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Polyphenols, flavonoids, carotenoids contents and mineral composition of *Bombax costatum* calyx: Their contribution to overall antioxidant

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Abstract
This study was designed within the of antioxidant extracts from *Bombax Costatum* calyx. Efficiency in the extraction of antioxidants (total phenolics, flavonoids and carotenoids) from calyces was assessed through different processes involving solvents (methanol, ethanol, acetone and water). Results indicated that extraction with methanol gave the highest yield for total phenolics, expressed as gallic acid equivalents (EAG) per g dry weight (DW) (84.57 ± 2.1 EAG), while the highest results for total flavonoids were found acetone extracts. Antioxidant activity of the extracts reported the highest reducing power for aqueous extracts with extraction, while methanol and ethanol samples exhibited the highest antioxidant activity by 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent. These interactions were found without effect on the oxidative stability of the emulsions. These study suggest that *Bombax Costatum* calyces have the potential to be used like food antioxidants.

Keywords: *Bombax costatum*, activity antioxidant, carotenoids phenolics Côte d'Ivoire

Introduction
Associated with reduced risk of several diseases such as cardiovascular diseases and certain types of cancer (Kaviarasan *et al.*, 2007; Kaisoon *et al.*, 2011) [32, 30]. Flavonoids are known to possess diverse biological activities including antioxidant activity (Izzreen and Fadzelly, 2013) [27]. The potant antioxidant activity of flavonoids, their ability to scavenge hydroxyl radicals. May be the most important function of flavonoids and underlies many of their actions in the body (Merfort *et al.*, 1996) [41]. Another pharmacological action of flavonoids is the protector effects on carcinogenesis by inhibiting the neoplastic effects of chemical carcinogens; their activity as antioxidants on microsomal mono-oxygenase promotes a detoxifying action with an antineoplastic effect (Elliott *et al*., 2000) [16]. The importance of the carotenoid compounds in the diet has been recognized, not only as precursors of vitamin A but also as antioxidants in cell protection and in the prevention of degenerative diseases (Stahl and Sies 2003) [50]. Carotenoids are fat soluble antioxidants found in many fruits and vegetables and are required for human epithetlial cellular differentiation (Zhang and Hamauzu 2003) [57]. The present study objective was to determine total the phenolic, Flavonoids and carotenoids as well as minerals levels of *Bombax costatum* calyx extracts. This study is expected to provide the potential of seaweed extract to be used as an antioxidant of the calyx extracts as a source of natural antioxidants for health benefits.

Materials and Methods
Material and chemicals. The calyxes of *B. costatum* used for this work were randomly harvested at maturity from a farm in Yamoussoukro, center portion of Côte d'Ivoire (West Africa) in November 2016. The raw materials were physically examined to ensure disease-free. Then, these calyxes were immediately transported to in laboratory INP-HB LAPISEN (Yamoussoukro, Côte d'Ivoire). These, flowers were stored under prevailing ambient conditions (25°C) for 24 h and carefully cracked. The flowers were cleaned of any adhering residue and hand-picked to eliminate damaged ones and the calyxes were separated to corolla and subsequently dried in the shade 30ºC. All the chemicals, reagents and solvents used in the experiments were of analytical grade. Polyphenols (gallic acid), flavonoids (catechins), carotenoids (*β*-carotene) and Folin-Ciocalteu standards were purchased from Redox Bucharest - Sigma Aldrich, Dako, Epp. Romania. Methanol, ethanol, acetone, petroleum ether, DPPH (1,1-diphenyl-2-picrylhydrazyl), potassium persulfate, nitric acids and percholoric acids were purchased from Merck Romania SRL.
Extraction and analytical method. Dried *B. costatum* calyxes were ground into fine powder using mortar and a sieve of diameter (250 µm) to obtain dried powder. This powder was stored in air tight plastic containers at 4°C in a refrigerator for analysis. Twenty gram of the sample was soaked with 250 mL of methanol, ethanol, acetone and water each for 72 h. The filtrate from each sample was concentrated using the rotary evaporator at 40°C. The dried extracts were weighed and stored frozen.

Determination of plant percentage yield
The percentage yield was obtained from this formula: (%) =

$[(W_2 – W_1) / W_0]$ x 100 where, W_2 is the weight of the extract and the container, W_1 is the weight of the container alone and W_0 is the weight of the initial dried sample.

Determination of total phenolic content:

Total extracted polyphenol content was determined according to the Folin-Ciocalteu method reported by Singleton and Rossi (1965) [48] and modified by Wood *et al.* (2002) [53]. To 30 µl sample extract. 2.5 ml of diluted Folin-Ciocalteu's phenol reagent (1/10) were added. The mixture was kept for 2 min in the dark at room temperature and 2 ml of calcium carbonate solution (75 $g.L^{-1}$) were added. The mixture was heated at 50°C for 15 min then cooled down. The absorbance was measured at 760 nm against water as blank. Analyses were performed in triplicate. Total polyphenols content was quantified as gallic acid equivalent per liter of extract equivalent Gallic acid (g/L Gallic acid Equivalent).

Determination of total flavonoid content

The total flavonoids content was determined using the Dowd method (Meda *et al.*, 2005) [40]. 5mL of 2% aluminium trichloride ($AlCl_3$) inmethanol was mixed with the same volume of the methanolic extract solution (0.4 mg/mL). After ten minutes the absorbance was measured at 415 nm using PerkinElmer UV-VIS Lambda. Blank sample consisting of a 5mL extract solution with 5mL methanol without $AlCl_3$. The total flavonoid content was determined using a standard curve with catechin (0–100mg/L) as the standard. Total flavonoids content is expressed as mg of catechin equivalents (CE)/100 g DW.

Determination of total carotenoid content

Total carotenoids were determined according to the procedure given by Yuan *et al.*, (2009) [56] as follows: 5g of calyxes were grinded and extracted with a mixture of acetone and petroleum ether (1:1, v/v) repeatedly using the mortar and pestle until a colorless residue was obtained. The upper phase was collected and combined with crude extracts after being washed for several times with water. The extracts were made up to a known volume with petroleum ether. Total carotenoids content was determined by recording the absorbance at 475 nm with a spectrophotometer (Spectrophotometer, Type JASCO UV-500, Japan). Total carotenoids were estimated by mg/100 g DW.

Determination of mineral element

The method of AOAC (2000) [5] was used to determine minerals. The minerals, that are, calcium, Potassium, iron, Phosphorus and magnesium were determined in a dilute solution of the ashed samples by atomic absorption spectrophotometer (VARIAN AA20 Techtron Pty. Ltd, Australia). The results are expressed as dry weight (DW) basis.

Measurement of antioxidant activity by DPPH

Extraction. Ten g (10 g) of calyxes and processed *B. costatum* flowers were homogenized with 100mL methanol for 1 min and centrifuged at 10 000 rpm for 15 min at 4°C.The clear supernatant was transferred to a glass bottle and measured immediately for total antioxidant activity using DPPH assay. 100 μL of sample was added to 5mL DPPH solution, and the absorbance of DPPH reagent was determined at 515nm after 30min of incubation (Brand-Williams *et al*., 1995) [8]. The inhibition percentage of the absorbance was calculated as follows:

Inhibition (%) = $[(Abs_{t0} – Abs_{t30}) / Abs_{t0}]$ x 100 where, Abs_{t0} was the absorbance of DPPH at time 0 and Abs_{t30} was the absorbance of DPPH after 30 min of incubation.

Statistical analyses. The mean values and standard deviations of each analysis are reported. Analysis of variance (ANOVA) was performed as part of the data analyses (SAS, 1989). When F-values were significant ($p<0.05$) in ANOVA, then least significant differences were calculated to compare treatment means.

Results and Discussion

Percentage Yield

The percentage yield of *Bombax Costatum* calyx in different solvents is shown in Table 1. The result showed that methanol extract of *Bombax costatum* calyx had the highest yield and significantly different ($p<0.05$) from ethanol, acetone and water. Since the antioxidant compounds found in plants have different polarities, different solvents are used to isolate antioxidants. Water, methanol, ethanol, and acetone are solvents commonly used in extraction processes (Aksoy *et al.*, 2013) [3].

Percentage yields ranged from 8.3% to 26.52%. Furthermore, our results showed that methanol extracts of all three tested parts were found to be higher than those obtained by the ethanol extraction, which is in agreement with several studies reporting the efficiency of methanol in polyphenols recovery compared to ethanol and other solvents of different polarity degrees (Anokwuru *et al.*, 2011; Ghasemzadeh *et al.*, 2011) [4, 21]. On the contrary, in the investigations of Gayatri and Sahu, (2010) [20] and Koffi *et al.*, (2010) [36], ethanol showed better extractive values than methanol and other examined solvents.

Total phenolic content: Phenols are very important plant constituents because of their radical scavenging ability due to their hydroxyl groups (Hatano *et al.*, 1989). Methanol has been reported by Yao *et al.* (2004) [54] to be the most suitable solvent in the extraction of polyphenol compounds from plant tissue due to its ability to inhibit the action of polyphenol oxidase that causes the oxidation of polyphenols.

The result of the Total Phenolic Content (TPC) of *Bombax Costatum* calyx in different solvents is shown in Table 1. The result of the total phenolic content of *Bombax Costatum* calyx in different solvents showed that methanol extract yielded 84.57 ± 0.56 $g.L^{-1}$EAG, ethanol extract yielded 82.23 ± 1.3 $g.L^{-1}$EAG, acetone extract yielded 57.12± 0.26 $g.L^{-1}$EAG and water extract yielded 24.25 ± 0.16 $g.L^{-1}$EAG. The result of this study showed that there was no significant difference between the phenolic content of the methanol and ethanol extract ($p<0.05$) but the two extracts were significantly different from water and acetone extract ($p<0.05$). Compared with the percentage yield of all the extract, there was a decline from methanol to ethanol and acetone but an increase in the phenolic content of water extract (Table 1). This result showed that alcohols are better solvents than acetone and water for the extraction of phenols from *Bombax Costatum* calyx. The aqueous TPC (24.2$g.L^{-1}$EAG) in this study, was

lower than the TPC of the aqueous extract of *Bombax malabaricum* calyx reported by Essam *et al.* (2014), (118.26 $g.L^{-1}$EAG). The methanol extracts TPC (32.4 ± 2 $g.L^{-1}$EAG) of *Bombax ceiba* flowers reported by Donipati and Subhasini. (2016) was also lower than the methanol extracts TPC in this study (84.57 ± 0.56 $g.L^{-1}$EAG).

The values (12, 24 $g.L^{-1}$EAG) of ethanol extracts of *Hibiscus sabdariffa* flowers reported by Christian and Jackson (2009) [10] were lower than the values reported for the ethanol extract in this study. Futhermore, in the extraction of phenolic compounds from different cultivars of green walnuts, Jakopic *et al.* (2009) [28] reported that the methanol extracted significantly higher amount of phenols compared to ethanol. However, a research conducted by Koffi *et al.* (2010) [36] indicated that ethanol was the best solvent for the extraction of phenols in the Ivorian plants under study. There was no found literature that compared all the four solvents used in this study. It has been reported by Singleton *et al.* (1999) [49] that the Folin-Ciocalteu assay has been shown not to be specific to just polyphenols but to any other substance that could be oxidised by the Folin reagent. In addition, phenolic compounds, depending on the number of phenolic groups they have, respond differently to the Folin-Ciocalteu reagent. Therefore, the total phenolic content of *Bombax Costatum* calyx in this study may not be the exact content of the phenols present.

Total Flavonoid Content (TFC)

The result of the Total flavonoid Content (TFC) of *Bombax Costatum* calyx in different solvents is shown in Table 1. The result of the total flavonoid content of *Bombax Costatum* calyx in different solvents showed that methanol extract yielded 18.10 ± 2.10 $g.L^{-1}$EQ, ethanol extract yielded 14.94 ± 1.90 $g.L^{-1}$EQ, acetone extract yielded 34.14 ±1.1 $g.L^{-1}$EQ and water extract yielded 11.03 ± 0.1 $g.L^{-1}$EQ.

The result of the total flavonoid content showed that the acetone extract had the highest flavonoid content (34.14 ± 1.1 $g.L^{-1}$EQ) and significantly different ($p<0.05$) compared to methanol, ethanol and water. This suggests that the flavonoids present in *Bombax Costatum* calyx were better extracted by acetone. These results have been reported by Anokwuru *et al.* (2011) [4] who mentioned that acetone was better solvent of total flavonoid contents of *Hibiscus sabdariffa* calyx.

Total carotenoid Content (TCC)

The result of the TCC of *Bombax Costatum* calyx in different solvents is shown in Table 1. Total carotenoid Content ranged from 22.71 ± 1.4% to 58.23 ± 0.7%. The result of the total carotenoids content showed that the water extract had the highest carotenoids content (24.71 ± 1.4%) and significantly different ($p<0.05$) compared to methanol, ethanol and acetone. This result showed the influence of solvents. The solubility of the solute into the solvent is different because of polarity differences between solvents. Factors that have been attributed to bringing variation include different solvents (Li *et al*., 2006) [38]. These results reveal that water could be used for the extraction of carotenoids from vegetable total as Yuan *et al.* (2009) [56] showed in their studies. According this study, TCC in water boiling extract yielded was 40.77%, which was higher than TCC obtained in this study.

Inhibition of DPPH radical

The DPPH radical-scavenging activity of the methanol, ethanol, acetone and water extract of *Bombax Costatum* calyx is shown in Fig. 1. Methanol extract inhibited 52.62 ± 2.1 %, ethanol extract inhibited 39.54 ± 1.1 %, acetone extract inhibited 19.12 ± 0.98 % and water extract inhibited 29.1 ± 1.3 % of the DPPH free radicals. The antioxidant activity of the extract and the yield depends on the selected solvent (Gong *et al*., 2012) [22, 23].

DPPH assay is a preliminary test to investigate the antioxidant potential of extracts (Kaur *et al.*, 2008) [31]. The result of the DPPH free radical scavenging activity (Figure 1) showed that methanol extract of *Bombax Costatum* calyx inhibited more free radicals than ethanol, acetone and water. However, it was only significantly different from only water and acetone extract. Due to different antioxidant potentials of different compounds, the antioxidant activity of extract is strongly dependents on the extraction solvent (Jang *et al.*, 2007) [29]. The methanol extract recorded the highest phenolic content and also had the highest antioxidant activity; however, the acetone extract that gave the highest flavonoid content recorded the lowest antioxidant activity. This suggests that the flavonoid contents of the acetone extract may be glycosylated making the flavonoids not freely available for antioxidant activity. There was a positive linear correlation between the antioxidant activity and total phenolic content of *Bombax Costatum* calyx. This result suggested that the phenolic compounds contributed significantly to the antioxidant capacity of the investigated plant species. This result was consistent with the findings of many research groups who reported such positive correlation between total phenolic content and antioxidant activity (Cai *et al.*, 2004; Tawaha *et al.*, 2007) [9, 51]. This value (39.54 ± 1.1 %) was higher than 31.63 ± 2.02 % the reported value for *Chorisia chodatii* (*Bombacaceae*) (Refaat *et al.*, 2013) [46] in ethanol extracts. A dose-dependent increase in antioxidant activity was found and no difference in activity was found between the water and ethanol extracts. These results corroborate the ones obtained in the studies of Dutra *et al.* (2012) [15], who worked with *Bombax ceiba* flower possess the significant antioxidant activity. Some studies suggest that to obtain the beneficial effects of the carotenoid (Parisi *et al.*, 2008; Satoh *et al.*, 2009) [44, 47]. Preliminary phytochemical screening revealed the presence of phenolics, flavonoids and carotenoid have been proved to be responsible for the antioxidant activity of various medicinal plants reported earlier (Kumar *et al.*, 2000) [37]. Hence, these may be responsible for the observed activity in both these species. The present study proved the antioxidant potential of *Bombax Costatum.*

Mineral Contents of Sampled calyx

(Figure 2) revealed that significant different occur in the mineral content across the sampled extracts. The result shows that *Bombax Costatum* has the highest phosphorus content of 67.87 ± 2.02 mg % followed by Calcium (60.27 ± 1.02 %) and Magnesium (48.7± 2.7 % mg), Sodium (29.27 ± 0.92 mg %) and iron (17.33 ± 0.92 mg %). Comparing this result with the reported value for *Bombax buonopozense,* its showed that it has the highest Magnesium (60.86 mg %) and Iron (80.37 mg %) (Adeyeye, 1999) [1]. The relatively high mineral content

obtained in the calyx *Bombax Costatum* investigated in this study has proved that indigenous calyx *Bombax Costatum* could be a cheap source of dietary mineral requirement for man and hence a good substitute for expensive cultivated varieties of vegetable with lower mineral content value. Minerals are very important in normal body functions. Mineral such as sodium has been reported to be very important in the maintenance of acid-base balance in the body (Adeyeye, 2002) [2]. The ratio of Na to Potassium is important to hypertensive patient while Calcium is essential for bone and teeth formation (Vunchi *et al*, 2011) [52]. Magnesium is an important element in the correction of diseases associated with circulatory system (Vunchi *et. al*, 2011) [52] while Iron is essential for blood formation and normal functioning of central nervous system (Adeyeye and Otoketi, 1999) [1].

Table 1: Percentage extracts yield, total phenolic content, total flavonoid content and carotenoids content.

Solvent	Yield (%)	TPC (g.L^{-1}EAG)	TFC (g.L^{-1}EQ)	TCC (%)
Methanol	26.52^{a}	84.57 ± 2.1^{a}	18.10 ± 2.10^{b}	58.23 ± 0.7^{a}
Ethanol	24.62^{a}	82.23 ± 1.3^{a}	14.94 ± 1.90^{c}	42.51 ± 1.1^{c}
Acetone	17.1^{b}	57.12 ± 0.26^{b}	34.14 ±1.1^{a}	26.32 ± 1.2^{b}
water	8.3^{c}	21.25 ± 0.16^{c}	11.03 ± 0.1^{d}	24.71 ± 1.4^{d}

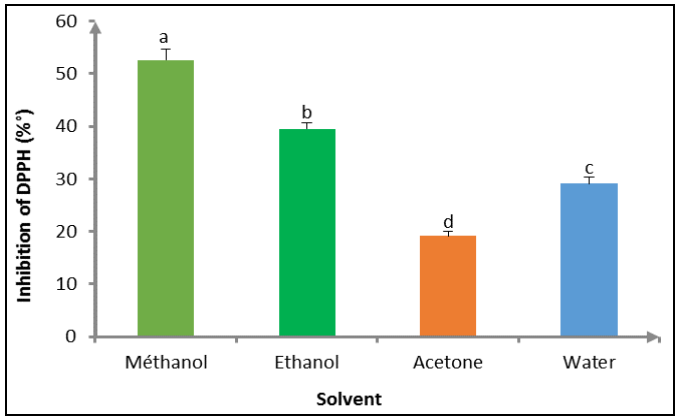

Fig 1: Percentage inhibition of the DPPH radicals by methanol, ethanol, acetone and water extracts of *Bombax Costatum* calyx.

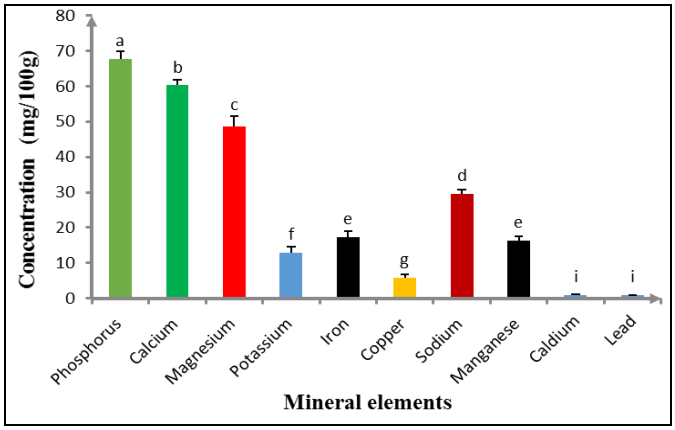

Fig 2: Mineral profile of dry powder into methanolic extract of Bombax Costatum calyx.

Conclusion

In the present study, methanolic extract of *Bombax Costatum* calyx gave the highest extraction yield, phenolic contents and antioxidant activity against DPPH free radicals. Acetone extract gave the highest flavonoid content but had the least inhibition to DPPH free radicals. The result also showed that a higher amount of flavonoids were extracted compared to phenols in all the solvents however; there was a stronger linear correlation between the phenolic contents and inhibition of DPPH free radicals than flavonoid contents. This suggests that phenolics contribute more to the antioxidant activity of *Bombax Costatum* calyx.

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