Impact of Processing and Extraction Methods on the Phytochemical Composition of Garcinia Kola Stem Bark and Stone Breaker Leaves

Abstract

This study investigates the effects of three processing methods; blanching, drying, and fermentation on the phytochemical properties of *Garcinia* kola stem bark and stone breaker leaves, both of which are traditionally used in medicine. The primary aim was to evaluate how these processing techniques enhance the phytochemicalprofiles of these plant materials and to determine the most effective extraction method for retaining their beneficial properties, particularly for cardiovascular health. The contents of saponins, flavonoids, oxalates, phytates, tannins, and phenolic compounds in aqueous and ethanolic extracts were quantified. The results demonstrated that the fermented samples exhibited the highest flavonoid content (3.90 mg/g in fermented stone breaker ethanolic extract (FSBE) and 4.17% in fermented stone breaker aqueous extract (FSBA)) and phenolic content (75.29 mg/g in FSBA and 75.92 mg/g in FSBE). Furthermore, fermentation yielded the lowest oxalate (1.35 mg/g in fermented *Garcinia* kola stem bark aqueous extract (FGBA) and 1.84 mg/g in fermented *Garcinia* kola stem bark ethanolic extract (FGBE)) and phytate levels (20.92 mg/g in FSBA and 22.75 mg/g in FSBE). In contrast, the dried samples contained the highest saponin levels ranging from 0.09% in blanched stone breaker ethanolic extract (BSBE) to 1.34% in blanched *Garcinia* kola stem bark aqueous extract (BGBA). Tannin content was consistently low across all samples, ranging from 0.01 to 0.05 mg/g. While aqueous extracts displayed higher values for some components, ethanolic extracts outperformed in others. The findings underscore the health benefits of these plant materials, particularly highlighting the superiority of fermented samples due to their enriched flavonoid and phenolic content, which may significantly contribute to overall well-being, especially for cardiovascular patients.

Keywords; Phenolic, Phytochemical, Extracts, Flavonoid, Cardiovascular

1. Introduction

“Plants are vital sources of nutrition for both humans and animals, offering a variety of primary and secondary metabolites. While primary metabolites are essential for plant growth and development, secondary metabolites, though not directly involved in the plant's internal functions, are significant due to their unique chemical properties” (Stone & Williams, 1992; Delgoda & Murray, 2017). “These secondary metabolites provide substantial nutritional and medicinal benefits, exhibiting antibiotic, antifungal, antioxidant, anti-inflammatory, and antiviral activities. Consequently, they have extensive applications in the pharmaceutical, food, cosmetics, and fine chemical industries” (Prasad *et al.*, 2015; Bor *et al.*, 2016; Widelski & Kukula-Koch, 2017; Shields, 2017; Pan *et al.*, 2018; Dhama *et al.*, 2018; Kallscheuer *et al.*, 2019). “Medicinal

plants have long played a crucial role in traditional medicine across various cultures, primarily due to their bioactive compounds such as saponins, flavonoids, tannins, and phenols, which contribute to their therapeutic effectiveness. *Garcinia* kola, commonly known as bitter kola, and *Phyllanthus niruri*, known as stone breaker, are widely used in African traditional medicine for treating liver disorders, infections, and cardiovascular diseases" (Iwu *et al.,* 1999; Akinmoladun *et al.,* 2007). "*Garcinia* kola is especially known for its anti-inflammatory, antimicrobial, and antioxidant properties, with bioactive compounds like saponins and flavonoids contributing to its health benefits" (Shraim *et al.,* 2021). "Likewise, *Phyllanthus niruri* has traditionally been used to treat ailments such as jaundice, kidney stones, and diabetes, thanks to its potent antioxidant and hepatoprotective properties" (Calixto *et al.,* 1998). Processing methods, including blanching, drying, and fermentation, have been shown to significantly influence the retention and bioavailability of these phytochemicals. Fermentation, for example, has been found to enhance the phenolic content and antioxidant activity of plant materials (Hur *et al.,* 2014), while drying, a common preservation technique, can concentrate certain nutrients but may also degrade sensitive compounds like flavonoids (Andrade *et al.,* 2021). "Blanching, commonly used to inactivate enzymes, can either increase or decrease phytochemical content depending on the conditions and plant material involved" (Duarte *et al.,* 2015). Considering the vital role of these compounds in promoting cardiovascular health and offering therapeutic benefits, understanding how different processing methods impact their concentration and activity is essential. This study evaluates the effects of blanching, drying, and fermentation on the saponin, flavonoid, tannin, phenolic, oxalate, and phytate contents of *Garcinia* kola stem bark and *Phyllanthus niruri* (stone breaker) leaves. The results will contribute to optimizing traditional remedies and improving extraction methods for pharmaceutical and nutraceutical applications.

2. Materials and Methods

2.1 Source of Materials

*Garcinia*kola (*Orogbo* bark) was obtained from Danjuma, Akure, Ondo State, Nigeria while *Phylanthus niruri* (Stone Breaker) leaves were obtained from Ijapo Estate, Akure, Ondo state, Nigeria. The raw materials were identified and authenticated in the Department of Crop Science and Pest Management, Federal University of Technology, Akure, Ondo State, Nigeria.Solvents, standards and reagents of analytical grade were used. Purified deionized water was prepared with the Milli-Q® water purification system (Millipore, Arlington, MA, USA).

2.2 Sample Preparation

Samples were prepared using the method described by Origbemisoye and Ifesan (2019). Briefly, the raw samples, *Garcinia* kola stem bark and Stone breaker leaves, were sorted and then washed under running water prior to further processing. They were subjected to three different processing and two extraction (Aqueous and Ethanol) methods—blanching, drying, and fermentation—and combined in different ratios to form eighteen samples. For the first treatment,

the samples were dipped in water for 5 minutes and wrapped in foil paper for two days at room temperature for fermentation. After fermentation, the samples were unwrapped for further processing. For the second treatment, the samples were blanched for 2 minutes at 88 °C, and the water was then drained for further processing. The third treatment involved drying the samples only. All treated samples were dried in a Quincy Lab oven (Model T9FB918776) at 50 °C for 96 hours, milled into fine powder using a Hamilton Beach blender (Model 58180C), and placed in vacuum-sealed bags prior to further processing. They were then labeled as follows: Sample 1, blanched *Garcinia* kola stem bark; Sample 2, blanched stone breaker leaves; Sample 3, a mixture of 50% blanched *Garcinia* kola stem bark and 50% blanched stone breaker leaves; Sample 4, dried *Garcinia* kola stem bark; Sample 5, dried stone breaker leaves; Sample 6, a mixture of 50% dried *Garcinia* kola stem bark and 50% dried stone breaker leaves; Sample 7, fermented *Garcinia* kola stem bark; Sample 8, fermented stone breaker leaves; and Sample 9, a mixture of 50% fermented *Garcinia* kola stem bark and 50% fermented stone breaker leaves.

The extraction process of the processed plant materials utilized purified water and 70% ethanol (v/v) with a raw material-to-extractant ratio of 1:10, based on Zhang *et al*. (2018). Extracts were derived from blanched, dried, fermented, and blended forms of Stone breaker leaves and *Garcinia* kola stem bark. For infusions and decoctions, processed plant material in powdered form was mixed with measured water. Infusions were prepared by pouring boiling water over the material, steeping for 15 minutes, then cooling for 45 minutes, while decoctions involved heating the plant material in cold water to boiling, followed by a 30-minute simmer and a 15-minute cool-down, in line with the European Pharmacopoeia (2007). Both were filtered using ashless filter paper (ash content 0.007%, retention 8–12 μm, 90 mm diameter). Macerates were created by combining the plant blend with a solvent, allowing aqueous macerates to steep for 5 days at 5 °C in darkness and ethanol macerates for 7 days at room temperature in darkness. Ultrasonic extraction was conducted in a bath at 45 °C for 60 minutes. All extracts were refrigerated overnight at 5 °C to settle, followed by filtering to remove ballast and macromolecular compounds, yielding purified plant extracts for further study. They were then labeled as follows: Sample 1, Blanched *Garcinia* Kola Stem Bark Aqueous Extract (BGBA); Sample 2, Blanched *Garcinia* Kola Stem Bark Ethanolic Extract (BGBE);Sample 3, Blanched Stone Breaker Aqueous Extract (BSBA); Sample 4, Blanched Stone Breaker Ethanolic Extract (BSBE); Sample 5, 50% BGBA& BSBA; Sample 6, 50% BGBE& BSBE; Sample 7, Dried *Garcinia* Kola Stem Bark Aqueous Extract(DGBA); Sample 8, Dried *Garcinia* Kola Stem Bark Ethanolic Extract (DGBE); Sample 9, Dried Stone Breaker Aqueous Extract (DSBA); Sample 10, Dried Stone Breaker Ethanolic Extract (DSBE); Sample 11, 50% DGBA&DSBA; Sample 12, 50% DGBE& DSBE; Sample 13, Fermented *Garcinia* Kola Stem Bark Aqueous Extract (FGBA); Sample 14, Fermented *Garcinia* Kola Stem Bark Ethanolic Extract (FGBE); Sample 15, Fermented Stone Breaker Aqueous Extract (FSBA); Sample 16, Fermented Stone Breaker Ethanolic Extract (FSBE); Sample 17, 50% FGBA& FSBA; Sample 18, 50% FGBE& FSBE.

2.3 Determination of phytochemical composition of processed *Garcinia* kola stem bark, Stone breaker leaves and their blends extracts.

2.3.1 Qualitative Analysisof processed *Garcinia* kola stem bark, Stone breaker leaves and their blends extracts.

2.3.1.1. Saponin determination

The ability of saponin to produce frothing in aqueous solution was used as a screening test for saponin. About 0.5g of extract was shaken with distilled water in a test tube frothing which persist on warming was taken as preliminary evidence for the presence of saponin (Gowri and Vasantha, 2010).

2.3.1.2 Tannin determination

About 0.5g of the flourextract was stirred with 100ml of distilled water, filtered and ferric chloride reagent was added to the filtrate a blue, black green or blue green precipitate was taken as evidence for presence of tannin (Gowri and Vasantha, 2010).

2.3.1.3 Phlobatannin determination

Deposition of red precipitate when 0.5g of the flour extract was boiled with 1% aqueous HCl was taken as evidence for the presence of phlobatannin (Tiwari *et al.*, 2011).

2.3.1.4 Anthraquinone determination

Borntrager's test was used for the detection of Anthraquinone. About 0.5g of the flour extract was shaken with 10ml of benzene, filtered and 5ml of 10% ammonia solution added to the filtrate. The mixture was shaken and the presence of pink, red or violet colour in the ammonia layer indicates the presence of free Anthraquinone (Gowri and Vasantha, 2010).

2.3.1.5 Flavonoid determination

About 0.5g of the flour extract was stirred with 20ml of dilute ammonia solution a yellow colouration was observed, the disappearance of the yellow colour after the addition of 1ml conc. H_2SO_4 indicates the presence of flavonoid (Tiwari *et al.*, 2011).

2.3.2 Quantitativeof processed *Garcinia* kola stem bark, Stone breaker leaves and their blends extracts.

2.3.2.1Determination of saponin content

The method described by Obadoni and Ochuko (2001) with slight modification was used to determine the saponin content. The prepared ethanolic and aqueous extracts were obtained by soaking the plant material in 70% ethanol and distilled water, respectively, in a ratio of 1:10. This was done over 72 hours with periodic shaking. The extracts were evaporated to dryness and dissolved in diethyl ether to remove fat. The remaining residue (defatted extract) was further processed. The defatted extract was treated with 20% ethanol, and the mixture was heated to 55 °C for 4 hours, followed by filtration. The filtrate was then concentrated and subjected to petroleum ether to remove pigments and other unwanted compounds. The aqueous layer was collected and evaporated to dryness. The residue (saponins) was weighed. The saponin residue was mixed with a known volume of distilled water and sulfuric acid (0.5 ml concentrated H□SO□ was added to the solution). The mixture was allowed to stand for 10 minutes, followed

by heating in a water bath for 10 minutes at 60 °C. After cooling, absorbance was measured using a spectrophotometer at 550 nm. The saponin content was calculated by comparing the absorbance to a saponin standard curve. The results were expressed as the percentage of saponin relative to the total sample weight.

2.3.2.2 Determination of Flavonoids

The method described by Chang *et al*. (2002), with slight modifications, was used to determine the flavonoid content. About 0.5 mL of the extract was mixed with 1.5 mL of methanol, 0.1 mL of 10% aluminum chloride solution, 0.1 mL of 1M potassium acetate, and 2.8 mL of distilled water, making a total volume of 5 mL. The mixture was vortexed and incubated at room temperature for 30 minutes. After incubation, absorbance was measured at 415 nm using a UV-Visible spectrophotometer against a methanol blank. A standard quercetin curve (50–300 µg/mL) was prepared under the same conditions. The total flavonoid content was expressed as a percentage of quercetin equivalents per gram of dry weight (% QE/g DW). The results were calculated by comparing the absorbance of the samples to the quercetin standard curve and then converting the concentration into a percentage based on the weight of the dried plant material used.

2.3.2.3Determinationof Oxalate content

The method described by Ukpabi and Ejidoh (1989) was used. 5 ml of samples was digested with 10 ml 6 M HCl for one hour and made up to 250 ml in a volumetric flask. The pH of the filtrate was adjusted with concentrated NH_4OH solution until the colour of the solution changed from salmon pink colour to a faint yellow colour. Thereafter, the filtrate was treated with 10 ml of 5% $CaCl_2$ solution to precipitate the insoluble oxalate. The suspension was centrifuged at 2500 x g, after which the supernatant was decanted. The precipitate was dissolved in 10 ml of 20% (v/v) H_2SO_4 and the solution was made up to 300 ml. An aliquot (125 ml) was heated until near boiling point and then titrated against 0.05 M standardized $KMnO_4$ solution to a faint pink colour which persisted for about 30 seconds after which the burette reading was taken and used to estimate the oxalate content.

$$Oxalate\ (mg/g) = \frac{(titre\ value\ x\ volume\ of\ KMnO_4\ x\ dilusion\ factor)/5}{Sample\ size} \qquad \text{Eqn. 2.1}$$

2.3.2.4Determination of Phytate Content

The determination of phytate in sample was done using the method described by Abulude (2004). 5 ml of the extract samples was dispersed in 200 ml of 2% HCl and extracted. Following extraction, the dispersion was filtered and 50 ml of the filtrate was mixed with 10 cm^3 of 0.3% ammonium cyanide (NH_4SCN) and diluted with 107 ml of distilled water. The extract was titrated against 0.00195 g/ml of Ferric chloride solution until a brownish yellow colour persisted. Phytate content was estimated with the expression:

$$Phytate\ Phosphorous = (Iron\ equivalent\ x\ 1.95\ g\ of\ titre)\ x\ 3.65\ g \qquad \text{Eqn. 2.2}$$

2.3.2.5 Determination of tannin content

The method described by Porter *et al.* (1986) with slight modifications was used to determine the tannin content. The extracts were filtered through filter paper into a 50 mL volumetric flask and evaporated to dryness using a rotary evaporator at a temperature not exceeding 40 °C. The dried extract was reconstituted in a known volume of distilled water to a final volume of 50 mL. A series of standard tannic acid solutions (0, 10, 20, 30, 40, 50 mg/L) were prepared for calibration. In a 100 mL beaker, 1 mL of the extract or standard solution was mixed with 5 mL of Folin-Denis reagent and 4 mL of distilled water, allowing the mixture to stand for 30 minutes at room temperature. Afterward, 10 mL of 1 M sodium carbonate solution was added to the mixture, which was left to react for an additional 30 minutes at room temperature. The absorbance of the resulting blue color was measured at 725 nm using a UV-Visible spectrophotometer, with distilled water as a blank. The tannin content was calculated based on a calibration curve plotted from the absorbance against the concentration of tannic acid standards. The results were expressed as mg of tannin equivalents per gram of dry weight (mg TAE/g DW) of the plant material.

2.3.2.6Determination of Total phenol content

The total phenol content (TPC) was determined by Folin–Ciocalteu assay (Singleton *et al.,* 1999) using gallic acid as standard. Fifty microliter of the extract solution containing 0.5 mg of aqueous extract was dispensed into a test tube, 50 µl of distilled water and 500 µl of Folin–Ciocalteu reagent was added respectively into the test tube and shaken thoroughly.After 3 min, 400 µl of 7.5% sodium carbonate solution was added and the mixture was incubated at 45 ^{o}C in a water bath for 40 min. Absorbance was measured at 765 nm against blank. The same procedure was repeated to all standard gallic acid solution (0.1 mg/ml). The blank is a mixture of 100 µl of distilled water, 500 µl of Folin-Ciocalteu reagent and 400 µl of 7.5% sodium carbonate. The total phenolic content was expressed as gallic acid equivalent per gram of sample (mg of GAE/g sample) through the calibration curve of gallic acid and calculated as follows;

$$Total\ phenolic\ content\left(mgGAE/g\right) = \frac{Abs_{\cdot sample} \text{x}\ Conc_{\cdot standard}\left(\frac{mg}{ml}\right)}{Abs_{\cdot standard} \text{x}\ Conc_{\cdot sample}\left(\frac{mg}{g}\right)} \qquad \text{Eqn. (2.3)}$$

2.4 Statistical Analysis

Results were expressed as the means of three separate determinations. The data were subjected to analysis of variance (ANOVA) using the statistical package for social statistics (SPSS version 13).

3. RESULTS AND DISCUSSION

3.1 Phytochemical Constituents of Processed *Garcinia* Kola Stem Bark, Stone Breaker Leaves Flour, its blends, and their extracts.

3.1.1 Phytochemical screening of Processed *Garcinia* Kola Stem Bark, Stone Breaker Leaves Flour, its blends, and their extracts.

The result of the preliminary phytochemical screening carried out on the methanolic extracts of all the samples in order to know the phytochemicals that were present in the samples before carrying out their qualitative analysis is presented in Table 1a-1c. The results revealed the presence of a wide range of phyto-constituents which include; saponins, flavonoids, Oxalate, Phytate, tannin and phenol were found to be present in all the samples, while phlobatanninand anthraquinone were absent in all of them.

3.1.2 Phytochemical composition of processed *Garcinia* Kola Stem Bark, Stone Breaker Leaves Flour, its blends, and their samples extracts.

3.1.2.1 Saponin content of blanched, dried and fermented *Garcinia* Kola Stem Bark, Stone Breaker Leaves Flour, its blends, and their extracts samples.

The saponin concentration in the six sample extracts was quantitatively determined and is summarized in Fig. 1a-1c. Statistical analysis revealed a significant difference ($p<0.05$) among the samples, with values ranging from 0.09% in Blanched Stone Breaker Ethanolic (BSBE) to 1.34% in Blanched *Garcinia* Kola Stem Bark Aqueous (BGBA). The blanched samples generally had lower saponin content than the dried samples, which ranged from 0.19% in DSBE to 2.77% in DGBA. Similarly, the fermented samples showed saponin levels between 0.19% in FSBE and 3.07% in FGBA. The high saponin content observed in the aqueous fermented samples could be due to its polarity, as polar compounds are known to extract well in aqueous solutions. The high values observed in the fermented samples could be attributed to microorganisms capable of modifying plant constituents. During fermentation, these microorganisms release chemically bound compounds, enriching the plant samples with phytochemicals that have enhanced bioavailability and bioactivity. This process also alters the ratio of nutritive to anti-nutritive components in the plants (Yeo and Ewe, 2015). Comparatively, the saponin content found in this study is significantly higher than the report by Moneim *et al.* (2019), who reported 0.25±0.01%, 2.31±0.00%, 2.35±0.01%, and 2.41±0.01% for aqueous extract, ethanol extract, methanol extract, and ethyl acetate extract respectively of *Jatropha curcas* leaf. Saponins are characterized by a bitter taste and foaming properties, and are poorly absorbed from the digestive tract in humans.

3.1.2.2 Flavonoid content of blanched, dried and fermented *Garcinia* Kola Stem Bark, Stone Breaker Leaves Flour, its blends, and their extracts samples.

The concentration of flavonoids found in the sample extracts was quantitatively determined, as shown in Figure 2a-2c. Results revealed a significant ($P<0.01$) difference between the samples. Notably, the fermented samples had the highest flavonoid content, ranging from 2.33% in FGBE to 4.17% in FSBA, followed by the dried samples, which ranged from 2.44% in DGBE to 3.90% in DSBA. The blanched samples had the lowest flavonoid content, ranging from 1.18% in BGBE to 1.88% in BSBE. Generally, it was observed that the aqueous stone breaker samples had the highest flavonoid values (1.88% in BSBA to 4.17% in FSBA) across all processing methods, while the ethanolic extract of *Garcinia* kola stem bark samples had the lowest values (1.18% in FGBA to 2.33% in FGBE). The high values observed in the fermented samples are of significant importance as flavonoids are known for their antioxidant properties, scavenging free radicals, reducing oxidative stress, and protecting cells from damage (Origbemisoye and Ifesan, 2019). Comparatively, the values observed in this study are lower than the results of Emmanuel *et al.*, (2022), who reported the aqueous and ethanolic extracts from *Boerhavia diffusa* L. and *Lonchocarpus sericeus* (Poir.) Kunth ex DC. leaves to be 14.14%-21.05% in ethanolic and 7.11%-12.00% in aqueous extracts, respectively.

3.1.2.3 Oxalate content of blanched, dried and fermented *Garcinia* Kola Stem Bark, Stone Breaker Leaves Flour, its blends, and their extracts samples.

Fig. 3a – 3c shows that the highest amount of oxalate was observed in the dried samples ranging from 1.92 mg/g in sample DGBE to 7.96 mg/g in sample DSBA followed by the blanched samples 1.80 mg/g in BGBE to 7.50 mg/g in BSBA while the fermented samples had the least value (1.35 mg/g in FGBA to 6.75 mg/g in FSBE), respectively. The results indicate that blanched samples, such as BSBA (Blanched Stone Breaker Aqueous) with 7.50 mg/g and BSBE (Blanched Stone Breaker Ethanol) with 7.41 mg/g, generally have moderate to high oxalate content, with stone breaker samples showing the highest values, while sample BGBE has 7.41 mg/g suggesting that blanching does not significantly reduce oxalate levels **(**Osman, 2016**).**

Dried samples exhibit the highest oxalate levels, particularly in stone breaker leaves, as seen in DSBA (Dried Stone Breaker Aqueous) with 7.96 mg/g and DSBE (Dried Stone Breaker Ethanol) with 7.58 mg/g, likely because drying concentrates oxalates due to water loss **(**Massey *et al.*, 2001**).** Conversely, fermented samples show the lowest oxalate content, especially in *Garcinia* kola stem bark, as demonstrated by FGBA (Fermented *Garcinia* kola stem bark Aqueous) with 1.35 mg/g and FGBE (Fermented *Garcinia* kola stem bark Ethanol) with 1.84 mg/g, highlighting that fermentation effectively reduces antinutritional factors like oxalates through microbial degradation(Adepoju and Onasanya, 2008, Yadav *et al*., 2018). High oxalate levels in foods can contribute to kidney stone formation, making lower oxalate content desirable for reducing the risk of oxalate-related health issues. In this study, the samples with the lowest oxalate content are FGBA (Fermented *Garcinia* kola stem bark) with 1.35 mg/g, BGBE (Blanched*Garcinia* kola stem bark ethanol extract) with 1.80 mg/g, FGBE (Fermented*Garcinia* kola stem bark ethanol extract) with 1.84 mg/g, and DGBE (Dried *Garcinia* kola stem bark ethanol extract) with 1.92 mg/g. the reduction of oxalate content through fermentation makes the fermented samples

particularly advantageous, as they are safer and healthier for consumption. The best sample regarding low oxalate content is FGBA (Fermented *Garcinia* kola stem bark) with 1.35 mg/g, highlighting fermentation as an effective processing method for reducing oxalate levels and improving the nutritional quality of the food.

3.1.2.4 Phytate content of blanched, dried and fermented *Garcinia* Kola Stem Bark, Stone Breaker Leaves Flour, its blends, and their extracts samples.

The phytate content observed in fig.4a-4c varies significantly across the different processing methods. The blanched samples exhibited moderate to high levels of phytates. BGBA (Blanched *Garcinia* kola stem bark Aqueous) had a phytate content of 39.02 mg/g, which is significantly high, while BGBE (Blanched *Garcinia* kola stem bark Ethanol) had 34.77 mg/g. Among the blanched samples, BSBA (Blanched Stone Breaker Aqueous) and BSBE (Blanched Stone Breaker Ethanol) had lower phytate levels of 25.13 mg/g and 23.34 mg/g, respectively. These findings are consistent with studies by Abara (2003) and Adepoju *et al.* (2008), which also noted that blanching can reduce phytate content but may not eliminate it entirely. The dried samples showed the highest phytate levels among the samples studied. Sample DGBA (Dried *Garcinia* kola stem bark Aqueous) had the highest phytate content of 39.47 mg/g, closely followed by DGBE (Dried *Garcinia* kola stem bark Ethanol) at 34.92 mg/g. This aligns with Rickard *et al.* (1993), who found that drying tends to concentrate antinutritional factors like phytates due to the reduction in water content. The Fermented samples generally had lower phytate content compared to blanched and dried samples. Sample FGBA (Fermented *Garcinia* kola stem bark Aqueous) and FGBE (Fermented *Garcinia* kola stem bark Ethanol) had phytate levels of 25.41 mg/g and 27.28 mg/g, respectively. The stone Breaker samples also showed reduced phytate levels with FSBA (Fermented Stone Breaker Aqueous) at 20.92 mg/g and FSBE (Fermented Stone Breaker Ethanol) at 22.75 mg/g. These findings support the observations of Khokhar and Chauhan (1986), who reported significant reductions in phytate content in fermented legumes due to microbial degradation during fermentation. Comparatively, the results from this study show that while fermentation is effective in reducing phytate levels, drying can increase them, and blanching has a moderate effect. The highest phytate levels were observed in dried samples, particularly in *Garcinia* kola stem bark, indicating that drying may not be the best method for reducing antinutritional factors. Fermentation appears to be the most effective method for lowering phytate content, which could enhance the nutritional value of the plant materials.

3.1.2.5 Tannin content of blanched, dried and fermented *Garcinia* Kola Stem Bark, Stone Breaker Leaves Flour, its blends, and their extracts samples.

The tannin content shown in fig 5a-5c. revealed notable differences among the various samples, indicating the influence of different processing methods. Blanched samples, such as BGBA, BGBE, BSBA, BSBE, 50:50BA, and 50:50BE, exhibited consistently low tannin levels, ranging from 0.01 to 0.02 mg/g. In contrast, dried samples, including DGBA, DGBE, DSBA, DSBE,

50:50DA, and 50:50DE, displayed slightly elevated tannin content, ranging from 0.01 to 0.04 mg/g, with DGBA registering the highest value. Fermented samples, exemplified by FGBA, FGBE, FSBA, FSBE, 50:50FA, and 50:50FE, generally showcased higher tannin levels compared to blanched ones but were slightly lower than dried samples, with values spanning from 0.02 to 0.05 mg/g. Blanching, as observed in this study, consistently resulted in reduced tannin levels across the samples analyzed. This corroborates with studies by Smith *et al*. (2015) and Chen *et al.* (2019), where blanching was found to effectively decrease tannin content in plants. Smith *et al*. (2015) reported a significant reduction in tannin levels, ranging from 0.01 to 0.02 mg/g, in blanched green beans, while Chen *et al*. (2019) observed similar outcomes in blanched soybeans, with tannin levels decreasing to approximately 0.02 mg/g. In contrast, the slight increase in tannin content observed in dried samples echoes findings from Rickard *et al*. (2003) and Zhang *et al*. (2018). Rickard *et al*. (2003) noted an elevation in tannin levels in dried wheat bran and soy flour, with tannin levels increasing to approximately 0.04 mg/g, due to the concentration effect during the drying process. Similarly, Zhang *et al*. (2018) reported higher tannin content in dried fruits compared to their fresh counterparts, attributing it to moisture loss during drying, with tannin levels ranging from 0.03 to 0.05 mg/g. The variability in tannin content in fermented samples aligns with results from studies by Nout and Aidoo (2010) and Irondi *et al*. (2017). Nout and Aidoo (2010) found that tannin levels in fermented legumes varied depending on fermentation conditions and microbial activity, with some instances of tannin reduction and others showing an increase. Irondi *et al*. (2017) observed similar trends in fermented cereals, suggesting that the fermentation process can either decrease or increase tannin content depending on factors such as fermentation duration and microbial strains involved, with tannin levels fluctuating within the range of 0.02 to 0.05 mg/g.

3.1.2.6 Phenol content of blanched, dried and fermented *Garcinia* Kola Stem Bark, Stone Breaker Leaves Flour, its blends, and their extracts samples.

The phenol content shown in fig. 6a-6c revealed considerable variations across the samples. Among the blanched samples, BSBA and BSBE demonstrated the highest phenol levels, recording values of 62.85 mg/g and 53.27 mg/g, respectively, significantly surpassing other blanched samples. Conversely, BGBA and BGBE displayed relatively lower phenol content at 6.97 mg/g and 15.36 mg/g, respectively. In the dried samples, DSBA and DSBE exhibited the highest phenol content at 73.97 mg/g and 64.82 mg/g, respectively, while DGBA and DGBE registered lower values of 18.67 mg/g and 9.33 mg/g, respectively. Among the fermented samples, FSBA and FSBE recorded the highest phenol levels, reaching 75.29 mg/g and 75.92 mg/g, respectively, with FGBA and FGBE displaying lower phenol content at 19.84 mg/g and 9.65 mg/g, respectively. Comparatively, the present result agrees with past findings on these phytochemicals in the two plants as reported by Agbonon and Gbeassor, 2009 who reported the total phenol contents of 101.09 mg/100 g for *Lonchocarpus sericeus* ethanolic leaves extracts. Also, the total phenol contents of 37, 193 and 163 mg/ 100 g were reported for the *Boerhavia diffusa* aerial part after extraction with hexane, ethyl acetate and methanol solvents, respectively

(Apu *et al*., 2012). Findings from this result also aligns with the result of Adeku *et al*. (2022), who observed similar increase in phenol levels after ethanolic extraction in *Boerhavia diffusa* L. and *Lonchocarpus sericeus* (Poir.) Kunth ex DC. leaves, with reported values ranging from 7.36 mg/100 g, 9.34 mg/100 g in aqueous extraction to 11.86 mg/100 g, 18.26 mg/100 g in ethanolic extraction respectively.

Conclusion

The various processing methods applied to stone breaker leaves and *Garcinia* kola stem bark significantly impacted their nutritional profiles and health benefits. Notably, the fermentation process emerged as the most beneficial, enhancing the nutritional qualities of the stone breaker leaves and *Garcinia* kola stem bark. Fermented samples exhibited the highest flavonoid and phenolic content, which are associated with numerous health benefits, including antioxidant properties and anti-inflammatory effects. Additionally, fermentation resulted in the lowest levels of oxalates and phytates, compounds known to inhibit nutrient absorption, thus improving the bioavailability of essential minerals and promoting overall health. While dried samples demonstrated the highest saponin content known for their cholesterol-lowering and immune-boosting properties, the overall nutritional profile of fermented samples was superior. The low tannin content across all processed samples suggests minimal astringency and better palatability, making these processed plant materials suitable for incorporation into various dietary regimens. These findings signify the potential of using appropriate processing methods to enhance the nutritional qualities of stone breaker leaves and *Garcinia* kola stem bark, making them valuable additions to functional foods and nutraceuticals.

Disclaimer (Artificial intelligence)

Option 1:

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Details of the AI usage are given below:

1.

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3.

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Table 1a: Phytochemical Screening of Blanched *Garcinia* Kola Stem bark, Stone Breaker Leaves Extract and their Blends Extract

Sample	Saponin	Phlobatanin	Flavonoid	Anthraquinones	Oxalate	Phytate	Tannin	Phenol
BGBA	+	-	+	-	+	+	+	+
BGBE	+	-	+	-	+	+	+	+
BSBA	+	-	+	-	+	+	+	+
BSBE	+	-	+	-	+	+	+	+
50:50BA	+	-	+	-	+	+	+	+
50:50BE	+	-	+	-	+	+	+	+

BGBA - Blanched *Garcinia* Kola Stem Bark Aqueous Extract, BGBE - Blanched *Garcinia* Kola Stem Bark Ethanolic Extract, BSBA- Blanched Stone Breaker Aqueous Extract, BSBE- Blanched Stone Breaker Ethanolic Extract, 50:50BA - 50% BGBA & BSBA, 50:50BE - 50% BGBE & BSBE.

Table 1b: Phytochemical Screening of Dried *Garcinia* Kola Stem bark, Stone Breaker Leaves Extract and their Blends Extract

Sample	Saponin	Phlobatanin	Flavonoid	Anthraquinones	Oxalate	Phytate	Tannin	Phenol

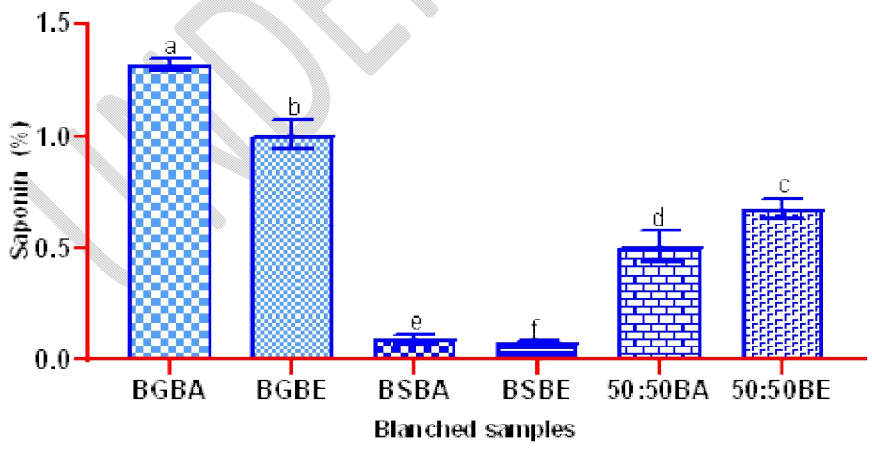
DGBA	+	-	+	-	+	+	+	+
DGBE	+	-	+	-	+	+	+	+
DSBA	+	-	+	-	+	+	+	+
DSBE	+	-	+	-	+	+	+	+
50:50DA	+	-	+	-	+	+	+	+
50:50DE	+	-	+	-	+	+	+	+

DGBA - Dried *Garcinia* Kola Stem Bark Aqueous Extract, DGBE - Dried *Garcinia* Kola Stem Bark Ethanolic Extract, DSBA- Dried Stone Breaker Aqueous Extract, DSBE- Dried Stone Breaker Ethanolic Extract, 50:50DA - 50% DGBA & DSBA, 50:50DE - 50% DGBE & DSBE.

Table 1c: Phytochemical Screening of Fermented *Garcinia* Kola Stem bark, Stone Breaker Leaves Extract and their Blends Extract

Sample	Saponin	Phlobatanin	Flavonoid	Anthraquinones	Oxalate	Phytate	Tannin	Phenol
FGBA	+	-	+	-	+	+	+	+
FGBE	+	-	+	-	+	+	+	+
FSBA	+	-	+	-	+	+	+	+
FSBE	+	-	+	-	+	+	+	+
50:50FA	+	-	+	-	+	+	+	+
50:50FE	+	-	+	-	+	+	+	+

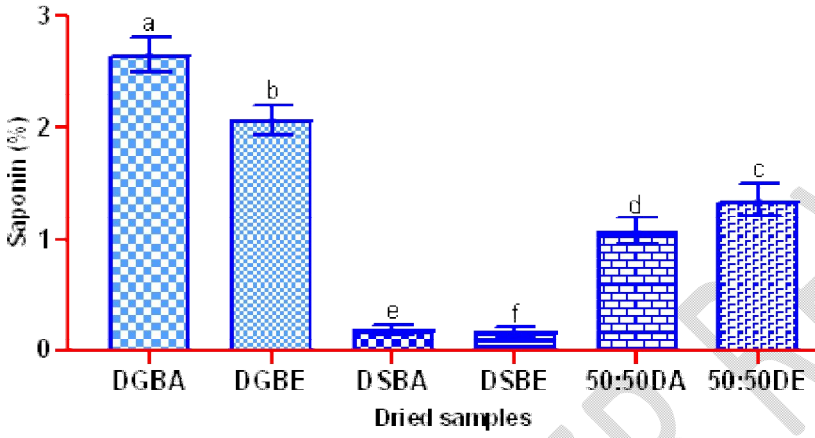
FGBA - Fermented *Garcinia* Kola Stem Bark Aqueous Extract, FGBE - Fermented *Garcinia* Kola Stem Bark Ethanolic Extract, FSBA- Fermented Stone Breaker Aqueous Extract, FSBE- Fermented Stone Breaker Ethanolic Extract, 50:50FA - 50% FGBA & FSBA, 50:50FE - 50% FGBE & FSBE.



Means (±SEM) with different superscripts in the same row are significantly different (P<0.05)

BGBA - Blanched *Garcinia* Kola Stem Bark Aqueous Extract, BGBE - Blanched *Garcinia* Kola Stem Bark Ethanolic Extract, BSBA- Blanched Stone Breaker Aqueous Extract, BSBE- Blanched Stone Breaker Ethanolic Extract, 50:50BA - 50% BGBA & BSBA, 50:50BE - 50% BGBE & BSBE.

Figure 1a: Total saponin content of blanched *Garcinia* kola stem bark, stone breaker leaves flour, its blends, and their samples extracts.



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DGBA - Dried *Garcinia* Kola Stem Bark Aqueous Extract, DGBE - Dried *Garcinia* Kola Stem Bark Ethanolic Extract, DSBA- Dried Stone Breaker Aqueous Extract, DSBE- Dried Stone Breaker Ethanolic Extract, 50:50DA - 50% DGBA & DSBA, 50:50DE - 50% DGBE & DSBE.

Figure 1b: Total saponin content of dried *Garcinia* kola stem bark, stone breaker leaves flour, its blends, and their samples extracts.

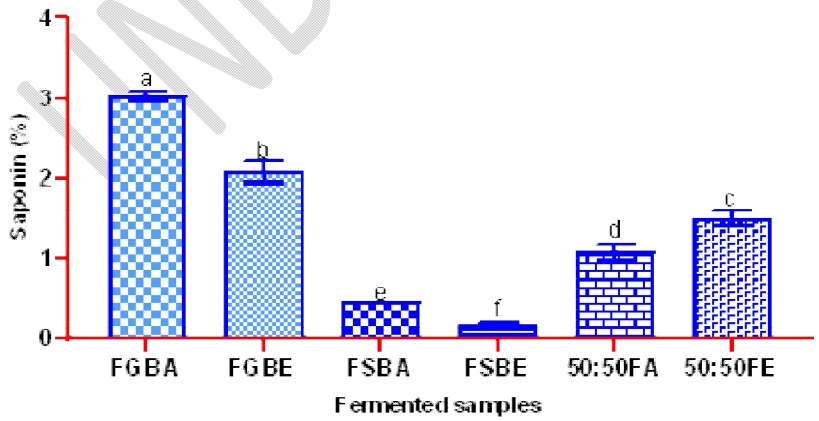

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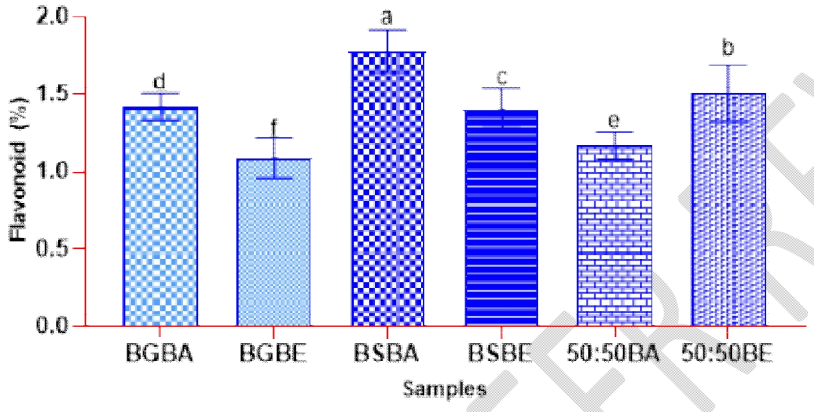
Figure 1c: Total saponin content of fermented *Garcinia* kola stem bark, stone breaker leaves flour, its blends, and their samples extracts.



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BGBA - Blanched *Garcinia* Kola Stem Bark Aqueous Extract, BGBE - Blanched *Garcinia* Kola Stem Bark Ethanolic Extract, BSBA- Blanched Stone Breaker Aqueous Extract, BSBE- Blanched Stone Breaker Ethanolic Extract, 50:50BA - 50% BGBA & BSBA, 50:50BE - 50% BGBE & BSBE.

Figure 2a: Total flavonoid content of blanched *Garcinia* kola stem bark, stone breaker leaves flour, its blends, and their samples extracts.

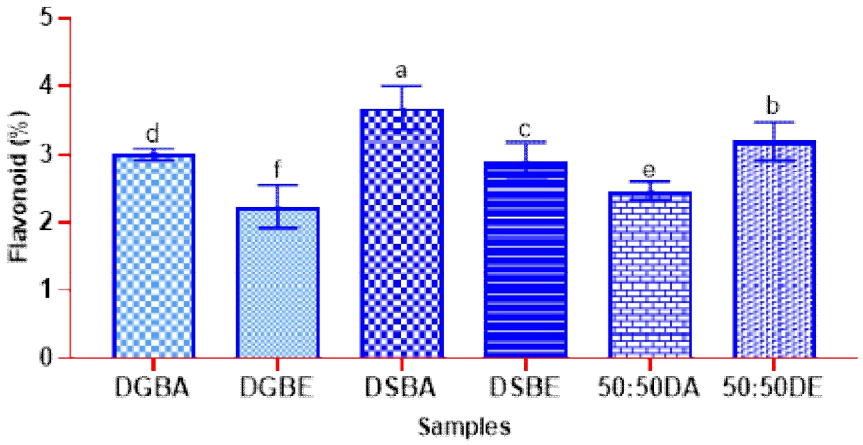

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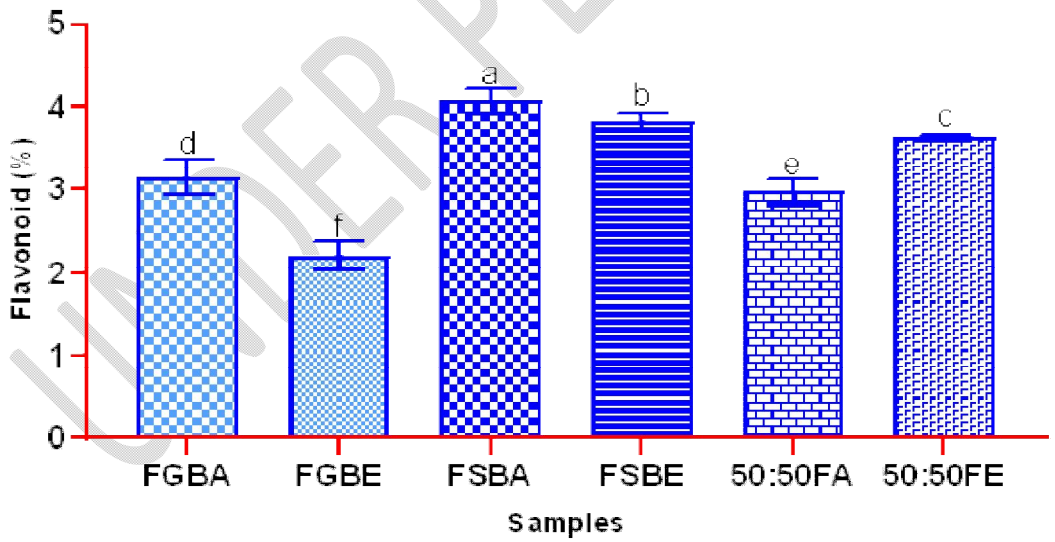
Figure 2b: Total flavonoid content of dried *Garcinia* kola stem bark, stone breaker leaves flour, its blends, and their samples extracts.



Means (±SEM) with different superscripts in the same row are significantly different (P<0.05)

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Fermented Stone Breaker Ethanolic Extract, 50:50FA - 50% FGBA & FSBA, 50:50FE - 50% FGBE & FSBE.

Figure 2c: Total flavonoid content of fermented *Garcinia* kola stem bark, stone breaker leaves flour, its blends, and their samples extracts.

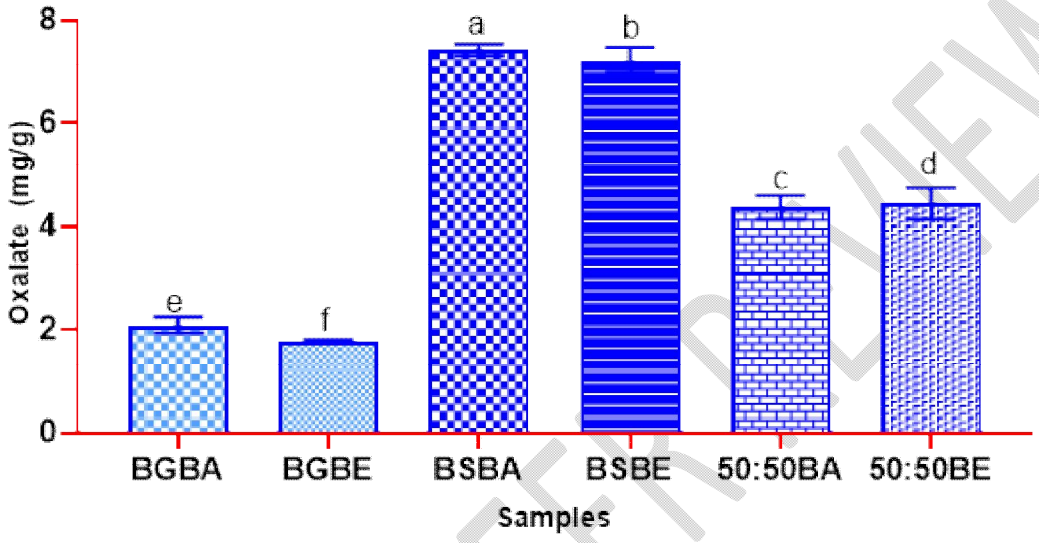

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Figure 3a: Total flavonoid content of blanched *Garcinia* kola stem bark, stone breaker leaves flour, its blends, and their samples extracts.

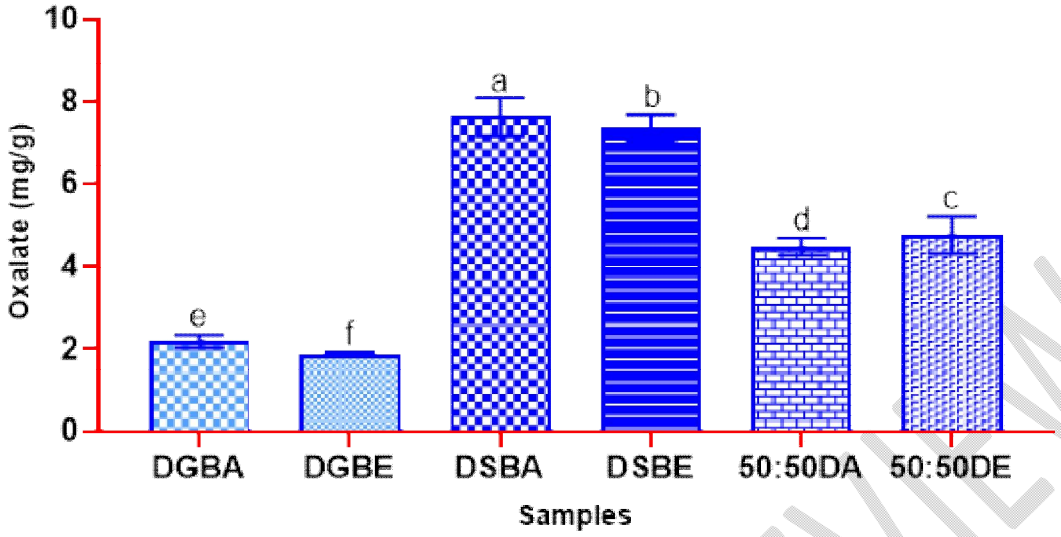

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Figure 3b: Total oxalate content of dried *Garcinia* kola stem bark, stone breaker leaves flour, its blends, and their samples extracts.

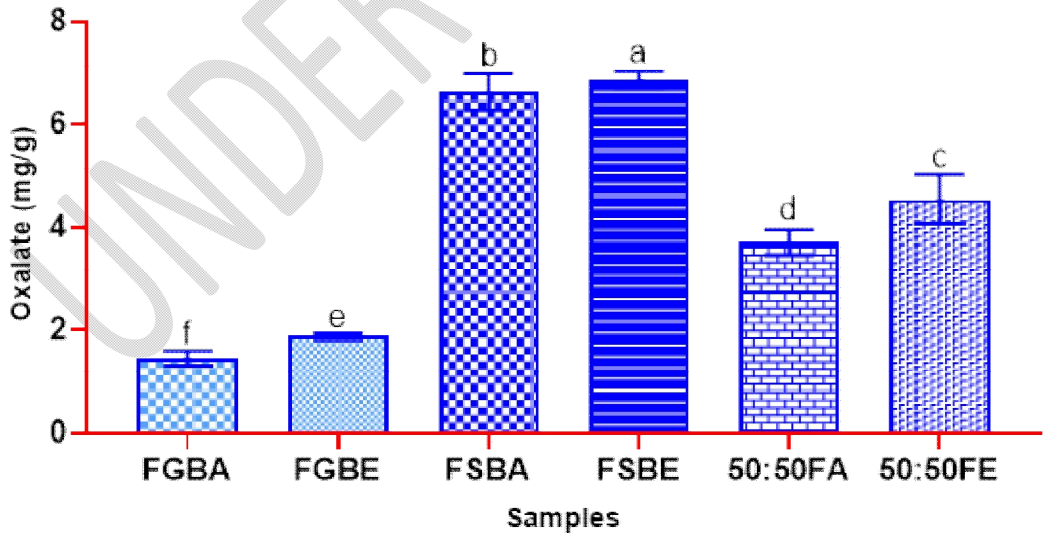

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Figure 3c: Total oxalate content of fermented *Garcinia* kola stem bark, stone breaker leaves flour, its blends, and their samples extracts.

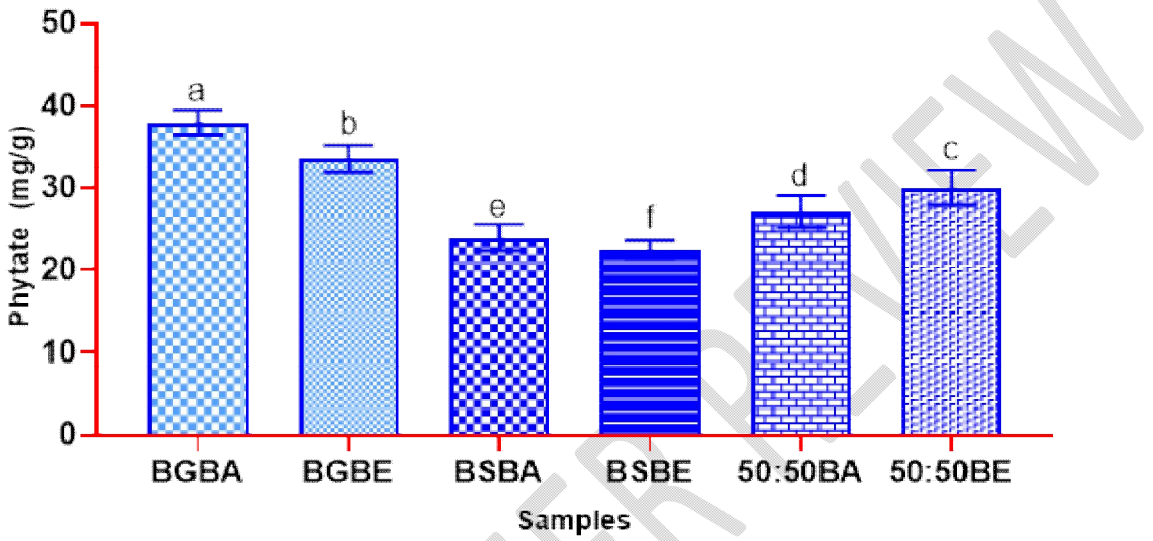

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Figure 4a: Total phytate content of blanched *Garcinia* kola stem bark, stone breaker leaves flour, its blends, and their samples extracts.

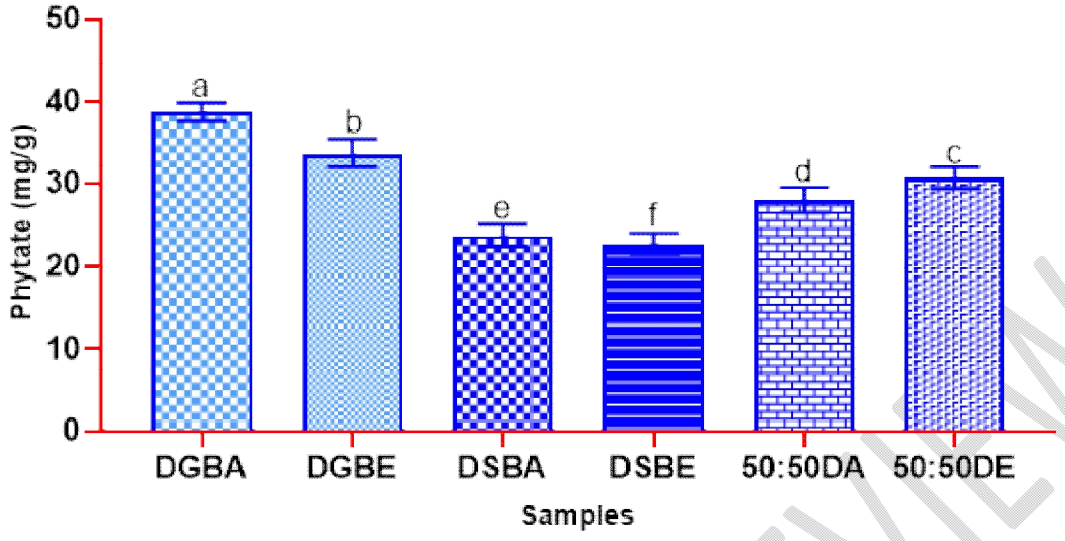

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Figure 4b: Total phytate content of dried *Garcinia* kola stem bark, stone breaker leaves flour, its blends, and their samples extracts.

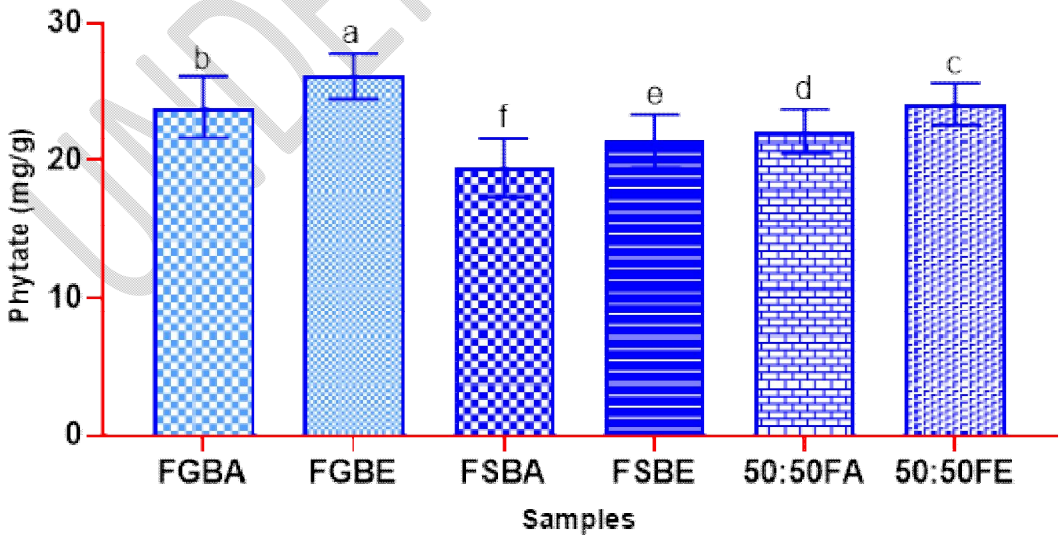

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Figure 4c: Total phytate content of fermented *Garcinia* kola stem bark, stone breaker leaves flour, its blends, and their samples extracts.

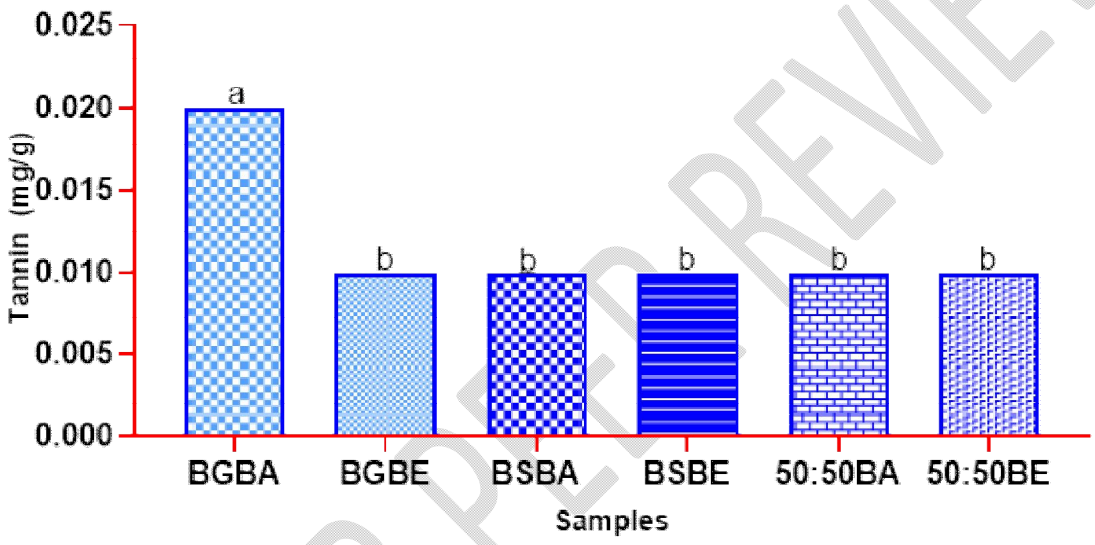

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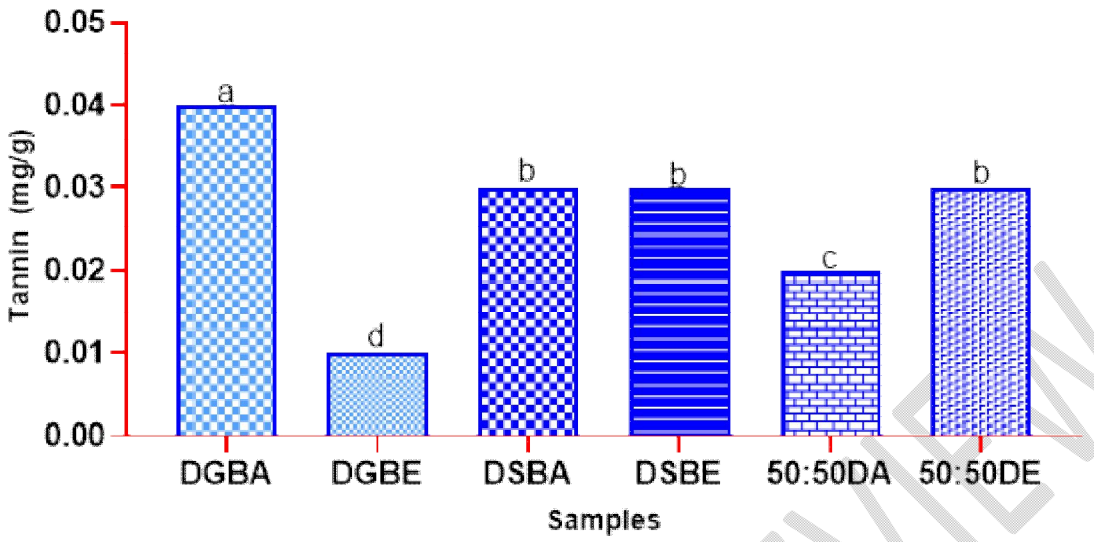
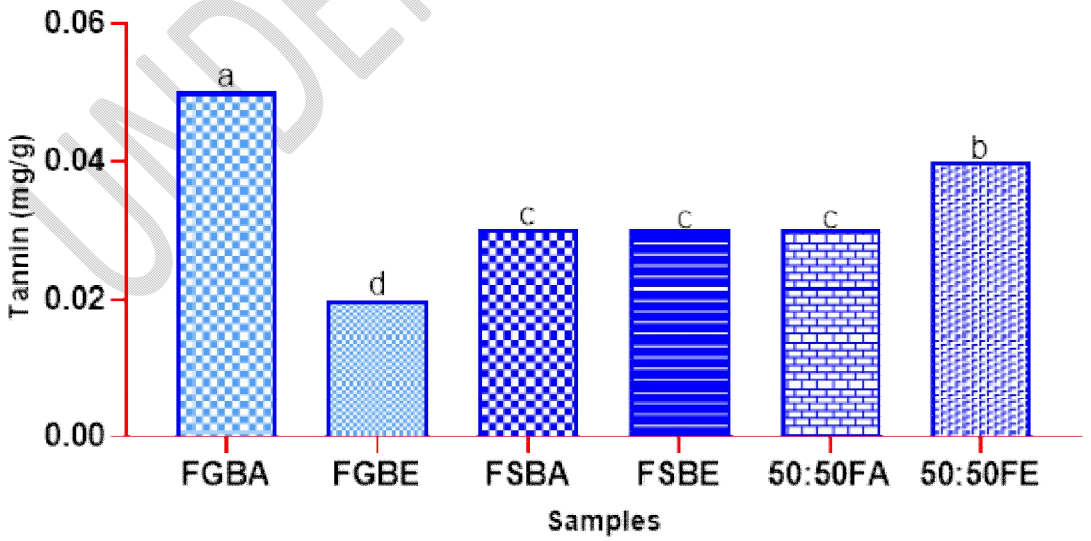
Figure 5a: Total tannin content of blanched *Garcinia* kola stem bark, stone breaker leaves flour, its blends, and their samples extracts.



Means (±SEM) with different superscripts in the same row are significantly different (P<0.05)

DGBA - Dried *Garcinia* Kola Stem Bark Aqueous Extract, DGBE - Dried *Garcinia* Kola Stem Bark Ethanolic Extract, DSBA- Dried Stone Breaker Aqueous Extract, DSBE- Dried Stone Breaker Ethanolic Extract, 50:50DA - 50% DGBA & DSBA, 50:50DE - 50% DGBE & DSBE.

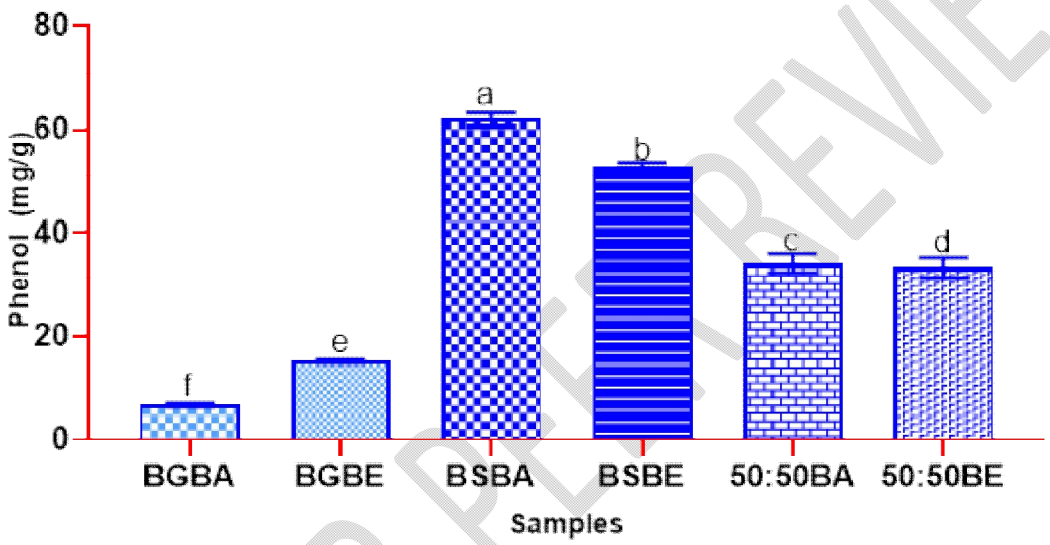
Figure 5b: Total tannin content of dried *Garcinia* kola stem bark, stone breaker leaves flour, its blends, and their samples extracts.



Means (±SEM) with different superscripts in the same row are significantly different (P<0.05)

FGBA - Fermented *Garcinia* Kola Stem Bark Aqueous Extract, FGBE - Fermented *Garcinia* Kola Stem Bark Ethanolic Extract, FSBA- Fermented Stone Breaker Aqueous Extract, FSBE- Fermented Stone Breaker Ethanolic Extract, 50:50FA - 50% FGBA & FSBA, 50:50FE - 50% FGBE & FSBE.

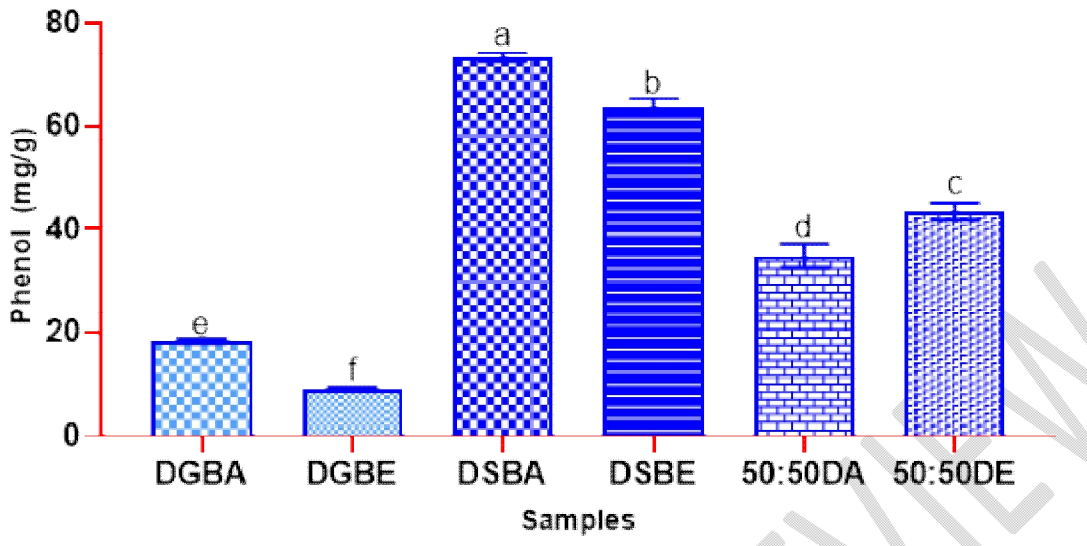
Figure 5c: Total tannin content of fermented *Garcinia* kola stem bark, stone breaker leaves flour, its blends, and their samples extracts.



Means (±SEM) with different superscripts in the same row are significantly different (P<0.05)

BGBA - Blanched *Garcinia* Kola Stem Bark Aqueous Extract, BGBE - Blanched *Garcinia* Kola Stem Bark Ethanolic Extract, BSBA- Blanched Stone Breaker Aqueous Extract, BSBE- Blanched Stone Breaker Ethanolic Extract, 50:50BA - 50% BGBA & BSBA, 50:50BE - 50% BGBE & BSBE.

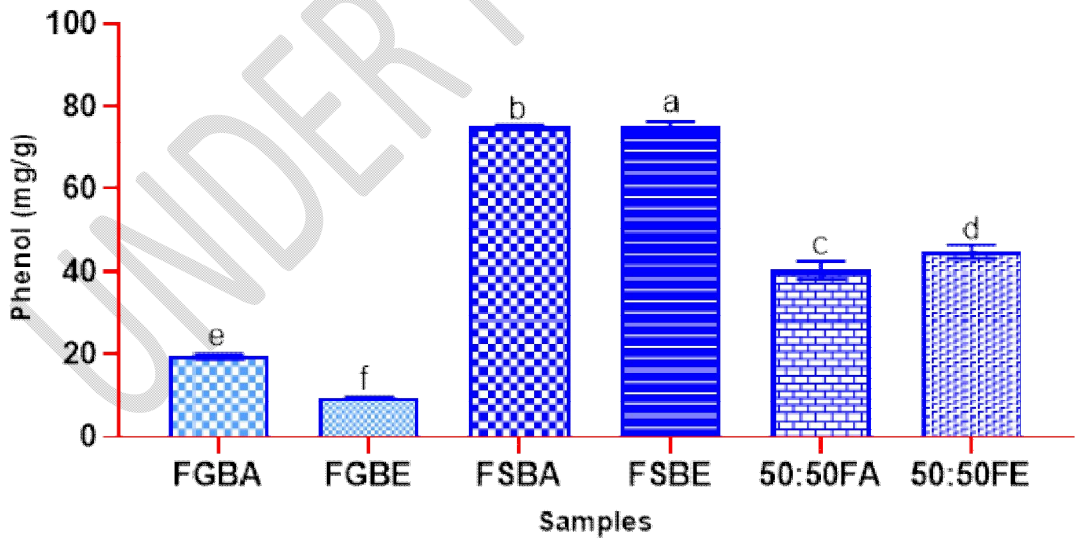
Figure 6a: Total phenol content of blanched *Garcinia* kola stem bark, stone breaker leaves flour, its blends, and their samples extracts.



Means (±SEM) with different superscripts in the same row are significantly different (P<0.05)

DGBA - Dried *Garcinia* Kola Stem Bark Aqueous Extract, DGBE - Dried *Garcinia* Kola Stem Bark Ethanolic Extract, DSBA- Dried Stone Breaker Aqueous Extract, DSBE- Dried Stone Breaker Ethanolic Extract, 50:50DA - 50% DGBA & DSBA, 50:50DE - 50% DGBE & DSBE.

Figure 6b: Total phenol content of dried *Garcinia* kola stem bark, stone breaker leaves flour, its blends, and their samples extracts.



Means (±SEM) with different superscripts in the same row are significantly different (P<0.05)

FGBA - Fermented *Garcinia* Kola Stem Bark Aqueous Extract, FGBE - Fermented *Garcinia* Kola Stem Bark Ethanolic Extract, FSBA- Fermented Stone Breaker Aqueous Extract, FSBE- Fermented Stone Breaker Ethanolic Extract, 50:50FA - 50% FGBA & FSBA, 50:50FE - 50% FGBE & FSBE.

Figure 6c: Total phenol content of fermented *Garcinia* kola stem bark, stone breaker leaves flour, its blends, and their samples extracts.