (model-TE-7 Tempette) at 38^0C for 92 hours for evaporation to take place until a gummy concentrate for analysis was obtained (Salie *et al.,* 1996).

Phytochemical Screening of Plant Extracts

Phytochemical screening of the plant(fresh and dried calyxes of red and green varieties of *Hibiscus sabdariffa)* extracts was carried out using methods of Trease and Evans, (2009); Sofowora, (1993). Bioactive compounds that were screened for include Alkaloids, Tannins, Saponins, Flavonoids, Steroids, Phlobatanins, Phenols, Terpenoids.

Collection of Test Organisms

Clinical isolates of *Salmonella typhi*and*Escherichia coli* were obtained from the microbiological unit, Benue State University Teaching Hospital, Makurdi. The microorganisms were sub-cultured and pure isolates of the resulting growth were confirmed.

Confirmatory Test on the Test Organisms

Test organisms were confirmed using Gram staining and biochemical tests such as Indole, oxidase, urease and carbohydrate fermentation as described by Cheesbrough, (2009).

Calibration of Inoculum using McFarland's Standard 1

The test organisms were calibrated using McFarland standard 1.A mixture of tetraoxosulphate (VI) acid 1% and barium chloride 1% was used. Exactly 9.9ml of H_2So_4 was added to 0.1ml of $BaCl_2$ in a test tube to form precipitate suspension. About 9.9ml of H_2SO_4 and 0.1ml of $BaCl_2$ correspond with 3 X 10^8 CFU/ml which is McFarland's standard 1. This was the turbidity standard for the test organisms (Cheesbrough, 2009).

Preparation of Cell Suspension

Each test organisms was sub-cultured on a selective media plates using streak plate method. The inoculated plates were incubated at 37^0C for 24 hours. Growth from pure cultures were transferred into test tubes containing 5ml of 0.9% sterile saline. The volume of the suspension was adjusted to attain a turbidity standard which matches McFarland's standard 1 (Cheesbrough, 2009).

Serial Dilution of Fresh and Dried Extracts (Green and Red varieties) of *Hibiscus sabdariffa*

One milliliter of each extracts was transferred into sterilized bijou bottles containing 9ml each of distilled water. The mixture was stirred using a glass rod. Serial dilution was made by measuring 1ml of the solution into another bijou bottle containing 9ml of sterile distilled water and was allowed to mix properly. The procedure was used to prepare 80mg/ml, 40mg/ml, 20mg/ml, 10mg/ml, and 5mg/ml.

Susceptibility Testing of the Organisms with Fresh and Dried *Hibiscus sabdariffa*Calyx Extracts (Green and Red varieties) using Agar well Diffusion Method

Salmonella-Shigella and Eosin methylene blue plates were inoculated with the suspension of test organisms by streaking. Using agar well diffusion method, wells were aseptically made with a sterile cork borer. About 0.1ml each of the solutions of different concentrations 80mg/ml, 40mg/ml, 20mg/ml, 10mg/ml and 5mg/ml of green, red and mixed varieties of

fresh and dried *Hibiscus sabdariffa* extracts were poured into labelled wells. Also, extracts of green and red varieties of *Hibiscus sabdariffa* were mixed at varying proportions of 1:4, 2:3, and 2.5:2.5, 3:2, 4:1 of green and red variety at different concentrations of 80mg/ml, 40mg/ml, 20mg/ml, 10mg/ml and 5mg/ml were poured into labelled wells.

About 0.1ml of 80mg/ml gentamycin was poured into one of the well to serve as control. Plates were allowed to stand for 1 hour for proper diffusion and were incubated at 37^0C for 24hours. After incubation, plates were examined for Inhibition Zone Diameter (IZDs) to determine the degree of susceptibility of the test organisms. Inhibition Zone Diameter were measured in millimetres and recorded by calculating the means of IZDs for plates in triplicate (Chinedu, 2011).

Statistical Analysis

Data obtained was analysed for statistical significance. Student T-test and analysis of variance (ANOVA) SPSS version 17.0 were used to verify the effect of fresh and dried calyx extracts of red and green varieties of *Hibiscus sabdariffa*on selected pathogens. These extracts were combined at different ratio to determine the effectiveness against the pathogens.

RESULTS AND DISCUSSION

Table 1. Phytochemical screening of fresh and dried green and red varieties of *Hibiscus sabdariffa* calyx extracts

Phytochemical Components	Green variety		Red variety	
	Fresh	Dried	Fresh	Dried
Akaloids	+	+	+	+
Tannins	+	+	+	+
Saponins	-	+	-	+
Flavonoids	-	-	+	+
Steroids	-	-	-	-
Phenols	+	+	+	+
Terpenoids	-	-	-	-
Phlobatanins	-	-	-	-

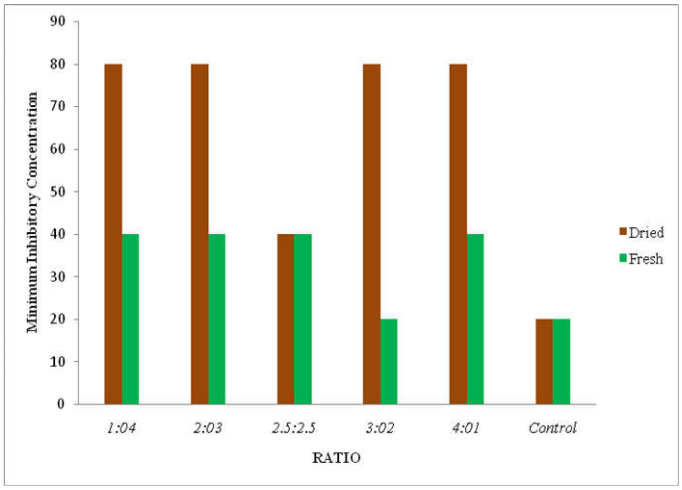

Figure 1. Minimum Inhibitory Concentration of mixed ratio of fresh and dried red and green varieties of *Hibiscus Sabdariffa* calyx extracts on *Salmonella typhi*

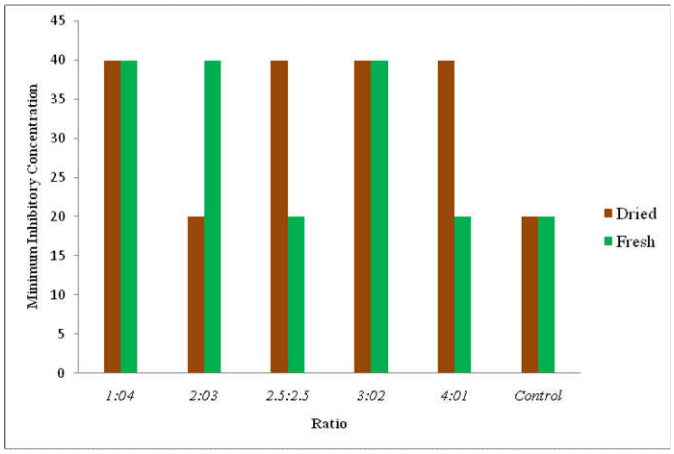

Figure 2. Minimum Inhibitory Concentration of mixed ratio of fresh and dried red and green varieties of *Hibiscus Sabdariffa* calyx extracts on *E.coli*

Table 2. Inhibition Zones (mm) of (Dried) Green and Red Variety Calyx Extracts of *Hibiscus sabdariffa* on *S.typhi* and *E.coli*

Test Organism	Conc/ml	Inhibition zone of RV(mm)	Conc/ml	Inhibition zone of GV(mm)	P-value
E.coli	20	3.70 ± 3.97	40	1.50 ± 2.17	0.010
Salmonella typhi	40	2.10 ± 2.99	AC	0.0 ± 0.0	0.054

($p \leq 0.05$)
Key- GV- green variety, RV-red variety, AC- all concentrations

Table 3. Inhibition Zones (mm) of (Fresh) Green and Red Variety Calyx Extracts of *Hibiscus sabdariffa* on *S.typhi* and *E.coli*

Test Organism	Conc/ml	Inhibition zone of RV(mm)	Conc/ml	Inhibition zone of GV(mm)	P-value
E.coli	2	04.70±4.76	AC	0.00±0.00	0.012
Salmonella typhi	20	5.80±5.94	40	2.50±3.56	0.006

($P \leq 0.05$)
Key- GV- green variety, RV-red variety, AC- all concentrations

DISCUSSION

The phytochemical screening of (dried) *Hibiscus sabdariffa* calyx carried out in this study shows that alkaloids, tannins, and phenols are the main phytochemicals present in the ethanolic extracts of both (dried) red and green varieties of *Hibiscus sabdariffa* calyxes. Saponins was absent in fresh green and red varieties but present in the dried calyxes of both varieties. This agrees with the report of Obouayeba *et al.* (2014). *E.coli* was susceptible to the (dried) red and green calyx extract while *Salmonellatyphi* was susceptible to the red calyx extract. This could be attributed to the presence of flavonoids in the (dried) red calyx extract which was absent in the (dried) green variety. Also phytochemical screening of (fresh) red and green calyx extracts showed the presence of alkaloids, tannins and phenols as the bioactive components. Although, flavonoid was also present in the (fresh) red variety but absent in the (fresh) green variety, saponins was also absent in the fresh green variety. Flavonoids include plant pigment called anthocyanins which are responsible for the red colour variation of the plant while acid taste is due to the presence of some organic acids which also contributed to the

red colour variation of the plant. This research also corresponds to the work of Olaleye (2007), as it was reported that the dark red coloured *Hibiscus sabdariffa* calyx has the highest content of anthocyanin followed by the light red coloured type, while the green coloured type has just traces or no anthocyanin. This study disagrees with the work of Adebisi and Ojokoh (2011) who reported that alkaloids and saponins were absent from the photochemical screening of extracts. This study showed that ethanol extract of *Hibiscus sabdariffa* calyx had effective antimicrobial activities. Both (dried) red and green calyxes inhibited *E.coli.* This corresponds to the report of Olaleye, (2007) as the study reported that *Hibiscus sabdariffa* calyx extracts may possess remarkable therapeutic action in the treatment of gastrointestinal infection and diarrhea in human. *Salmonella typhi* was inhibited by the (dried) red variety only, the (dried) green variety showed no inhibition against *Salmonella typhi* at all concentrations. This could be attributed to the presence of some bioactive components such as flavonoid which was presentin the red variety but absent in the green variety. The result further showed that only the (fresh) red variety inhibited *E. coli* at concentration of 20mg/ml and zero activity for fresh green variety. The fresh red variety inhibited *Salmonella typhi* at 20mg/ml while the green variety inhibited *S.typhi* at 40mg/ ml. Statistical analysis revealed that there was significant difference ($p<0.05$) in effectiveness between (fresh) red and (fresh) green variety. The non-inhibition of the (fresh) green calyx extracts to *E.coli*may be due to the absence of bioactive compounds such as flavonoids and saponins which is present in both (dried) red and green varieties. Combining (dried) red and green ethanolic extracts of *Hibiscus sabdariffa* at different ratio revealed that there was no significant difference ($p>0.05$) between concentration and ratio of red to green variety on the growth of the tested organisms. *Salmonella typhi* which recorded no inhibition when tested against the green variety was susceptible at a combined ratio of red to green, which was unable to inhibit test organisms when it was applied singly. Flavonoids are known to be effective antimicrobial agent against a wide array of microorganisms. Fresh red and green varieties were combined at different ratio and the result showed that there was no significant difference ($p>0.05$) between concentration and ratio of red to green calyx extracts on the growth of the tested pathogens.

Conclusion

It has been concluded that fresh and dried *Hibiscussabdariffa* calyx extracts (red and green varieties) showed inhibition of bacterial growth (invitro) at different concentrations as a result of some bioactive components that has anti-microbial effects. *Hibiscus sabdariffa* calyx extracts of both fresh and dried redvariety has more antimicrobial activity as compared to the green variety. The green variety can be improved by establishing a combined state with the red variety to improve its effectiveness and should be used as an alternative source of antimicrobial agent due to the increased rate of resistant organisms.

REFERENCES

Adabayo-Tayo, B.C., Samuel U.A. 2000. Microbial Quality and Proximate Composition of Dried *Hibiscus sabdariffa* Calyces in Uyo, Eastern Nigeria. *Microbiological Journal*, 5. (1): 13-18.

Adebisi, O., Ojokoh, A.O. 2011. Antimicrobial Activities of Green and Red Calyx Extracts of *Hibiscus sabdariffa* on Some Microorganisms. *Journal of Agriculture and Biological Sciences*, 2(2):38-42.

Books Limited, Ibadan, Nigeria. Pp 289. *Medicinal Journal,* 188(4):54-60.

Cheesbrough, M. 2009. District Laboratory Practice in Tropical Countries. 2nd Edition. Cambridge University Press. Pp 157-160.

Chinedu, P.A., Ijeoma, E., Olusola, A., Ayobani, O.A. 2011. Polyphenolic Content and Antioxidant Activity of *Hibiscussabdariffa,*Calyx. *Research Journal of Medical Plant,* 5:557-565

Doughari, J. H., Mahmood, A. M., Tyoyina, J. 2011. Antimicrobial Activity of Leaf Extracts of *Sennsobtusifolia*. *Journal of Pelgia Research,* 9(2):34-37.

Ines, D.R., Bernd, B., Michael, H. 2014. *Hibiscus sabdariffa L.)* A Phytochemical and Pharmacological Review. *Journal Food Chemistry*, 19(12):424-445.

Llondu, E.M., Lloh, A.C. 2007. Inhibition of Three Fungal Isolates from Sorrel Drinks. zobo) Using Hurdle Technique. *World Journal of Agricultural Science*, 3(3): 341-343.

Morton, J. F. 1987. Fruits of warm climates. Florida Flair Books, Miami. Pp.286.

Oboueyaba, A.P. 2014. Phytochemical and Antioxidant Activity of Roselle (*Hibiscus sabdariffa L.*) Petal Extracts. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, 5(2):1453-1459.

Olaleye, M.T. 2007. Cytotoxicity and Antibacterial activity of Methanolic extracts of *Hibiscus sabdariffa*. *Medicinal Plant Research*, 10(1):7-13.

Onyeagba, R.A., Ugbogu, D.C., Iroakazi, O. 2004. Studies on the Antimicrobial Effects of Garlic. *Allimzingiberofficinale roscoe*) and Lime. *Citrus aurantifolis Linn*). *African Journal of Biotecnology,* 5. 3): 254-257.

Salie, F. Eagles, P. F. K. and Lens, H. M. J. 1996. Preliminary antimicrobial screening of four South African Asteraceae species. *Journal of. Ethnopharmacology*., 52(1):27-33.

Sofowora, A. 1993. Medicinal Plants and Traditional Medicine in Africa. 2nd Edition, Spectrum

Trease, G.E., and Evans, W.C. 2009. Pharmacognosy. 16th Edition. Sauders Elsevier Limited, New York. Pp 658.

Yang, M.Y., Huang, C.J., Peng, C.H., Chan, K.C., Yang, Y.S., Wang, C.J. 2005. The Hypolipidemic Effect of *Hibiscussabdariffa* Polyphenols via Inhibiting Lipogenesis and Promoting Hepetic Lipid Clearance. *Journal of Agriculture and Food Chemistry*, 58(19): 850-856

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