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Original Research Article

Assessment of Antioxidant and Polyphenol Profile of *Chrysobalanus icaco* from Southern Nigeria

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ABSTRACT

Due to the distinguished medicinal value of *Chrysobalanus icaco*, it was of great interest to conduct phytochemical and antioxidant investigations on it. In this study, the polyphenol profile and antioxidant activity of *C. icaco* methanol crude extract and fractions were investigated. The crude extract of *C. icaco* was subjected to fractionation using methanol, n-hexane, ethyl acetate, and n-butanol as solvents. 2, 2-diphenyl-1picryl-hydrazyl (DPPH), 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and ferric ion reducing power (FRAP), ascorbate oxidase, total phenolic content, total flavonoids and polyphenol profile was determined using standard methods. A significant increase was observed in the DPPH radical scavenging activity, total flavonoids, total phenol, FRAP, ABTS, TAC and ascorbate oxidase of the crude extract when compared to methanol, ethyl acetate, n-hexane, n-butanol, and aqueous fractions. Polyphenols such as kaempferol, chlorogenic acid, catechin, quercetin, galic acid, pomolic acid, rutin, epicatechin, stigmasterol, sitosterol and caffeic acid were found in the different fractions. The total polyphenols content of different fractions of *C icaco* extract were as follows; methanol fraction > n-hexane fraction > ethyl acetate fraction > n-butanol fraction > aqueous fraction. The findings revealed that *C icaco* kernel could be used as a potent source of molecules with positive health attributes.

***Keywords*:** Antioxidant; *Chrysobalanus icaco*; Methanol; Phytochemical; Polyphenol

Introduction

Plants, particularly restorative herbs, have been utilized for the avoidance and/or treatment of several illnesses since exceptionally ancient times.[1] The use of the plant as a primary source for healthcare is the highest in developing countries, which constitute about 80% of the world population. [1] Among all the chemical constituents of plants, phenolic compounds possess an imperative place with a few properties such as antioxidant action.[2] The high cost of medicines, the detachment of health care and the accessibility of therapeutic plants are pushing populaces towards home grown medication.[3]

Currently, there is an increase in the number of studies aiming to identify new sources of natural antioxidants that are important for health care benefits.[4-7] Polyphenols are of tremendous significance owing to their different organic activities, such as antimicrobial, anticoagulant, anticancer and vasodilatory impacts.[8] These activities have been ascribed to their decreasing control that relates to the portability of phenolic hydrogen atoms, which permits human cells to guard against oxygen-induced harm.[8] In expansion, numerous infections including diabetes are treated palatably and at a lower cost by medicinal plants. Radicals are involved in oxidative stress through the alteration of antioxidant defence mechanism.[9-12] However, medicinal plants and balance diet consumption remain the main sources of antioxidant phytochemicals that may fight free radical damage.[6,12-15]

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Chrysobalanus icaco (*Chrysobalanaceae*) is a medicinal plant popularly known as ''Gbofilo'' and characterized as a medium-sized shrub.[16] The kernel which shakes freely inside is removed and ground for inclusion in pepper soups. In Nigeria, the seeds of *C. icaco* are consumed in the form of soup. Its fruits are edible, and in many countries, the fruit is commonly sweet-pickled. The aim of this study was to evaluate the antioxidant activities and polyphenols contents of the methanol extract and fractions of *C. icaco.*

Materials and Methods

Collection and identification of Chrysobalanus icaco

Chrysobalanus icaco 'Gbofilo' is a large, egg-shaped fruit with a rough sandpaper-like surface (Plate 1). The fruits of the *C. icaco* were purchased from Igbudu main market Warri, Delta State, November 2019, and were identified in the Department of Plant Biology and Biotechnology, University of Benin with herbarium number of UBH-C437.

Ethical approval

Experimental protocols and procedures used in this study were permitted by the Delta State University, Faculty of Science (Abraka) (Approval No. REL/FOS/21/017).

Extraction of Chrysobalanus icaco

A portion of four hundred grams (400 g) of the pulverized Gbofilo kernels was suspended in 2 L of methanol for 48 hours in large amber bottles with intermittent shaking. At the end of the extraction, the crude methanol extract was filtered using a muslin cloth and then concentrated in a water bath maintained at 45^0C. The crude extract subjected to fractionation.

Fractionation of crude extract of C. icaco

The crude extract of *C. icaco* was subjected to liquid-liquid partition separation to separate the extract into different fractions. The crude

extract (50 g) was reconstituted with 250 ml of methanol, n-hexane, ethylacetate, n-butanol solvents and 250 ml of water 1:1 (v/v) separately in a separating funnel and then rocked vigorously. The sample was allowed to stand for 30 minutes for each of the solvents on the separator funnel until a fine separation line appear indicating the supernatant from the sediment before it was eluted sequentially. The process was repeated three times to get an adequate quantity for each fraction. The methanol, n-hexane, ethylacetate, n-butanol and the aqueous residue fractions were evaporated to dryness in a water bath to obtain five fractions respectively. Dimethyl sulfoxide (DMSO) was used as a solvent to dissolve various fractions. Antioxidant and polyphenol analyses were conducted on the fractions.

Determination of antioxidant activities in the various fractions of C. icaco extract

2,2-diphenyl-1-picrylhydrazyl

The free radical scavenging ability of the sample extracts against DPPH free radical was estimated using the method described by Ursini et al.[17] The sample (50 µg) was diluted with 3 ml ethanol and mixed with 3 ml DPPH solution. The reaction mixture was shaken, and then incubated in dark for 30 minutes. The absorbance of the solution was measured against a blank at 517 nm.

Percentage inhibition of DPPH was calculated using the following equation:

% Inhibition = [(A0 - A1)/A0] x 100

Where A0 = the absorbance of the blank sample and

A1 = the absorbance of the tested sample.

Total flavonoid assay

Total flavonoid was determined with colourimetric aluminium chloride methods as described by Ebrahimzadeh *et al.*[18] Five millilitres (5 ml) of 2% $AlCl_3$ in methanol was mixed with 0.2 ml of sample. Absorption was read at 415 nm after 10 min against a blank sample consisting of 5 ml extract solution with 5 ml methanol without $AlCl_3$.

Total phenolic content

The determination of total phenolic content was carried out according to the method described by Dewanto *et al.*[19] Zero point five millilitres (0.5 ml) of the sample was dissolved in 100 µl of Folin-Ciocalteau reagent and 6 ml of distilled water. It was vortexed for 1 minute, 2 ml of 15% Na_2CO_3 was added and the mixture vortexed once again for thirty seconds (30 seconds). The solution was made up to 10 ml with distilled water. After 1 hour, thirty minutes, the absorbance of the samples was read at 750 nm with a UV spectrophotometer.

Ferric reducing antioxidant power assay

The ability to reduce ferric ions was measured using the method described by Benzie and Strain.[20] The FRAP reagent was prepared by mixing 300 mM sodium acetate buffer (pH 3.6), 10.0 mM TPTZ (tripyridyltriazine) solution and 20.0 mM $FeCl_3.6H_2O$ solution in a ratio of 10:1:1 in volume. Precisely 0.5 ml of samples at different concentrations was then added to 3 ml of FRAP reagent. The reaction mixture was incubated at 37°C for 30 min. The increase in absorbance at 593 nm was measured.

Ferric reducing antioxidant power = (A1 – A2)/A1] x 100

Where; A1 is the absorbance of the control. A2 is the absorbance of the sample.

ABTS radical scavenging activity

ABTS radical-scavenging activity was determined according to the method described by Re *et al.* [21] Sample solution (1ml) was added to 9 ml ABTS+ solution, and the mixture was allowed to stand at room temperature for 6 minutes. Thereafter, the absorbance of the sample and blank were recorded at 734 nm. The inhibition percentage was calculated using the formula below.

ABTS scavenging activity = (A0 - A1)/A0] x 100

Where; A0 is the absorbance of the control. A1 is the absorbance of the sample.

Total antioxidant capacity (TAC)

The Total antioxidant capacity of the samples was estimated by the method described by Prieto *et al.* [22] Sample (0.1 ml) was added to1 ml of reagent solution (28 mmol/L Na_3PO_4, 4 mmol/L ammonium molybdate and 0.6 mol/L H_2SO_4) in test tubes. The tubes were incubated in a thermal block at 95ºC for 90 min. The mixture was allowed to cool at room temperature. The absorbance was measured at 695 nm against blank. Antioxidant capacity was stated as mg gallic acid equivalent per gram dry weight (mg GAE/g DW).

Assay of ascorbate oxidase activity

The assay of ascorbate oxidase activity was carried out using the method of Vines and Oberbacher.[23] The samples were mixed [1:5 (v/v)] with phosphate buffer (0.1 M/ pH 6.5) and centrifuged at 3000 g for 15 min at 5ºC. The supernatant obtained was used as the enzyme source. The enzyme extracts (0.1 ml) were added to 3.0 ml of the substrate solution (8.8 mg ascorbic acid in 300 ml phosphate buffer, pH 6.5) and the change in absorbance was determined at 265 nm in every 30 seconds for a period of 4 minutes. The enzyme activities were expressed as U/ml. One unit is the amount that produce1 µmole of dehydroascorbic acid per minute.

Determination of polyphenols

The polyphenols were determined using high-performance liquid chromatography (HPLC) as described by Seal. [24] The mobile phase contains 1% aqueous acetic acid solution (Solvent A) and acetonitrile (Solvent B), and 2ml/min was adjusted as the flow rate. The column was controlled at 28^0C and the sample injection volume was 5 µL. The total analysis time per sample was 65 minutes. The HPLC chromatograms were spotted using a photo diode array UV detector at three dissimilar wavelengths: 272, 280 and 310 nm according to compounds analysed. The Compounds were identified based on their retention time and by spiking with the standards under the same conditions. Sample quantification was done by measuring the integrated peak area and the content was calculated. Total polyphenols and individual polyphenols content were calculated as follows;

$$\text{Concentration of polyphenols (mg/g)} = \frac{\text{Peak area}}{\text{Standard peak area}} \times \text{Standard concentration}$$

Standard conc. = 19.20 mg/g, Standard peak area= 4388.1550 mg/g

Individual polyphenol standard conc. = 1.745 mg/g, and individual polyphenol standard peak area= 398.9231mg/g.

Statistical analysis

All the results were expressed in means bars and all data were analyzed using analysis of variance (ANOVA). Significant difference between means were determined at $p < 0.05$ confidence level using Least Significant Difference (LSD).

Results and Discussion

The results in Figure 1-7 showed that DPPH radical scavenging activity, flavonoids, total phenol, FRAP, ABTS, TAC and ascorbate oxidase of the crude extract was significantly higher compared to methanol, ethyl acetate, n-hexane, n-butanol, water fraction and BHT. However, as the concentration of the sample increases (100 -400 µg/ml) the antioxidant properties increases. Trends of the antioxidants (flavonoids, total phenol, FRAP, ABTS, and ascorbate oxidase) were as follows; crude extract > methanol fraction > n-hexane fraction > n-butanol fraction > ethyl acetate fraction > water fraction. DPPH and TAC had the following trends: crude extract > methanol fraction. In the present study, the polarity of solvents plays a vital role in the extraction process. Interestingly, whenever charged species are present in the extraction medium the reaction barrier is highly dependent on the polarity of the solvent.[25] Solvent may help in the stabilization of the reactants to increase the reactivity of the extraction process.[1]

The polyphenols content of *C icaco* methanol fraction (Figure 8) were as follows: kaempferol > chlorogenic acid > catechin > quercetin >

galic acid > pomolic acid > rutin > epicatechin >stigmasterol > sitosterol > caffeic acid. *C icaco* n-hexane fraction polyphenols (Figure 9) were: kaempferol >chlorogenic acid > catechin > quercetin > galic acid > pomolic acid > epicatechin > rutin > stigmasterol. Figure 10 illustrates the polyphenol content of *C icaco* ethyl acetate fraction and they were given as follows: kaempferol > chlorogenic acid > catechin > quercetin > galic acid >pomolic acid > epicatechin > stigmasterol >rutin. *C icaco* n-butanol fraction polyphenol content was: chlorogenic acid > kaempferol >catechin > quercetin>galic acid > pomolic acid> caffeic acid (Figure 11). The water fraction of *C icaco* polyphenols had the following trends: chlorogenic acid > kaempferol > quercetin > catechin > galic acid (Figure 12).The total polyphenols content of different fractions of *C icaco* extract were as follows; methanol fraction > n-hexane fraction > ethyl acetate fraction> n-butanol fraction > water fraction (Figure 13). The increase in chlorogenic acid and kaempferol when compared with other polyphenol compounds observed in this study could be a result of their polar hydroxyl group. Studies have shown that polarity increases extraction yield, antioxidant activity [26], and free radical scavenging activity of plant extracts and this may be attributed to the high affinity of polyphenol compounds towards more polar solvents as compared to non-polar ones.[1]

Plate 1: Picture of *Chrysobalanus icaco*

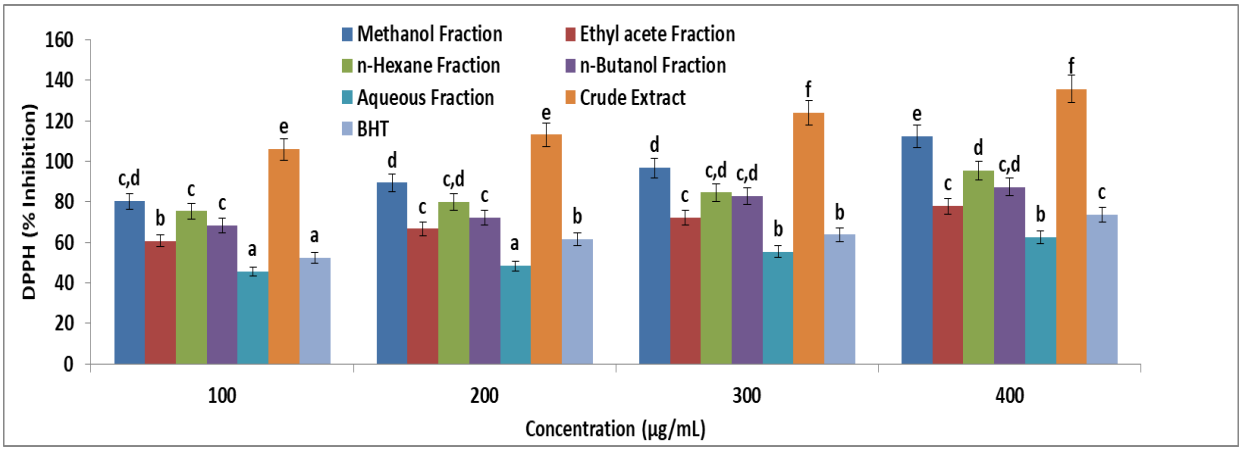

Figure 1: DPPH radical scavenging activity of C. icaco different fractions and crude extract.
Bars represent means of triplicates values. Bars with different alphabet (a-e) differ at p<0.05.

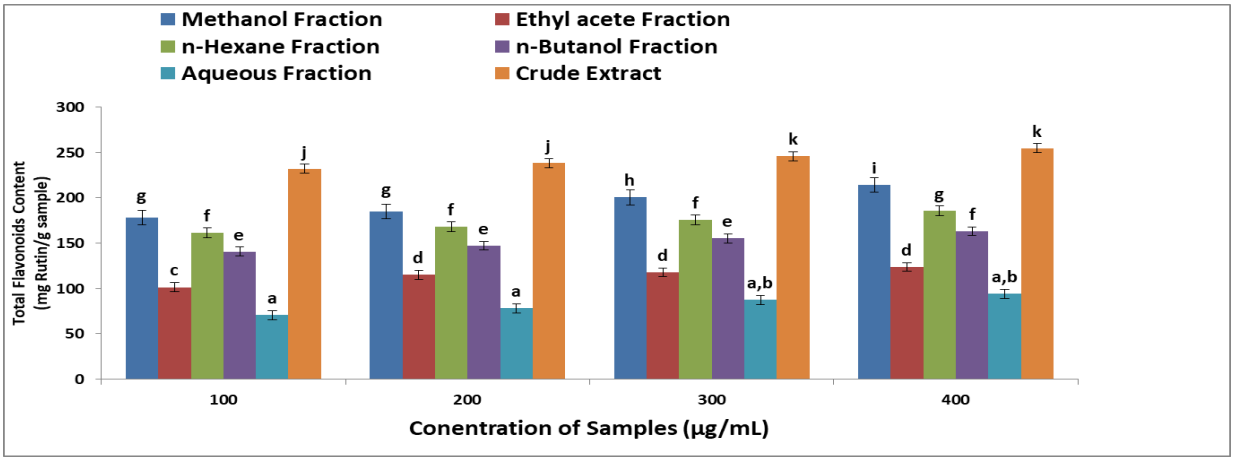

Figure 2: Total flavonoids of *C icaco* different fractions and crude extract. Triplicates values were represented in mean bars. Bars with different scripts (a-k) differ at p<0.05

Plant polyphenols are secondary metabolites recognized by one or more hydroxyl groups authoritative to one or more aromatic rings. A few thousand polyphenolic atoms have been considered in higher plants, including consumable ones.[1] Plant polyphenols are classified into two major groups, flavonoids and non-flavonoids. Examples of flavonoids are: flavanols, flavonols, anthocyanidins, flavones, flavanones, and chalcones. Non-flavonoids include stilbene, phenolic acids, saponins, and tannins. Studies have appeared that plant polyphenols can be utilized as antioxidants against distinctive oxidative stress-induced diseases. [27,28]

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Conclusion

Based on the present results, extract and fractions of *C icaco* exhibited high quantities of powerful antioxidant activities. The HPLC analysis of the fractions provided a tentative identification of major polyphenols. The presence of these polyphenols metabolites is probably the main contributor to the antiradical capacity of *C icaco*

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kernel, as well as supporting its uses in folk medicine to treat certain illnesses related to oxidative stress.

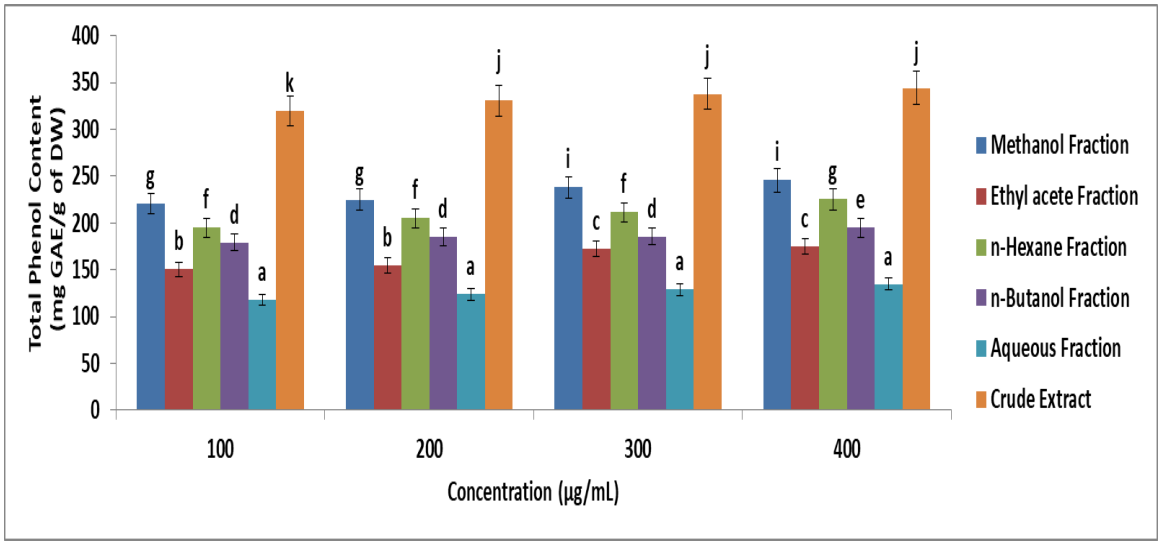

Figure 3: Total phenol content of *C icaco* different fractions and crude extract . Triplicates values were represented in mean bars. Bars with different letters (a-k) differ at $p<0.05$.

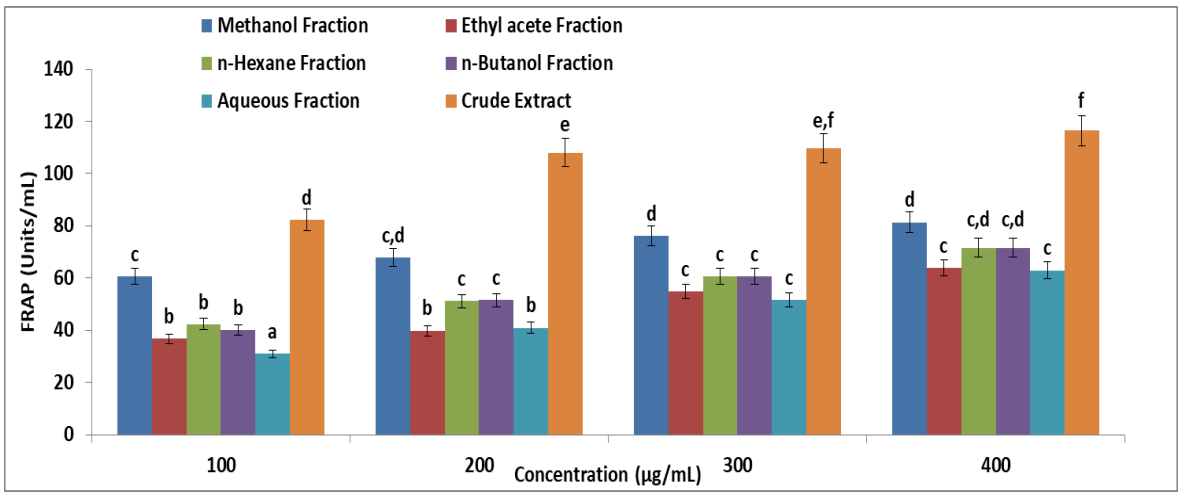

Figure 4: FRAP of *C icaco* different fractions and crude extract. Values (triplicates) were represented in mean bars. Bars with different alphabet (a-f) differ at $p<0.05$.

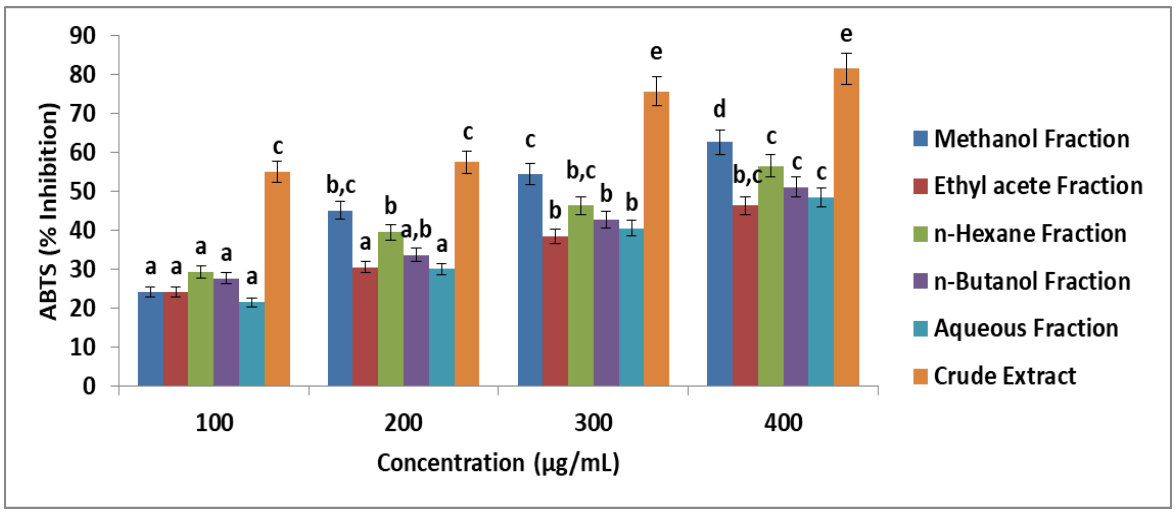

Figure 5: ABTS of *C icaco* different fractions and crude extract. Triplicates values were represented in mean bars. Bars with dissimilar letters (a-e) differ at p<0.05.

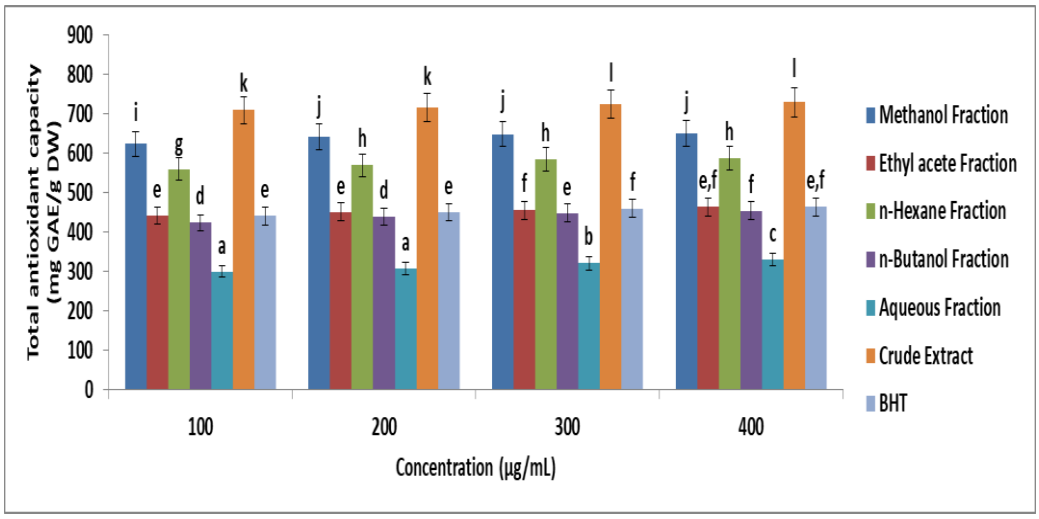

Figure 6: Total antioxidant activities of *C icaco* different fractions and crude extract. Triplicates values were represented in mean bars. Bars with unlike alphabet (a-k) differ at p<0.05.

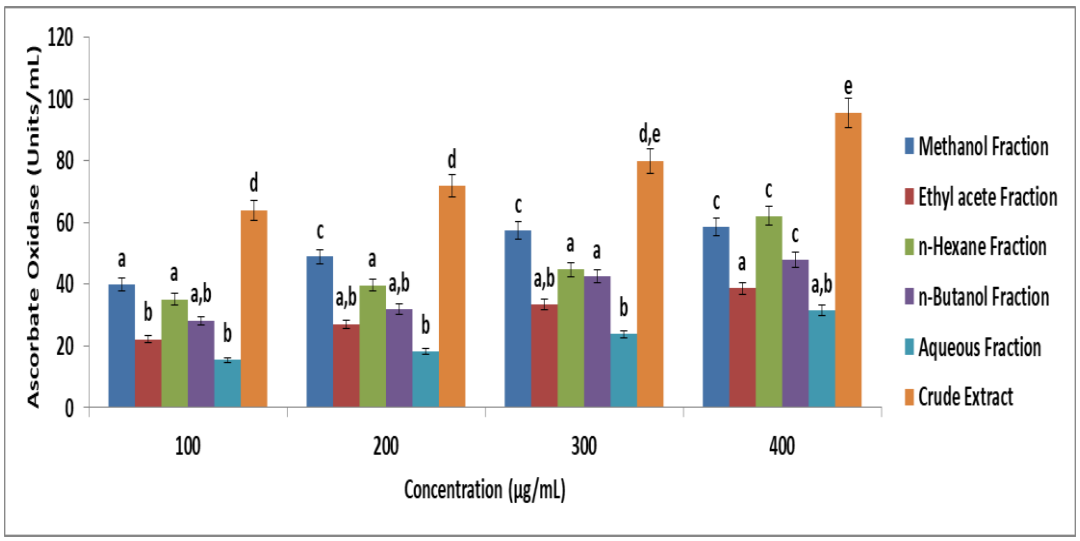

Figure 7: Ascorbate oxidase activity of *C icaco* crude extract and different fractions. Triplicates values were represented in mean bars. Bars showing different character sets (a-e) differ at p<0.05

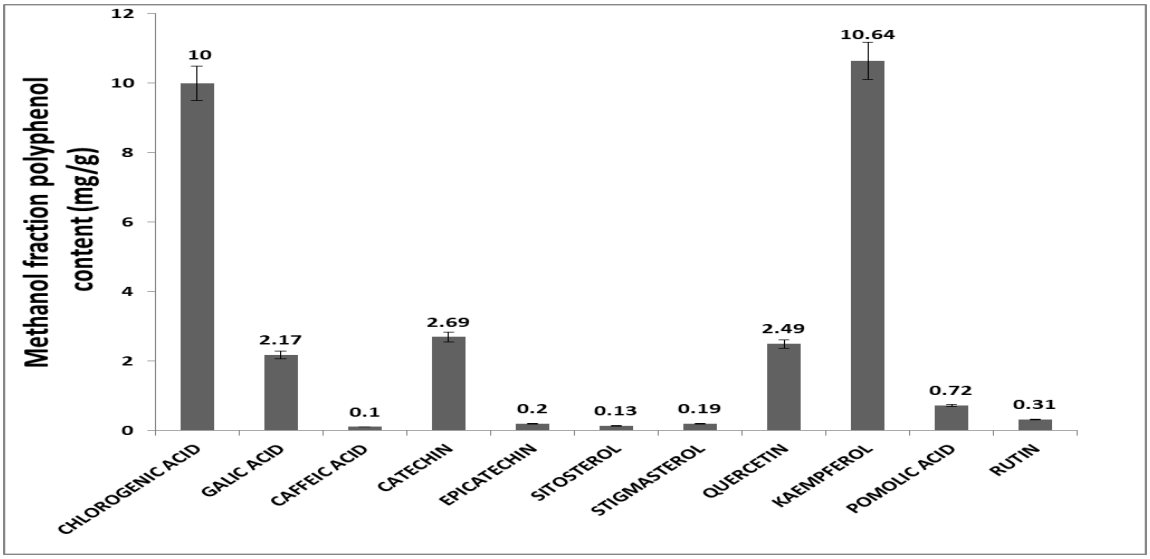

Figure 8: The polyphenol content of *C icaco* methanol fraction

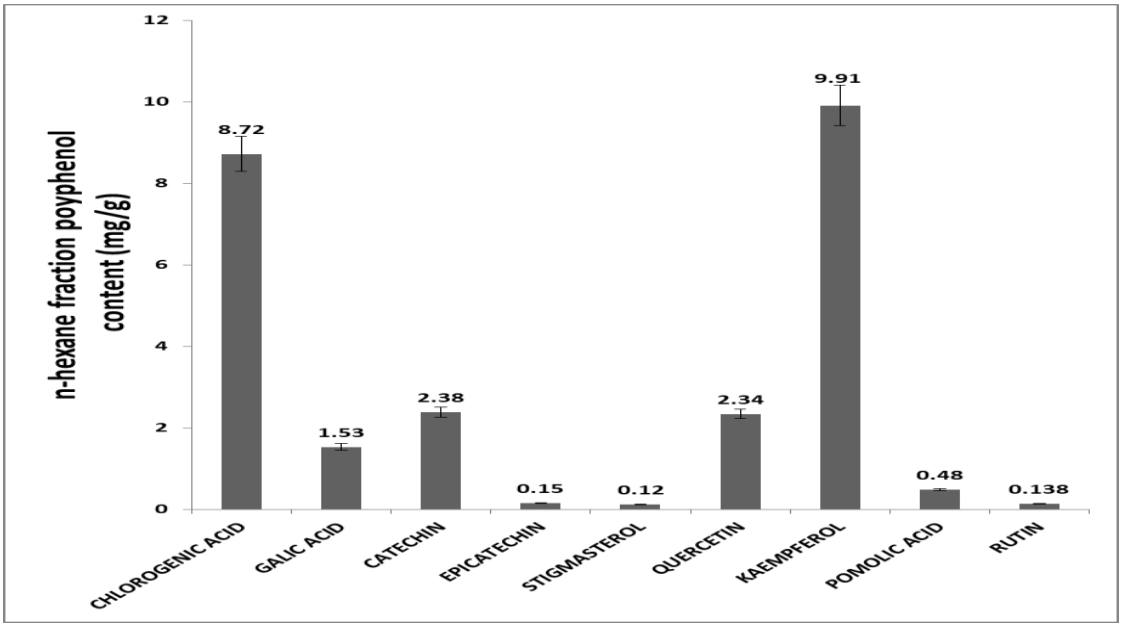

Figure 9: The polyphenol content of *C icaco* n-hexane fraction

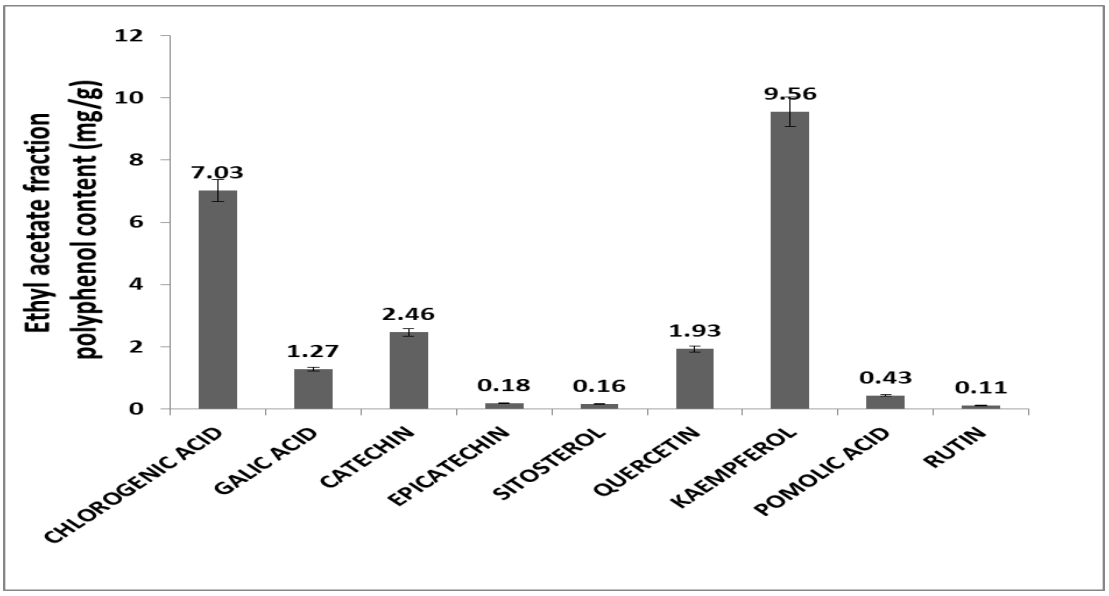

Figure 10: The polyphenol content of *C icaco* Ethyl acetate fraction

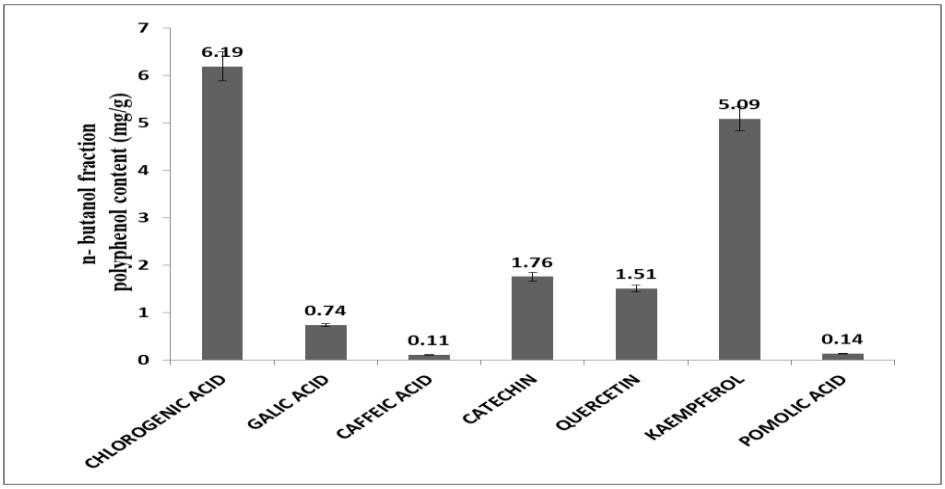

Figure 11: The polyphenol content of *C icaco* n-butanol fraction

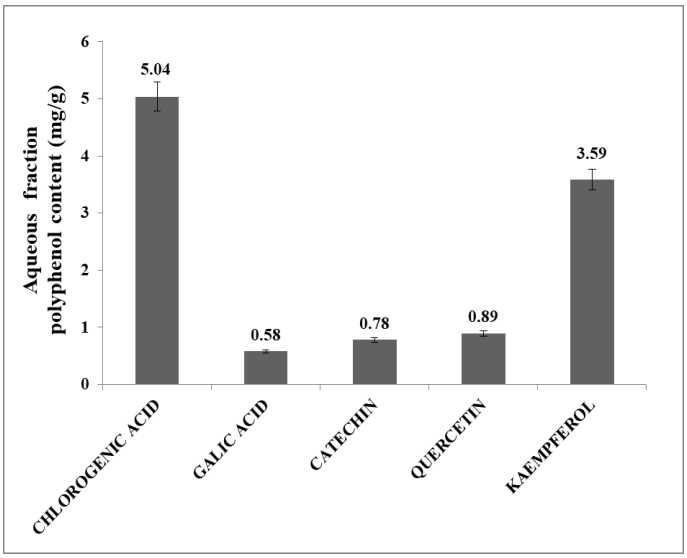

Figure 12: The polyphenol content of *C icaco* aqueous fraction

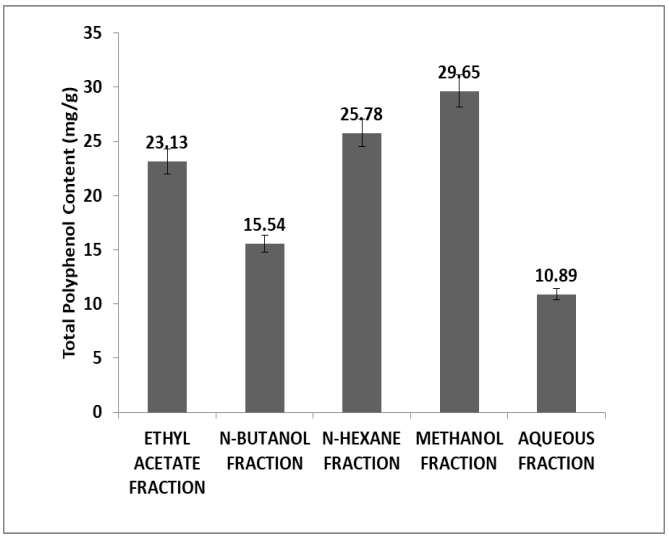

Figure 13: Total polyphenol content of different fractions of *C icaco* extract.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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