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Phytochemicals compound and antioxidant activity study using different solvents and drying mode of flower *Bombax costatum* Pellgr and Vuillet (Bombacaceae) from Côte d'Ivoire

Toma Eugene Zan Bi[1], Thierry Yapo Monnet[2*], Kouame Claude Ya[3], Elvis Serge Gbocho Ekissi[4], Jean Bedel Fagbohoun[5], Patrice Lucien Kouame[6]

[1, 2, 4, 6] Laboratory of Biochemistry and Food Technology, University Nangui Abrogoua, 02 BP 801 Abidjan 02, Côte d'Ivoire

[3] Department of Biochemistry and Microbiology, Agroforestry unit, University Jean Lorougnon Guede, BP 150 Daloa, Côte d'Ivoire

[5] Department of Biochemistry-Genetics, University Peleforo Gon Coulibaly, BP 1328 Korhogo, Côte d'Ivoire

Abstract
The best levels of total flavonoids and carotenoids in the dry extracts of the calyx and corolla of *Bombax costatum* are obtained when these plant tissues are dried in the shade and the extraction carried out with respectively hydro-ethanol solvents (30/70, v/v) and hydro-acetone (30/70, v/v). As for polyphenols, the same plant tissues must be dried in an oven and the extraction must be made with the solvent hydro-acetone (30/70, v/v). The hydroethanolic dry extracts of calices and corollas of the oven dried *Bombax costatum* flower have the highest antioxidant activity regardless of the method used.

Keywords: Bombax costatum, drying mode, type of solvent, antioxidant activities, phytochemicals

1. Introduction
Bombax costatum is a plant species of wooded savannas and Sahelo-Sudanian woodlands (Belem *et al*., 2008)[5]. Its range extends from Senegal to Cameroon to the Central African Republic (Assogba *et al*., 2017) [4]. It is also spreading in the Guinean zone (Assogba *et al*., 2017) [4]. This wild plant prefers sandy-clay soils but supports stony and lateritic soils. In Sudanese parks, the species is not far from the villages. It is resistant to bush fires and drought (Belem *et al*., 2008) [5]. In Côte d'Ivoire, the plant *Bombax costatum* is found in shrub savannas (Tiebré *et al*., 2016) [30]. The flowers (especially the calices) of *Bombax costatum* are used as ingredients in gelatinous sauces (Ouattara, 2016) [23]. Immature fruits and sometimes flowers are added as thickeners in sauces (Ouattara, 2016) [23]. Young fruits, cut into slices and dried, are used in cooking (Belem, 2008) [5]. In traditional practices, solar drying is the most used. But, drying in the shade is also practiced (Ouafi *et al*, 2015) [22]. However, in laboratory, drying in an oven is the most prominent method. The most used solvent for extracting active components from plants by rural people is water. It is used as an extraction solvent most often because of its availability, low cost and non-toxicity. However, solvents such as ethanol, methanol and acetone exist. They are most often used in the laboratory in their simple or mixed forms (water / organic solvent). It seemed to be that these conditions can influence the phytochemical compounds and the antioxidant activity of the dry extracts of the calyx and corolla of *Bombax costatum*. The present study objective was to evaluate the effect of drying mode and extraction solvents on some phytochemical compounds and the antioxidant activity of calyx and corolla *Bombax costatum* Pellgr. and Vuillet (Bombacaceae) extracts.

2. Materials and methods
2.1 Material
The calyxes and corollas of *B. costatum* used for this work were randomly harvested at maturity from a farm in Yamoussoukro, Centre 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 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 at 30 °C.

2.2 Preparations of the raw powders
Raw powders were obtained according to the method of El Abdali (2015) [10] slightly modified. Batches of calyx and corolla were divided into three parts (A, B and C for each batch). Part A drying was carried out in sun,7 hours per day respectively during 3 and 4 days for calyxes and corollas. In the shade (Part B) of the laboratory, calyx and corolla were dried 7 hours per day for 4 and 5 days respectively. The last part (Part C) drying was carried out in a ventilated oven (MEMMERT) at 60 °C for 8 h for the calyxes, and 8 h 30 min for the corollas. The dried calyxes and corollas obtained were respectively ground in a wooden mortar for 2 hours for and 1 hour 20 minutes. The different crude powders obtained were sieved using an AFNOR sieve with a mesh size of 250 µm. The raw powder samples were packaged in different glass vials previously dried in a ventilated oven (MEMMERT) at 45 °C for 24 hours and labelled. They were then hermetically sealed and stored in a desiccator (Basic Line) at 25 °C.

2.3 Phytochemical compound extraction of the raw powders

Extraction was made following method of Collin *et al*. (2011) [7] slightly modified. Raw powders of calices and corolla of *Bombax costatum* were macerated in a 500 ml of different solvents such as water, ethanol, methanol, acetone and mixtures of water + ethanol (70:30, v / v), water + methanol (70:30, v / v) and water + acetone (70:30, v / v) in a beaker. The mixture was filtered with Whatman filter paper no. 42 to the removal of dust.

The evaporation of solvents was carried out using rotavapor at 40 °C. Filtered and dried extract was packaged in glass tubes hermetically sealed and kept at 25 °C temperature for further use.

2.4 Preparation of aqueous plant extract

14 g of dried calyx or corolla extract of *Bombax costatum* flower was dissolved in 100 ml of distilled water. The resulting solution was homogenized by manual stirring for 2 min at room temperature (25 °C) to obtain an aqueous plant extract.

2.5 Determination of total phenol content

Total extracted polyphenol content was determined according to the Folin-Ciocalteu method reported by Singleton and Rossi (1965) and modified by Wood *et al.* (2002) [34]. 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).

2.6 Determination of total flavonoid content

The total flavonoids content was determined using the Dowd method (Meda *et al.,* 2005) [20]. 5 mL of 2 % aluminum trichloride ($AlCl_3$) in methanol 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 5 mL extract solution with 5 mL 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.

2.7 Determination of total carotenoid content

The total carotenoid levels of the dry vegetable extracts of calices and corolla of the Bombax costatum flower were determined according to the method of Herrero-Martinez *et al*. (2006) [14]. Ten (10) g of the plant dry extract was dissolved in 20 mL of tetrahydrofuran (1.25 %). The mixture, well homogenized by vortex for 2 min, was centrifuged at 1500 rpm for 5 min. Then, fifteen (15) mL of dichloromethane and 15 mL of sodium chloride (10 %, w / v) were added to the collected supernatant in a 100 mL vial. After 2 minutes of manual stirring at room temperature (25 °C), the organic layer was collected, and the solvent was evaporated under nitrogen vapor. The extraction was repeated twice. The residues were dissolved in 5 mL of tetrahydrofuran (1.25 %) and diluted 1/40 with distilled water. The absorbance of this reaction medium was read at 461 nm with Spectronic 20 spectromhotometer genesys TM. The total carotenoid content was expressed in mg / 100 g of carotenoids with reference to a calibration curve obtained from a solution of β-carotene (3 µM).

2.8 Measurement of antioxidant activity by DPPH

Determining antioxidant activities of solids calyxes and corollas of Bombax costatum flower was performed according to the method Marinova and Bachvarov (2011) [19] using 1,1-diphenyl -2 -picrylhydrazyl (DPPH). A volume of 2.9 mL of DPPH was added to an aliquot of 100 µL of an ethanolic solution (5 mg / 250 mL, w / v) of the plant dry extract. The mixture, vigorously vortexed at room temperature (25 °C), was incubated at this location in the dark for 5 minutes. The discoloration of the DPPH was translated by a decrease in the absorbance measured at the JASCO V-530 UV-Vis spectrophotometer at 515 nm up to the plateau stage. A blank test was performed with ethanol (95 %). The rate of DPPH was determined as follows:

$$\text{DPPH (\%)} = \frac{([A]t - [A]\ \text{extract})}{A0} \times 100$$

A_0 = initial absorbance of DPPH,

$[A]\ \text{extract}$ = initial absorbance of the extract diluted in ethanol,

$[A]t$ = absorbance of the DPPH read at time t.

The inhibition percentages thus determined made it possible to calculate the EC50 (effective concentration) which represents the concentration of the substance (extract or butylhydroxyanisole) necessary to reduce by 50 % the free radicals in the reaction medium. The EC50 were calculated by linear regression where the abscissa was represented by the concentration of the test compounds and the ordinate by percent inhibition (PI %).

2.9 Measurement of antioxidant activity by ABTS

The Trolox Equivalent Antioxidant Capacity (TEAC) test described in Teow *et al*. (2007) [29] was used to assay the antioxidant activities of dry extracts of calyxes and corolla of *Bombax costatum*. The ABTS + radical cation was produced by mixing a solution of ABTS (8 mmol. L^{-1}) and a solution of $K_2S_2O_8$ (3 mmol. L^{-1}) (1 / 0.5, v / v). The mixture was then incubated for 16 h in the dark at room temperature (25 °C). Then, 0.1 mL (standard or extract) diluted in methanol (1/10, v / v), was added to 3.9 mL of the diluted ABTS solution. The mixture was vigorously vortexed for 2 min following by incubation for 6 min in the dark at room temperature (25 °C). The absorbance of the mixture was read in the Jasco V-530 UV-visible spectrophotometer at 734 nm. The results were expressed in µmol. g^{-1} TE (Trolox Equivalents). The percent degradation of ABTS by Trolox was compared to that of the sample. Percentage degradation A (%) of ABTS was expressed by using following mathematical formula,

$$\text{A (\%)} = \frac{\text{A(blank)} - \text{A(extrait)}}{100} \times \text{A(blank)}$$

A(blank) = blank absorbance after incubation;
A(extrait) = extract absorbance at 734 nm after incubation

2.10 Statistical analysis
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.

3. Results
3.1 Drying method and type of solvent effect on the rate of polyphenols and total flavonoids of calyx dry extracts
The methanolic extracts, ethanolic, acetone, aqueous, hydro-methanolic, hydro-ethanolic and hydro-acetone of calyx dried in the shade, direct sunlight and in an oven at 60 °C contain polyphenols and flavonoids rates significantly ($p < 0.05$) different (Table 1). The hydro-acetonic extracts of the calices dried in the shade and in the oven contain the highest levels of total polyphenols among all the extracts of these floral organs dried in these different places and obtained with all the solvents studied. They are respectively 58.62 ± 1.2 g / L Eq AG and 101.27 ± 2.2 g / L Eq AG. The hydro-methanol extract of sun-dried calyxes contains more total polyphenols (98.23 ± 1.3 g / L Eq AG). Hydro-ethanolic extracts calyxes dried in the shade and in an oven containing more than total flavonoids (respectively 41.82 ± 0.9 g / L Eq quercetin and 18.42 ± 0.9 g / L quercetin Eq) than those dried floral organs in the same places and obtained with other solvents investigated.

The rate of total polyphenols aqueous extracts calyxes dried in the shade to sun and the oven were respectively 40.21 ± 1.6 g / L Eq AG, of 27.31 ± 0.8 g / L Eq AG and 31.49 ± 0.33 g / L Eq AG. Those of total flavonoids are respectively 21.87 ± 0.7 g / L Eq quercetin, 11.67 ± 0.2 g / L Eq quercetin and 5.32 ± 0.3 g / L quercetin Eq. The hydro-methanol extracts, hydro and hydro-ethanolic acetone contain more polyphenols and total flavonoids than those obtained with methanol, ethanol, acetone and water regardless of the drying method. Addition of 30 % water to these solvents has improved the extraction capacity of the polyphenols and flavonoids calyx of *Bombax costatum* flower dried in the shade to sun and the oven.

Table 1: Influence of the type of solvent and the drying method on the levels of polyphenols and total flavonoids of the dry extracts of the calices of the *Bombax costatum* flower

Solvent	Shadow		Sun		Oven	
	Total Polyphenols (g / L Eq AG)	Total Flavonoids (g / L Eq Quercetin)	Total Polyphenols (g / L Eq AG)	Total Flavonoids (g / L Eq Quercetin)	Total Polyphenols (g / L Eq AG)	Total Flavonoids (g / L Eq Quercetin)
Methanol	44.75 ± 0.45^{e}	28.14± 1.1^{e}	85.12 ± 0.6^{d}	17.44±0.4^{e}	93.33 ± 1.3^{d}	9.27 ± 0.61^{e}
Ethanol	48.33 ± 1.1^{d}	26.74 ± 0.3^{f}	63.27 ± 1.4^{e}	14.88 ± 0.8^{f}	91.58 ± 0.9^{e}	8.94 ± 0.18^{f}
Acetone	41.67 ± 0.26^{f}	34.22±0.1^{d}	44.33 ± 0.32^{f}	30.22±0.3^{d}	53.27 ± 0.7^{f}	15.87±0.2^{d}
Water	27.31 ± 0.8^{g}	21.87 ± 0.7^{g}	31.49 ± 0.33^{g}	11.67 ± 0.2^{g}	40.21 ± 1.6^{g}	5.32± 0.3^{g}
Methanol/Water	50.24 ± 0.9^{c}	36.14±0.3^{c}	98.23 ± 1.3^{a}	34.86±0.1^{b}	99.42±0.77^{b}	17.14 ± 0.4^{b}
Ethanol / Water	54.47 ± 1.8^{b}	41.82 ± 0.9^{a}	90.86 ± 1.1^{c}	32.21 ± 1.1^{c}	97.29 ± 1.1^{c}	18.42 ± 0.9^{a}
Acetone / Water	58.62 ± 1.2^{a}	39.65 ± 0.4^{b}	93.31 ± 0.9^{b}	36.42 ± 0.7^{a}	101.27±2.2^{a}	16.47±0.1^{c}

3.2 Influence of the type of solvent and the drying method on the rate of polyphenols and total flavonoids dry extracts of *Bombax costatum* flower petals
Shade, sun and oven drying at 60 °C of *Bombax costatum* flower petals yielded methanol, ethanolic, acetone, aqueous, hydro-methanolic, hydro-ethanolic hydro-acetone extracts, containing polyphenols and flavonoids rates significantly ($p < 0.05$) different (Table 2). The hydro-acetone extracts of the dried petals in the shade and in the oven contain rates highest total polyphenol extracts from all these dried floral organs at all locations and obtained with all studied solvents. These rates are respectively 58.18 ± 0.9 g / L Eq AG and 69.33 ± 1.2 g / L Eq AG. The hydro-methanolic extract of the sun-dried corollas contains more than total polyphenols (61.28 ± 1.4 g / L Eq AG). The highest total polyphenol content is obtained with dried corollas in an oven whose phytochemicals are extracted with the aqueous solvent-acetone. The hydro-ethanolic extracts of sun-dried and oven-dried corollas contain more total flavonoids (respectively 37.33 ± 0.92 g / L EQ quercetin and 19.75 ± 0.2 g / L EQ quercetin) than those of these same floral organs dried in the same places and obtained with the other solvents studied. While the results of the aqueous acetone extract dried one in the shade show 44.22 ± 1.1 g / L Eq AG of total flavonoids. Thus, highest total flavonoids rate is obtained with the corollas dried in the shade with phytochemical compounds extracted with aqueous-acetone solvent. The acetonic and aqueous extracts of dried corollas in the shade contain the lowest levels of polyphenols (25.42 ± 0.27 g / L Eq AG) and flavonoids (25.41 ± 0.44 g / L Eq quercetin), extracted from dried parts in the same place and obtained with the other solvents studied. The lowest total polyphenols (34.49 ± 0.27 g / L Eq AG) and the total flavonoids (8.04 ± 0.2 g / L EQ quercetin) of the oven dried corolla are obtained with acetone extract. Phytochemicals extracted from sun-dried corollas with acetone and water had the lowest concentrations of polyphenols (30.27 ± 0.43 g / L Eq AG) and total flavonoids (13.98 ± 0.3 g / L EQ quercetin) among the extracts of these same floral organs dried at the same place and obtained with the other solvents studied. The levels of polyphenols (25.42 ± 0.27 g / L Eq AG) and total flavonoids (8.04 ± 0.2 g / L EQ quercetin) are obtained with the shade-dried corollas and the oven ones, when the phytochemicals are extracted with the acetone solvent. The hydro-methanol extracts, hydro and hydro-ethanolic acetone contain more polyphenols and flavonoids than those obtained with methanol, ethanol, acetone and water regardless of the drying mode. The addition of 30 % water to these solvents improved the ability of polyphenols and flavonoids to extract sun-dried and oven dried *Bombax costatum* flower corollas. Influence of the type of solvent and the drying method on the total carotenoid contents of the dried extracts of the calices of the *Bombax costatum* flower.

Table 2: Total polyphenol and flavonoid levels of dry extracts of *Bombax Costatum* corollas by solvent type and drying mode

	Shadow		Sun		Oven	
Solvent	Total Polyphenols (g / L Eq AG)	Total Flavonoids (g / L Eq Quercetin)	Total Polyphenols (g / L Eq AG))	Total Flavonoids (g / L Eq Quercetin)	Total Polyphenols (g / L Eq AG))	Total Flavonoids (g / L Eq Quercetin)
Methanol	34.28±0.44^{e}	30.48 ± 0.7^{e}	51.62± 0.74^{e}	18.22±0.23^{e}	60.82 ± 0.7^{d}	13.74 ± 0.3^{d}
Ethanol	32.37 ± 0.8^{f}	29.27 ± 0.9^{f}	49.38 ± 0.6^{f}	15.48± 0.18^{f}	56.61 ± 1.4^{f}	10.95±0.11^{f}
Acetone	25.42±0.27^{g}	36.33 ± 0.3^{d}	30.27±0.43^{g}	20.61±0.47^{d}	34.49±0.27^{g}	8.04± 0.2^{g}
Water	38.22 ± 0.3^{d}	25.41±0.44^{g}	49.74 ± 1.1^{d}	15.42 ± 0.3^{g}	57.62 ± 1.3^{e}	11.04±0.14^{e}
Methanol/Water	53.15 ± 0.6^{c}	38.81±0.32^{c}	57.36 ± 0.9^{c}	31.18±0.12^{c}	62.75± 0.42^{c}	15.45±0.17^{c}
Ethanol / Water	55.54 ± 1.1^{b}	41.94 ± 0.7^{b}	61.28 ± 1.4^{a}	37.33±0.92^{a}	66.21±0.49^{b}	19.75± 0.2^{a}
Acetone / Water	58.18 ± 0.9^{a}	44.22 ± 1.1^{a}	59.38 ± 0.8^{b}	33.26 ± 0.4^{b}	69.33 ± 1.2^{a}	17.28 ± 0.5^{b}

Drying in the shade, in the sun and in an oven at 60 °C of the calices of the Bombax costatum flower yielded methanolic, ethanolic, acetonic, aqueous, hydro-methanolic, hydro-ethanolic and hydro- acetone containing carotenoids at significantly different ($p < 0.05$) levels (Table 3). The hydro-acetonic extracts of the calices dried in an oven and in the shade contain the highest levels of carotenoids among all the extracts of these floral organs dried in these different places and obtained with all the solvents studied. These levels are respectively 50.62 ± 1.2 mg / g DM and 72.32 ± 0.8 mg / g DM. The hydro-ethanolic extract of sun-dried calices contains more carotenoids (64,12 ± 0,71 mg / g DM) than those of these same floral organs dried at this same location and obtained with the other solvents studied. The highest carotenoid level is obtained with the shade-dried calyxes whose phytochemicals are extracted with the hydro-acetone solvent. The carotenoid levels (20.17 ± 0.9 mg / g DM, 24.71 ± 0.9 mg / g DM and 28.44 ± 0.13 mg / g DM) are obtained with dried calices respectively in the oven, in the sun and in the shade from which the phytochemicals are extracted with the aqueous solvent. The hydro-methanolic, hydro-ethanolic and hydro-acetonic extracts contain more carotenoids than those obtained with methanol, ethanol, acetone and water regardless of the drying mode. The addition of 30 % water to these solvents improved the carotenoid extraction capacity of dried, sun and oven dried *Bombax costatum* flower calices.

Table 3: Total carotenoid rates of dry extracts of *Bombax costatum* calices by solvent type and drying mode

	Shadow	Sun	Oven
Solvent		Total carotenoids (mg / g DM)	
Methanol	46.70 ± 1.15^{e}	44.23 ± 0.7^{d}	29.40 ± 1.4^{e}
Ethanol	48.53 ± 1.7^{d}	42.51 ±1.1^{e}	30.18 ± 1.8^{d}
Acetone	37.14 ± 0.33^{f}	26.32 ± 1.2^{f}	27.32 ± 1.1^{f}
Water	28.44 ± 0.13^{g}	24.71 ± 0.9^{g}	20.17 ± 0.9^{g}
Methanol/Water	66.32 ± 0.44^{c}	61.24 ± 1.47^{b}	45.21 ± 1.34^{c}
Ethanol / Water	69.16 ± 1.4^{b}	64.12 ± 0.71^{a}	48.43 ± 0.8^{b}
Acetone / Water	72.32 ± 0.8^{a}	59.75 ± 1.6^{c}	50.62 1.2^{a}

3.3 Influence of the type of solvent and the drying method on the rate of total carotenoids dry extracts of petals of the flower of *Bombax costatum*

Drying in the shade, in the sun and in an oven at 60 °C of *Bombax costatum* flower corolla gave methanolic, ethanolic, acetonic, aqueous, hydro-methanolic, hydro-ethanolic and hydro-acetone extracts containing carotenoids at significantly different ($p < 0.05$) levels (Table 4). The hydro-acetonic extracts of oven-dried and oven-dried corollas contain the highest levels of carotenoids among all the extracts of these floral organs dried in these different places and obtained with all the solvents studied. These levels are respectively 37.38 ± 0.42 mg / g DM and 50.23 ± 0.52 mg / g DM. The hydro-ethanolic extract of sun-dried corollas contains more carotenoids (41.12 ± 0.9 mg / g DM) than those of these same floral organs dried at this same location and obtained with the other solvents studied. The highest carotenoid level is obtained with the shadow-dried corollas whose phytochemical compound are extracted with the hydro-acetone solvent. The aqueous extracts of dried, sun-dried and oven-dried corollas contain the lowest levels of total carotenoids respectively (18.29 ± 0.43 mg / g DM, 20.14 ± 0.71 mg / g DM and 24.61 ± 0.42 mg / g DM) among the extracts of these same fabrics dried at the same location and obtained with the other solvents studied. The hydro-methanol extracts, hydro and hydro-ethanolic acetone contain more total carotenoids than those obtained with methanol, ethanol, acetone and water regardless of the drying method. Addition of 30 % water to these solvents improved removal efficiency of carotenoid petals of the flower of *Bombax costatum* dried in the shade, direct sunlight and in an oven 60 °C.

Table 4: Total carotenoid rates of dry extracts of *Bombax costatum* corollas by solvent type and drying mode

	Shadow	Sun	Oven
Solvents		Total carotenoids (mg / g DM)	
Methanol	40.17 ± 1.7^{e}	38.27 ± 1.4^{d}	26.14 ± 0.3^{e}
Ethanol	41.23 ± 0.41^{d}	36.33 ± 1.7^{e}	28.31 ± 0.8^{d}
Acetone	39.18 ± 0.8^{f}	29.47 ± 0.6^{f}	21.19 ± 0.7^{f}
Water	24.61 ± 0.42^{g}	20.14 ± 0.71^{g}	18.29 ± 0.43^{g}
Methanol/Water	47.30 ± 0.4^{c}	40.72 ± 1.2^{b}	33.18 ± 0.4^{c}
Ethanol / Water	48.75 ± 1.1^{b}	41.12 ± 0.9^{a}	35.75 ± 1.4^{b}
Acetone / Water	50.23 ± 0.52^{a}	39.72 ± 0.7^{c}	37.38 ± 0.42^{a}

3.4 Influence of the type of solvent on the antioxidant activities of the dried extracts of the calices of the dried oven dried *Bombax costatum* flower at 60 °C

The antioxidant activities of solids calyx of the flower of *Bombax costatum* oven dried and obtained with different solvents was evaluated by scavenging DPPH radicals (free radical 1,1-diphenyl-2-picrylhydrasyl) and ABTS (acid 2,2'Azion-bis (3-ethylbenzthiazoline sulfonic acid-6) (Figure 1). The aqueous-ethanolic extract dry calyx of the flower of *Bombax costatum* dried in an oven at 60 °C was the most active of the dry extracts obtained irrespective of the solvent and the assay method used. Thus, it appeared a strong inhibition of the DPPH radical with a percentage of 59.62 ± 1.7 % while that of the assay using ABTS is 42.48 ± 1.9 %. Acetone extract has the weakest antioxidant activity. Those obtained with DPPH and ABTS assay tests are respectively 23.12 ± 0.98% and 12.43 ± 0.43%. Whatever the test used; the decreasing order of antioxidant activities is as follows: antioxidant activities of the dry extract (AAOES) hydro-ethanol > AAOES hydro-acetonique > AAOES hydro-methanol > ethanol AAOES > AAOES methanolic > aqueous AAOES > AAOES acetone. The antioxidant activities of the hydro-acetonic, hydro-methanolic, ethanolic, methanolic and aqueous dry extracts when the DPPH is used in the assay method are respectively 55.64 ± 2.1 %, 52.62 ± 2.1 %, 41.64 ± 1.8 %, 39.54 ± 1.1 % and 25.1 ± 1.3 %. When DPPH is replaced by ABTS, the antioxidant activities are respectively 41.85 ± 1.2 %, 40.37 ± 1.6 %, 30.53 ± 0.9 %, 28, 62 ± 0.9 % and 15.47 ± 0.72 %. The antioxidant activities obtained with the free radical inhibition test using DPPH are higher than those revealed by the same test using ABTS. Statistical analysis revealed that the inhibition rates are different ($p < 0.05$) from each other.

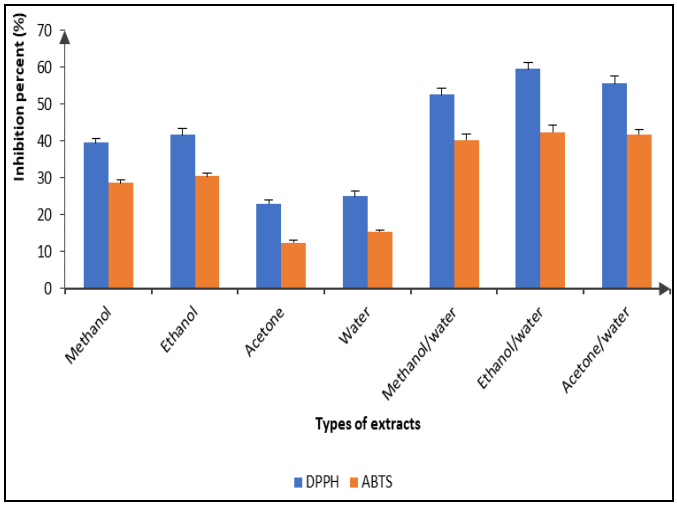

Fig 1: Influence of the type of solvent on the antioxidant activities of the dry extracts of calices of the bombax flowers dried in an oven at 60 °C

3.5 Influence of the type of solvent on the antioxidant activities of the extracts of the corollas of the *Bombax costatum* flower dried in an oven at 60 °C

The antioxidant activities of the dry extracts of petals of the flower of *Bombax costatum* dried in an oven at 60 °C and obtained with different solvents were also evaluated by scavenging DPPH radicals (free radical 1,1-diphenyl-2-picrylhydrasyl) and ABTS (2,2'Azion acid-bis (3-ethylbenzthiazoline sulfonic acid-6) (Figure 2). hydro-ethanolic dry extract of the petals of the flower of *Bombax costatum* dried in an oven at 60 °C proved to be the most active of the dry extracts obtained irrespective of the solvent and the assay method used. Thus, it appeared a strong inhibition of the DPPH radical with a percentage of 43.72 ± 1.6 % while that of the assay using ABTS is 36.48 ± 0.8 %. The aqueous extract has the weakest antioxidant activity. Those obtained with DPPH and ABTS assay tests are respectively 19.47 ± 0.32 % and 11.75 ± 0.65 %. Whatever the test used; the decreasing order of antioxidant activities is as follows: antioxidant activities of the dry extract (AAOES) hydro-ethanol > AAOES hydro-acetonic > AAOES hydro-methanol > ethanol AAOES > AAOES methanolic > AAOES acetonic > Aqueous AAOES. The antioxidant activities of the hydro-acetonic, hydro-methanolic, ethanolic, methanolic and acetonic dry extracts when the DPPH is used in the assay method are respectively 42.86 ± 1.4 % and 41.32 ± 1, 1 %, 33.87 ± 0.9 %, 30.27 ± 0.8 % and 21.30 ± 0.52 %. When DPPH is replaced by ABTS, the antioxidant activities are respectively 35.69 ± 1.7 %, 34.27 ± 1.1 %, 24.54 ± 0.72 %, 22.86 ± 0.76 % and 13.54 ± 0.37 %. The antioxidant activities obtained with the free radical inhibition test using DPPH are higher than those revealed by the same test using ABTS. Statistical analysis revealed that the inhibition rates are different ($p < 0.05$) from each other.

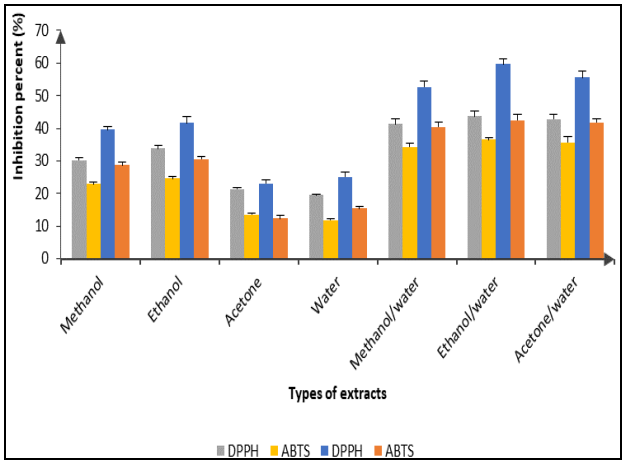

Fig 2: Influence of the type of solvent on the antioxidant activity of the extracts of the corollas of dried *Bombax costatum* flower dried in an oven at 60 °C

3.6 Influence of the drying mode on the total antioxidant activities of the hydroethanolic dry extract of the calices of the *Bombax costatum* flower

The development of the inhibition of the free radical 1,1-diphenyl-2-picrylhydrasyl (DPPH) by butylhydroxyanisole (BHA), a reference antioxidant and the hydro-ethanolic extracts of dried flower calices Bombax costatum to shade, sun and oven at 60 °C shows that the tissues studied have antioxidant activities. These biological parameters vary with the type of drying (Figure 3). At 40 µg / ml (a low concentration), the anti-radical effect of the various extracts of the calices of the *Bombax costum* flower in descending order is as follows: anti-radical effect of BHA > anti-radical effect of the extract hydro-ethanol production of oven-dried calices > anti-radical effect of the hydro-ethanolic extract of sun-dried calices > anti-radical effect of the hydro-ethanolic extract of the shade-dried calices. At the concentration of 30 µg / ml, the percentages of inhibition exceed 50 % except for hydroethanolic extracts of calices dried in the shade (44.25 %). The hydro-ethanolic extract of oven-dried calices has the best inhibitory activity (65.30 %). It is followed by the sun-dried calyx extract (60.45 %). At 100 µg / ml, the anti-radical effect of the various extracts of the calices of the *Bombax costatum* flower in descending order is the same as already obtained. Analysis of variance indicated that the drying method has a significant influence (at the 0.05 level) on the antioxidant activity.

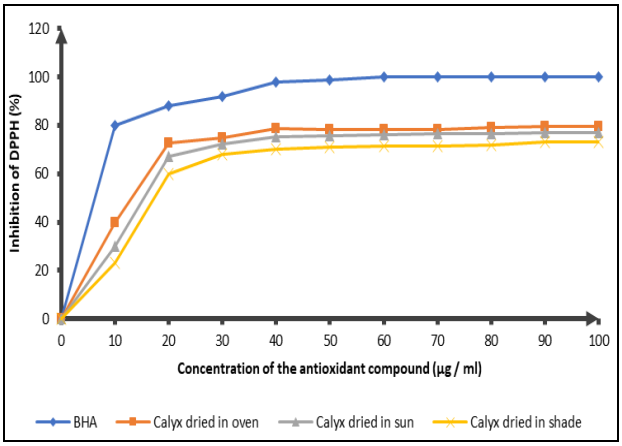

Fig 3: Influence of the drying mode on the total antioxidant activity of the hydroethanolic dry extract of the calices of the *Bombax costatum* flower

3.7 Influence of the drying mode on the total anti-oxidative activities of the hydro-ethanolic dry extract of the corolla of the *Bombax costatum* flower

The hydro-ethanolic extracts of the shade-dried, sun-dried and oven-dried corolla of the *Bombax costatum* flower influence the development of the inhibition of the free radical 1,1-diphenyl-2-picrylhydrasyl (DPPH). This situation shows that the tissues studied have antioxidant activities. These biological parameters therefore vary with the type of drying (Figure 4). At 40 µg / ml (a low concentration), the anti-radical effect of the various extracts of the corolla of the *Bombax costatum* flower in descending order is as follows: anti-radical effect of BHA > anti-radical effect of the extract hydro-ethanolic drying of oven-dried corollas > anti-radical effect of the hydro-ethanolic extract of sun-dried corollas > anti-radical effect of the hydro-ethanolic extract of the shade-dried corollas. At the concentration of 30 µg / ml, the inhibition percentages exceed 50 % except for the hydro-ethanolic extract of the shade-dried corollas (36.35 %). The hydro-ethanolic extract of the oven dried corollas at 60 °C has the best inhibitory activity (58.30 %). It is followed by the extract of sun-dried corollas (56.85 %). At 100 µg / ml (at high concentration), the anti-radical effect of the various extracts of the corolla of the *Bombax costatum* flower in descending order is the same as those already obtained. Analysis of variance indicated that the drying mode has a significant influence (at the 0.05 level) on the antioxidant activity.

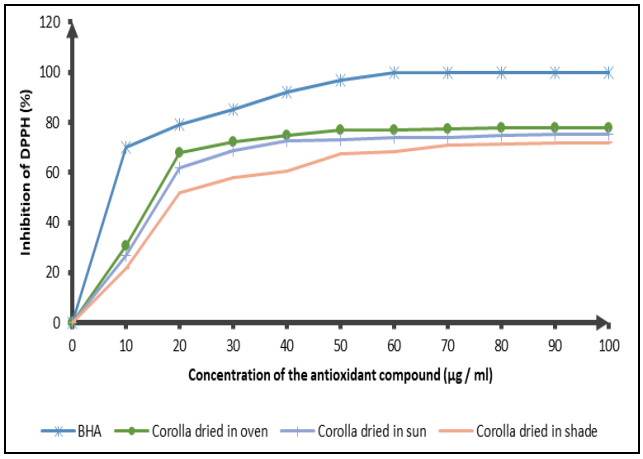

Fig 4: Influence of the drying mode on the total antioxidant activity of the hydroethanolic dry extract of the corollas of the *Bombax costatum* flower

3.8 Influence of the drying mode on the total antioxidant activity of the aqueous dry extract of the calices of the *Bombax costatum* flower

The development of the inhibition of the free radical 1,1-diphenyl-2-picrylhydrasyl (DPPH) by butylhydroxyanisole (BHA), a reference antioxidant and aqueous extracts of calices of the flower *Bombax costatum* dried at shade, the sun and the oven show that the studied tissues have antioxidant activities. These biological parameters vary with the type of drying (Figure 5). At 40 µg / ml (a low concentration), the anti-radical effect of the various extracts of the calices of the *Bombax costum* flower in descending order is as follows: anti-radical effect of BHA > anti-radical effect of the extract aqueous solution of oven-dried calices > anti-radical effect of the aqueous extract of sun-dried calices > anti-radical effect of the aqueous extract of shade-dried calices. At the concentration of 50 µg / ml, the percentages of inhibition exceed 50 % except for aqueous extracts of calices dried in the shade (48.6 %). The aqueous extract of calices dried in an oven at 60 °C has the best inhibitory activity (63.40 %). It is followed by the extract of sun-dried calices (59.30 %). At 100 µg / ml, the anti-radical effect of the different extracts of the calices of the *Bombax costum* flower in descending order is the same as those already obtained. Analysis of variance indicated that the drying mode has a significant influence (at the 0.05 level) on the antioxidant activity.

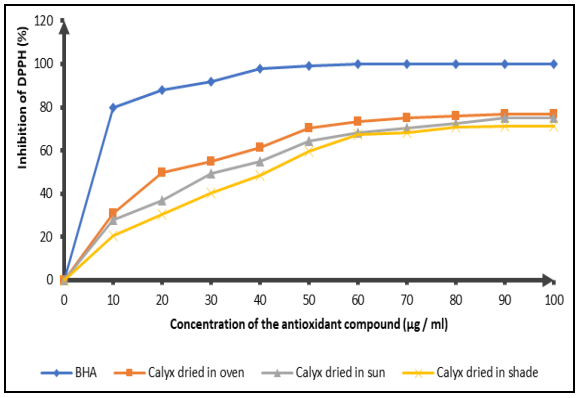

Fig 5: Influence of the drying mode on the total antioxidant activity of the aqueous dry extract of the calices of *Bombax costatum* flower

3.9 Influence of the drying mode on the total antioxidant activity of the aqueous dry extract of the corollas of the *Bombax costatum* flower

The aqueous extracts of the corollas of the *Bombax costatum* flower dried in the shade, in the sun and in the oven at 60 °C. have a different influence on the development of the inhibition of the free radical 1,1-diphenyl-2-picrylhydrasyl (DPPH). This situation shows that the tissues studied have antioxidant activities. These biological parameters therefore vary with the type of drying (Figure 6). At 40 µg / ml (a low concentration), the anti-radical effect of the various extracts of the corolla of the *Bombax costatum* flower in descending order is as follows: anti-radical effect of BHA > anti-radical effect of the extract hydro-ethanolic drying of oven-dried corollas > anti-radical effect of the hydro-ethanolic extract of sun-dried corollas > anti-radical effect of the hydro-ethanolic extract of the shade-dried corollas. At the concentration of 50 µg / ml, the percentages of inhibition exceed 50 % except for the aqueous extract of the corollas dried in the shade (46.30 %). The aqueous extract of oven-dried corollas has the best inhibitory activity (61.70 %). It is followed by the extract of sun-dried corollas (54.22 %). At 100 µg / ml (at high concentration), the anti-radical effect of the different extracts of the corolla of the *Bombax costatum* flower in descending order is the same as the others already. Analysis of variance indicated that the drying mode has a significant influence (at the 0.05 level) on the antioxidant activity.

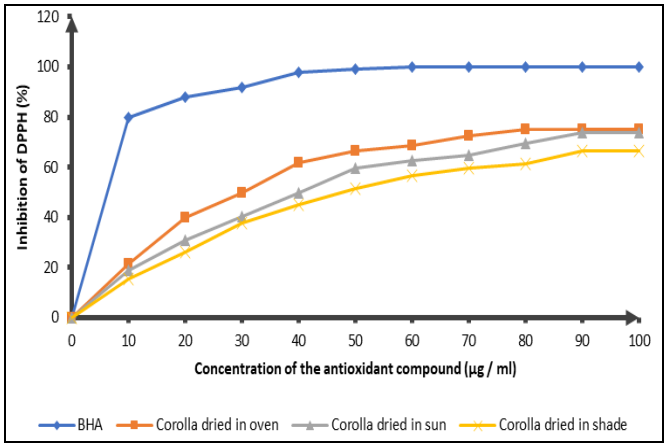

Fig 6: Influence of the drying mode on the total antioxidant activity of the aqueous dry extract of the corollas of the *Bombax costatum* flower

3.10 Influence of the drying mode on the EC50 of the hydro-ethanolic dry extract of the calices of the *Bombax costatum* flower

Drying in the shade, in the sun and in an oven at 60 °C significantly ($p < 0.05$) affect the EC50 of the hydro-ethanolic extracts of the calices of the *Bombax costatum* flower (Figure 7). BHA (butylhydroxyanisole) has a lower EC50 (4.8 ± 0.7 µg / ml) than the hydro-ethanolic extracts of the calices studied. The hydro-ethanolic dry extract of calices dried in an oven at 60 °C has an EC50 (8.5 ± 0.82 µg / ml) lower than that of the same fabrics dried in the sun and in the shade of which EC50 are respectively 15 ± 1 µg / ml and 35 ± 1.19 µg / ml. The hydroethanolic dry extract of the shaded calyx has the highest EC50 (35 ± 1.19 µg / ml) among all the extracts studied. Analysis of variance indicated that the drying mode has a significant influence (at the 0.05 level) on the EC50.

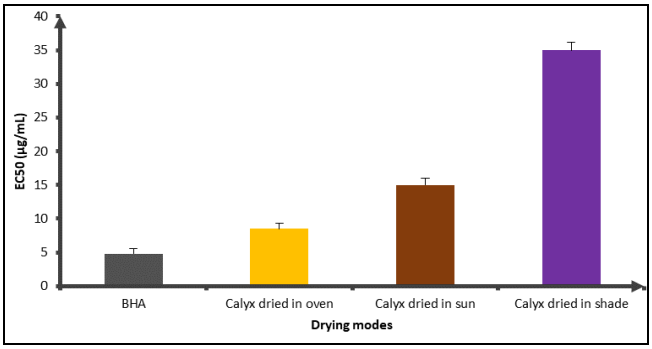

Fig 7: Influence of the drying mode on the EC50 of the hydroethanolic dry extract of the calices of the *Bombax costatum* flower

3.11 Influence of the drying mode on the EC50 of the hydroethanolic dry extract of the corollas of the *Bombax costatum* flower

Drying in the shade, in the sun and in an oven at 60 °C significantly ($p < 0.05$) affect the EC50 of the hydro-ethanolic extracts of the corolla of the *Bombax costatum* flower (Figure 8). BHA (butylhydroxyanisole) has a lower EC50 (4.8 ± 0.7 µg / ml) than the hydroethanolic extracts of the studied corollas. The hydro-ethanolic extract of the oven-dried corollas at 60 °C has a lower EC50 (12.5 ± 0.95 µg / ml) than the same sun-dried and shade-dried tissues are respectively 21 ± 1.1 µg / ml and 43 ± 1.7 µg / ml. The hydroethanolic extract of the shade-dried corollas has the highest EC50 (43 ± 1.7 µg / ml) among all the extracts studied. Analysis of variance indicated that the drying mode has a significant influence (at the 0.05 level) on the EC50.

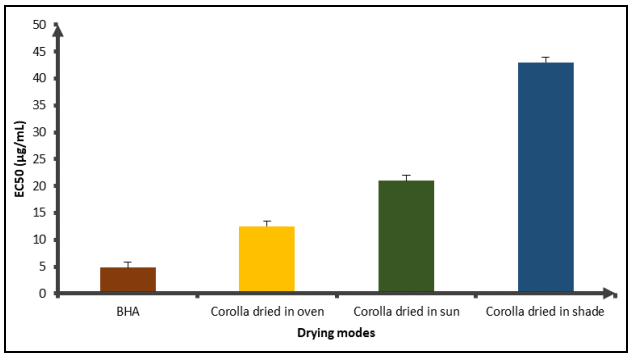

Fig 8: Influence of the drying mode on the EC50 of the hydroethanolic dry extract of the corollas of the *Bombax costatum* flower

4. Discussion

The drying method (in the shade, in the sun and in the oven at 60 °C) had an influence on the levels of the polyphenols, flavonoids and total carotenoids of the calyxes and corollas of the *Bombax costatum* flower, whatever the type of solvent (methanol, ethanol, acetone, water, water-acetone, water-methanol and water-ethanol). This situation suggests that the drying of these plant tissues should not be done at random. It must consider not only the molecule sought, but also the available solvent and the plant to be dried. This result is like that found by El Abdali (2015) [10] who worked on the flower *Matricaria chamomilla* of the family Asteraceae.

The link between the desired molecule, the type of plant, the available solvent and the place of drying can be explained by the fact that the dry hydro-acetonic extracts of the calices dried in the shade and in the oven at 60 °C contain the highest levels of polyphenols and carotenoids among all the extracts of these floral organs dried in these different places and obtained with all the solvents studied. But if the drying is done in the sun to obtain a better rate of total polyphenols and carotenoids, it is necessary to use respectively hydro-methanolic and hydro-ethanolic solvents. When the calyxes are replaced by the corollas of the same flower, to obtain a better rate of total polyphenols, these tissues must be dried in an oven at 60 °C.

The hydroethanolic dry extracts of dried calyxes in a shade and oven at 60 °C contain the total flavonoid levels. The promotion of the extraction of phenolic compounds, flavonoids and carotenoids from calices and corollas of *Bombax costatum* flower dried in the shade, in the sun and in the oven in hydro-methanolic, hydro-ethanolic and hydro-acetonic than in the solvents methanol, ethanol, acetone and water makes it possible to say that the addition of 30 % water to these organic solvents has made it possible to improve their extraction capacities. This dilution made it possible to transform the unfavorable media into highly favorable media for the solubilization of polyphenols, flavonoids and carotenoids (Mohammedi and Atik, 2011) [21]. This situation could be explained by the presence of water in mixed solvents which would increase the permeability of plant tissues and promote the phenomenon of mass diffusion in the extraction environment (Arimboor and Arumughan 2011, Trabelsi *et al.* , 2010) [3, 31] very important molecules such as polyphenols and carotenoids. This result is similar to those obtained by Mohammedi and Atik (2011) [21] and Bourgou *et al.* (2017) [6] who worked on the leaves of *Tamarix aphylla* and *Euphorbia helioscopia* respectively.

Among the ineffective solvents (methanol, ethanol, acetone and water) for the extractions of polyphenols and flavonoids from the calices and corollas of the *Bombax costatum* flower dried in the shade, in the sun and the oven at 60 °C, the Water is the bad solvent while it is widely used in households for extractions of the active ingredients of many plants including the calyxes and corolla of the flower *Bombax costatum*. Its high utilization is related not only to its availability but also to its relatively low cost and non-toxicity compared to other solvents. His choice is not usually based on any chemistry principle. Yet, according to Mahmoudi *et al.* (2013) [18], most polyphenols are poorly water soluble. Their extractions therefore require appropriate organic solvent mixtures with water. As for Saha *et al.* (2015) [27], they revealed that the solubility of carotenoids in organic solvents such as alcohol, in general, is high compared to the solubility in water, since most of these substances are insoluble or poorly soluble in water. water. In general, these phenolic compounds contained in plants are polar compounds, which are usually extracted with polar solvents such as aqueous ethanol (Wissam *et al.*, 2012) [33]. Despite this essential knowledge on the extractions of phenolic compounds and carotenoids, it has been noted that the water solvent has made it possible to extract phenolic compounds and carotenoids. The latter are therefore water-soluble but not predominant in the plant cells tested. This situation suggests that the calyx and corolla of the *Bombax costatum* flower contain a variety of polyphenols and carotenoids with different polarities. This situation is supported by the profile of molecules highlighted in this work. The low solubility of active principles of certain plants in water has already been demonstrated by Penchev (2010) [25] and Hamia *et al.* (2014) [13] who studied respectively the leaves of Melissa and the stems and flowers of *Rhanterrium adpressum*.

The levels of polyphenols and total flavonoids of the highest corollas are obtained with the hydro-acetone solvent. This result suggests that the hydro-acetone solvent is the best solvent for extracting polyphenols and flavonoids from the corolla of the *Bombax costatum* flower. In the case of calyxes of the same flower, a contrary result has been obtained. Polyphenols and flavonoids do not have the same best solvent. Their best extraction solvents are respectively hydro-ethanol and hydro-acetone. This situation shows that the extraction of the molecules of the floral organs of

Bombax costatum varies not only according to the floral organ but also according to the desired molecule and the solvent. The solvent which is a very important influence parameter will thus be added to those already highlighted by Aganga (2001) [1] and Pedneault *et al*. (2001) [24] and which are extrinsic factors such as geographical and climatic factors, genetic factors, but also the degree of maturation of the plant and the duration of storage. This result has already been reported by Allam (2015) [2] and Ebrahimi *et al*. (2008) [9] who respectively studied the leaves of two species *Hyoscyamus albus* and *Hyoscyamus muticus*.

For this study, like many others already carried out in this thesis, several solvents could be retained. These are hydro-ethanol, hydro-methanol and hydro-acetone solvents because of their high extraction performance of the molecules studied. Of these, the hydro-methanol solvent is very toxic. His handling requires a lot of precautions that sometimes can escape the manipulators. In addition, it is just like acetone, a pollutant. For further work, hydro-ethanol solvents and water were retained. This choice is explained by the fact that ethanol and water have the advantage of being non-polluting, less expensive and non-toxic compared to other solvents such as methanol (Jokić *et al*., 2010) [17]. In addition, water, even though it is not among the best carotenoid and polyphenol extraction solvents for the calyx and corolla of the Bombax costatum flower, remains the most widely used solvent in laboratories and households.

Given the complexity of the oxidation processes and the diverse nature of the antioxidants, with both hydrophilic and hydrophobic compounds, there is not a universal method by which the antioxidant activity can be quantitatively measured in a very precise way. Most often, the responses of different and complementary tests must be combined to give an indication of the antioxidant capacity of the test sample (Tabart *et al*., 2009; Hua *et al*., 2009) [28 ,15].

Thus, the antioxidant activities of the dry extracts of calices and corolla of the *Bombax costatum* flower were evaluated using two methods: The discoloration of the radical cation ABTS (2'2-acid, azino-bis- (3-ethylbenzothiazoline) 6-sulfonic acid) and the 2,2'-diphenyl-1-picyl hydrazyl radical test (DPPH). Thus, it has been noted that the dried hydro-ethanolic extracts of calices and corollas of the sun-dried *Bombax costatum* flower have more antioxidant activities than the hydro-acetonic and hydro-methanolic dry extracts very rich in polyphenols, flavonoids and total carotenoids irrespective of the dosing method used. This situation suggests that the hydroethanolic dry extracts of the calyx and corolla of the *Bombax costatum* flower contain in addition to the polyphenols, flavonoids and carotenoids assayed in this work other molecules with antioxidant activities and which are specifically hydro ethanosolubles. This result agrees with that reported by Cristina *et al*. (2009) [8] who worked on grape seeds. The development of the inhibition of the free radical DPPH by BHA and by the hydroethanolic dry extracts of the calyxes and corolla of the *Bombax costatum* flower at different drying modes was studied. The control used in this study is BHA, a molecule that is practically pure commercially available. Its antioxidant activity exceeds those of the hydro-ethanolic dry extracts of the calices and corollas of the *Bombax costatum* flower, which are crude extracts. A partial purification of these hydro-ethanolic dry extracts could exalt the inhibitory activities of the free radical DPPH. At concentrations of less than or equal to 40 µg / ml (a low concentration), the anti-radical effect of the various extracts of the calyx and corolla of the *Bombax costum* flower in descending order is as follows: anti-radical effect of hydro extracts -ethanolic dry calyxes and oven-dried corollas) anti-radical effect of dry hydro-ethanol extracts of sun-dried calices and corollas (anti-radical effect of hydro-ethanolic extracts of calices and dried corollas 'shadow. This result, supported by the values of the effective concentration (EC50) of the hydro-ethanolic extracts of the calyxes and corolla of the *Bombax costatum* flower, shows that the drying in the shade of the calyxes and corolla of the *Bombax costum* flower does not is not a good technique for getting good antioxidant activity. In households without an oven, it is best to use sun drying. Otherwise, the best method of drying is that of the oven at 60 °C which was still good for obtaining large amounts of total polyphenols in extracts of plant tissues studied. This situation shows that in general, the antiradical effect of an extract increases with the increase of the total polyphenol content in the extract, which leads to say that the antioxidant effect of a plant is in relation with the amount of polyphenols present as pointed out by Jayaprakasha and Patil (2007) [16] and Falleh *et al*. (2008) [11]. Indeed, according to Falleh *et al*. (2008) [11], there is a very significant correlation between the content of polyphenols (total polyphenols, flavonoids and condensed tannins) and the anti-free radical activity against the DPPH radicals, of the methanolic extract of *Cynara cardunculus*, a plant of the family Asteraceae. This correlation has also been demonstrated between the polyphenol levels and the antioxidant activities of grape seed extracts (Popovici *et al*., 2009) [26]. These extracts have EC50 which are lower than 50 µg / ml, so they are considered as better antioxidants according to Gulcin *et al*. (2012) [12]. Referring to these EC50, the hydroethanolic dry extracts of the calyx and corolla of the *Bombax costatum* flower can be considered as excellent antioxidants more powerful than those obtained with the methanolic extracts of the dried *Bombax ceiba* flower in the sun (EC50 = 87 µg / ml; (Vieira *et al*., 2009) [32]).

6. Conclusion

The drying method (shade, sun and oven at 60 °C) had an influence on the levels of polyphenols, flavonoids and total carotenoids of calices and corollas of the *Bombax costatum* flower. whatever the type of solvent (water, acetone, methanol, ethanol, water-acetone, water-methanol and water-ethanol). This situation suggests that the drying of these plant tissues should not be done at random. It must consider not only the molecule sought, but also the available solvent and the plant to be dried. The best levels of total flavonoids and carotenoids in the dry extracts of the calyx and corolla of *Bombax costatum* are obtained when these plant tissues are dried in the shade and the extraction carried out with respectively hydro-ethanol solvents (30/ 70, v/ v) and hydro-acetone (30/ 70, v/ v). As for polyphenols, the same plant tissues must be dried in an oven and the extraction must be made with the solvent hydro-acetone (30/ 70, v/v). The hydroethanolic dry extracts of calices and

corollas of the oven dried *Bombax costatum* flower have the highest antioxidant activity regardless of the dosing method used.

6. References

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