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Antioxidant activity of phenolic and flavonoid fractions of *Cleome gynandra* and *Maerua angolensis* of Burkina Faso

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ABSTRACT

Cleome gynandra L. and *Maerua angolensis* DC (Forsk) are two species of Capparidaceae family and of which the traditional uses are directed much against the inflammatory diseases, cancer and cellular ageing. The aim of this study is to measure the anti-FRAP, anti-ABTS and anti-DPPH activities through the n-hexane (n-H), dichloromethanolic (DCM), acetonitrile (ACN), ethyl acetate (EA), methanolic (MeOH) and n-butanol (n-BuOH) fractions of each species. We thus quantified the total phenolic and total flavonoids in different fractions. The whole of the tests were evaluated with a sample concentration at 100 µg/mL. Thus, three methods i.e., FRAP, ABTS and DPPH were used to estimate the total antioxidant capacity of the plants fractions. The total phenolic and total flavonoid were also determined spectrophotometrically using Folin-Ciocalteu and $AlCl_3$ reagents, respectively. Butanolic fractions were give the bests anti-FRAP (535.961 µmol AAEAC/g of fraction), anti-ABTS (155.868 µmol TEAC/g of fraction) and anti-DPPH (81.109 µmol QEAC/ g of fraction) activities. The best phenolic content was obtained with n-butanol fraction (133.023 mg GAE/g of fraction, for *C. gynandra*), followed of methanolic fraction of *M. angolensis* (128.043 mg GAE/g of fraction). *C. gynandra* was give the best flavonoids contents with DCMF (50.09 %), followed of EAF (19.464 %). The obtained results allow justifying the traditional uses of these plants in the above-mentioned diseases.

INTRODUCTION

The oxidative stress defines itself as being a loss of the balance between oxidizing and antioxidants within a cell (Cardey, 2007). The responsibility of these oxidative stresses belongs to amputees to the free radicals (Coulidiati, 2010). The latter are a member of reactive species of the oxygen or of some nitrogen, which play a very important role in diverse pathologies as the inflammatory cryptogenetic diseases of the bowel, atherosclerosis, the cancer and the cellular ageing (Wu et Ng, 2008; Gagliardi et al., 2009; Havlik et al., 2010; Bangou et al., 2011). The previous investigations show that among the recognized biological potentialities of plants, comes first of all the antioxidizing activity in front of the arsenal of the free radicals which are produced in the body (Bangou, 2012). The compound antioxidants found in plants play an important role in the treatment and the prevention of the oxidative stress diseases (Bangou, 2012). *C. gynandra* and *M. angolensis* are two species of plants belonging to the family of Capparidaceae (Leroy, 1982; Millogo-Rasolodimby, 2005). The pharmacological investigations show that these plants are worldwide used for these anticarcinogenic, antioxidizing, stimulating properties of the humoral, antiinflammatory immunity (Muchuweti et al., 2007; Narendhirakannan et al., 2007; Bala et al., 2010; Kumar et al., 2012) and more particularly *C. gynandra*. In the Burkina Faso, Nacoulma (1996) listed the diverse uses of these two plants (Table 1). We note very little study on *M. angolensis*. Phytochemical investigations are showed that *Cleome gynandra* is a rich source of nutrients, especially vitamins A and C, minerals (calcium and iron) and protein (Ekpong, 2009). Other authors are showed that this plant content Hexacosanol, kaempferol (Kumar et al., 2012). In the same way, methanolic extract content free gallic acid, gallotannins, Saponins and iridoid (Moyo et al., 2013). These polyphenolic compounds are well known for their antioxidizing properties (Li et al., 2003; Gülçin, 2006; Meda, 2010; Bangou et al., 2012).

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Table. 1: Traditional use and part used of *M. angolensis* and *C. gynandra*

Species	Traditional use	Part used	References
C. gynandra	-Foods poisonings, Rheumatism, sexual asthenia, headaches, otitis, stimulant, antibacterial, snake bite.	S-L, R	Nacoulma, 1996
	-Disinfectants	L	
	-Antihelmintic, antiinflammatory	Se, Wp	Bala et al., 2010
	-The juice extracted from the leaves is dropped into the ear to get relief from toothache	L L	Narendhirakannan *et al*., 2007 Hebbar *et al*., 2004
M. angolensis	-Stomach aches, asthenia	L	Nacoulma, 1996
	-Fever, aches and general malaise	L	Mothana *et al.*, 2009
	-Skin rashes, sores, womb cleansing, Sexually transmitted infection	R	Okatch *et al*., 2012

However the quality and the quantity of the secondary metabolites of plants are function of geographical conditions and of the origin of the plant (Millogo, 2008). The objective of our study is to measure: (1) the antioxidizing activity of the different fractions of these plants through the FRAP, ABTS and DPPH methods; (2) to quantify the phenolic and the flavonoids contents in six types of fractions.

MATERIALS AND METHODS

Chemicals and reagents

All reagents were of analytical grade. Folin-ciocalteu reagent, NaH_2PO_4, Na_2HPO_4, sodium carbonate, aluminium trichloride and gallic acid were purchased from Sigma-Aldrich Chemie (Steinheim, Germany).

2,2-diphenyl-picrylhydrazyl (DPPH), 2,2'-azinobis (3-ethylbenzothiazoline-6-sulphonate) ABTS, trichloroacetic acid, potassium persulfate, acetonitrile, methanol, acetone, n-hexane, n-butanol, dichloromethane and ethyl acetate were supplied by Fluka Chemie (Buchs, Switzerland). Potassium hexacyanoferrate $[K_3Fe(CN)_6]$ was from Prolabo (Paris, France) and ascorbic acid was from Labosi (Paris, France).

Plants materials

The whole plant of *Cleome gynandra* L. and leaves of *Maerua angolensis* DC were collected in May 2011 at Gampella, 25 Km East from Ouagadougou (Burkina Faso).

The plant was botanically identified by Professor Millogo-Rasolodimby from the plants Biology Department of the University of Ouagadougou. Voucher specimens (MR_03 and MR_04) was deposited in the OUA herbarium of the CIB (Centre d'Information sur la Biodiversité), UFR/SVT of the University of Ouagadougou.

Extraction and fractionation

The dried and powdered leaves of *M. angolensis* (50 g) and whole plant of *C. gynandra* (50 g) were extracted with 500 mL of acetone 80% for 24 h under mechanical agitation (SM 25 shaker, Edmund BÜHLER, Germany), at room temperature. After filtration, acetone was removed under reduced pressure in a rotary evaporator (BÜCHI Rotavapor R-200, Switzerland) at 40°C. The aqueous extracts were subjected to sequential liquid-liquid extraction with n-hexane, dichloromethane, acetonitrile, ethyl acetate, methanol and n-butanol. Each fraction was then collected and concentrated to dryness under reduced pressure to obtain n-hexane fraction (n-HF), dichloromethane fraction (DCMF), acetonitrile fraction (ACNF), ethyl acetate fraction (EAF), methanol fraction (MeOHF) and n-butanol fraction (n-BF).

ANTIOXIDANT ACTIVITY DETERMINATION

Iron (III) to iron (II) activity (FRAP)

The FRAP assay was done using the method as described by Meda et al. 2010. Absorbencies were read at 700 nm and Ascorbic acid was used to produce the calibration curve ($y = 0.0094x + 0.082$; $r^2 = 0.9983$; $p<10^{-4}$). The iron (III) reducing activity determination was performed in triplicate and expressed in µmol ascorbic acid equivalent (AAEAC) per g of fraction.

ABTS radical cation decolorization assay

The radical scavenging capacity of antioxidants for the ABTS (2,2'-azinobis-3- ethylbenzothiazoline-6-sulphonate) radical cation was determined as described by Meda et al., 2010 with some modifications. 20 µL of the diluted extracts or fractions (100 µg mL-1 in methanol) was allowed to react with 190 µL of fresh $ABTS^{\bullet+}$ solution and the absorbance was taken 15 min after initial mixing. Ascorbic acid was used as standard ($y = -0.0138x + 0.7539$; $r^2 = 0.9980$; $p<10^{-3}$) and the capacity of radical cation scavenging was expressed as µmol trolox equivalent (TEAC) per g of fraction.

DPPH radical scavenging activity

The ability of the extract to scavenge the radical DPPH (2,2-diphenyl-1-picrylhydrazyl) was evaluated as described in the literature (Lamien-Meda et al., 2008). The antioxidant content was determined using a standard curve of quercetin ($y = -0.0508x + 0.3938$; $r^2 = 0.9972$; $p<10^{-4}$). The results were expressed as µmol of quercetin equivalent (QEAC) per g of fraction.

PHYTOCHEMICAL ANALYSIS

Determination of total phenolics and total flavonoids

Total phenolics

Total phenolics of fractions were determined by Folin-ciocalteu reagent method (Meda et al., 2010). The standard

calibration curve was plotted using gallic acid (y = 0.0045x + 0.061; r^2 = 0.9996; $p<10^{-4}$). The absorbencies were measured at 760 nm against a water blank using an Epoch microplate rader (BIOTEK Instruments, USA). The mean of three readings was used and the results expressed as mg of gallic acid equivalents (GAE) per 100 mg of fraction.

Total flavonoids

The total flavonoids were evaluated by the aluminium trichloride method (Meda et al., 2010). Quercetin was used as reference compound to produce the standard curve (y = 0.0251x + 0.088; r^2 = 0.9998; $P<10^{-4}$). The absorbencies reading at 415 nm were taken after 10 min against a blank using an Epoch microplate rader (BIOTEK Instruments, USA). The results were expressed as mg of quercetin equivalent (QE) per 100 mg of fraction.

Statistical Analysis

The data were expressed as Mean ± Standard deviation (SD) of three determinations. Statistical analysis (ANOVA with a statistical significance level set at $p<0.0001$ and linear regression) was carried out with XLSTAT 7.1.

RESULTS AND DISCUSSION

Antioxidant investigations

Since the 1970s, the oxidative stress (oxidizers > antioxidants) was considered as a key phenomenon which the doctors and the biologists implied in numerous human diseases (Coulidiati, 2010). This imbalance appears during an overproduction of the reactive oxygenated species or is by failure of the antioxidant system (Dickinson and Forman, 2002). Thus, we estimated the antioxidant activity of our various fractions of plants through the methods FRAP, ABTS and DPPH.

FRAP method allows to measure the fitness of the phenolic to reduce the ions Fe (III) to Fe (II). At this level the results varied from 119.572 to 535.961 µmol AAEAC /g of fraction. The figure (1A) showed that the best reducing activities per gram of fraction were obtained with the n-butanol fraction of *C. gynandra* (535.961 µmol AAEAC) and methanol fraction of *M. angolensis* (435.798 µmol AAEAC). The weakest activity is given by the acetonitrile fraction (119.572 µmol AAEAC) of *C. gynandra*, followed by the DCM fraction (150.723 µmol AAEAC) of *M. angolensis*.

The antiradicalaire activity by the ABTS method such described by Meda et al. (2010) was used to determine the ability of our various fractions to scavenge the free radicals. The values of the antioxidant capacity ranged from 29.538 to 155.868 µmol TEAC/g of fraction for *C. gynandra* and that of *M. angolensis* fractions were 22.947 to 117.947 µmol TEAC/g of fraction (Figure 1B).). Among the tested fractions, the best activities "ABTS cation radical scavenger" were obtained (*C. gynandra*) in the n-butanol fraction and ethyl acetate fraction with 155.868 and 140.949 µmol TEAC /g of fraction, respectively. As regards the fractions of *M. angolensis*, the best activities are detained by the n-butanol fraction (117.947 µmol TEAC /g of fraction) followed by the methanol fraction (105.101 µmol TEAC /g of fraction). The weakest activities for two plants are given the dichloromethane and n-hexane fractions. Compared with the activities obtained by FRAP method, the n-butanol fraction gave the best antiradicalaire activity. Finally we estimated the antioxidant power of our fractions by the DPPH method as described by Meda et al. (2010). The results varied from 3.784 (*M. angolensis*) to 81.109 µmol QEAC/g of fraction (*C. gynandra*) in different fractions both plant species (Figure 1C).

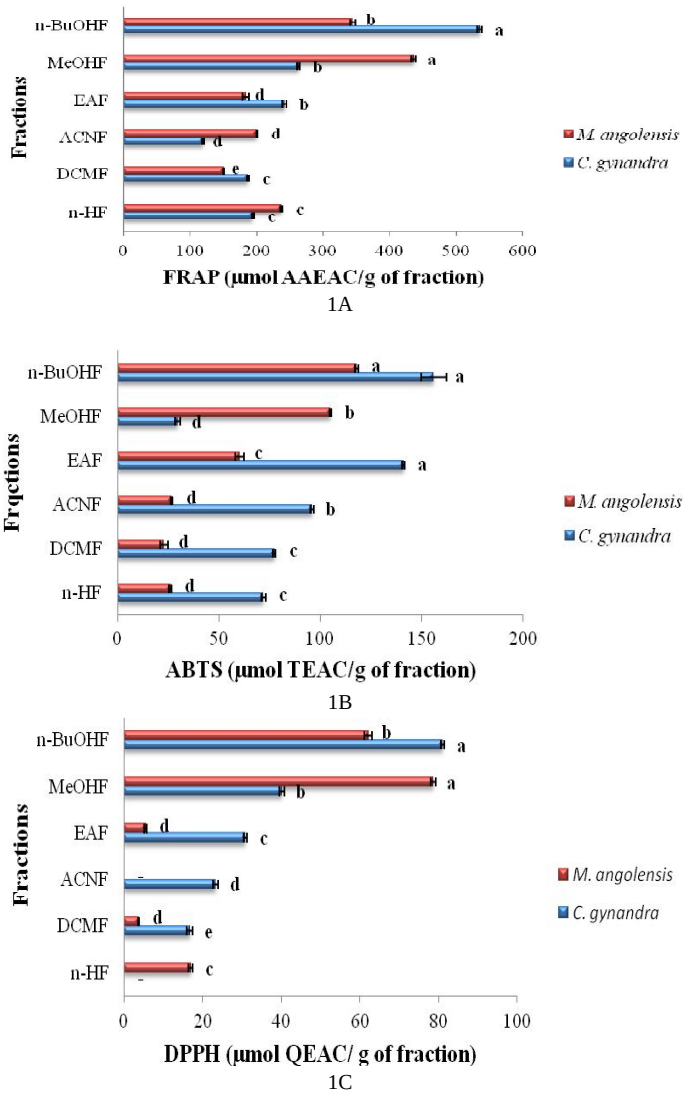

Fig. 1: Antioxidant activities obtained using the FRAP (Figure 1A), ABTS (Figure 1B) and DPPH (Figure 1C) methods on *Cleome gynandra* and *Maerua angolensis* fractions. n-HF: n-hexane fraction; DCMF: dichloromethane fraction; ACNF: acetonitrile fraction; EAF: ethyl acetate fraction; MeOHF: methanol fraction; n-BF: n-butanol fraction. Values are mean ± SD (n = 3). Different letters indicate significant difference ($p < 0.0001$).

The strongest activities were obtained by the n-butanol and methanol fractions with values superior or equal to 40 µmol QEAC /g of fraction. The others fractions have given the values lower or equal to 30 µmol QEAC /g of fraction. In the same way, Tatsimo et al. (2012) are investigated kaempferol rhamnoside derivatives anti-DPPH activity against MeOH, AtOAc and hexane

extracts. They found the best anti-DPPH activity with MeOH extract and the weak ones with hexane extracts (Tatsimo et al., 2012). Our results corroborate for what concerning the best type of extract.

By comparing the activities obtained by these three antioxidants methods of analysis, it emerges that the best results were obtained by the butanolic fractions and methanolic fractions, and it for both plant species. On the other hand, the fractions of *C. gynandra* gave the bests activities. To understand these results of analysis, we were anxious to quantify total phenolic and total flavonoids in the various fractions.

Phytochemical investigations

The polyphenolic compounds such as the total phenolic and total flavonoids are implied in various biological activities. They are the antioxidants activities (Djeridane et al., 2006; Lamien-Meda et al., 2008; Cèspedes et al., 2010), the antimicrobial activities (Shan et al., 2007; Meda, 2010), antitumor (Bangou et al., 2011) and in general way used against cardiovascular diseases (Gagliardi et al., 2009). These compounds were proportioned in six types of extracts of the two plants of the study.

Table. 2: Total phenolics and flavonoids of *Cleome gynandra* and *Maerua angolensis* fractions

Samples	Total phenolics (mg GAE/g of fraction)	Total flavonoids (mg QE/g of fraction)
Cleome gynandra		
n-HF	20.717 ± 0.702^{f}	0.648 ± 0.247^{e}
DCMF	37.380 ± 1.715^{e}	18.724 ± 1.327^{a}
ACNF	69.690 ± 0.550^{d}	6.647 ± 0.147^{d}
EAF	97.897 ± 2.552^{b}	19.055 ± 0.507^{a}
MeOHF	85.907 ± 1.232^{c}	11.179 ± 0.090^{c}
n-BuOHF	133.023 ± 2.205^{a}	16.704 ± 0.235^{b}
Maerua angolensis		
n-HF	13.473 ± 1.917^{e}	8.454 ± 0.261^{b}
DCMF	40.810 ± 3.077^{d}	3.516 ± 0.609^{c}
ACNF	3.750 ± 1.072^{f}	2.190 ± 0.220^{c}
EAF	69.510 ± 1.447^{c}	0.936 ± 0.105^{d}
MeOHF	128.043 ± 2.690^{a}	10.397 ± 0.304^{a}
n-BuOHF	107.553 ± 4.663^{b}	11.994 ± 0.074^{a}

n-HF: n-hexane fraction; ACNF: acetonitrile fraction; DCMF: dichloromethane fraction; EAF: ethyl acetate fraction; MeOHF: methanol fraction; n-BF: n-butanol fraction. Values are mean ± SD (n = 3). Different letters in the same column indicate significant difference ($p < 0.0001$).

The results obtained in *Cleome gynandra* fractions show that the total phenolics have varied from 20.717 to 133.023 mg GAE/g of fraction (Table 2). The fractions having extracted the highest total phenolics are: n-butanol fraction (133.023 mg GAE/g of fraction), ethyl acetate fraction (97.897 mg GAE/g of fraction) and methanol fraction (85.907 Mg GAE/g of fraction). The lowest contents of total phenolics were obtained with the n-hexane fraction (20.717 mg GAE/g of fraction), followed by dichloromethane fraction (37.380 mg GAE/g of fraction).

However the strongest contents of total flavonoids were obtained by the ethyl acetate fraction (19.055 mg QE/g of fraction), followed by dichloromethane fraction (18.724 mg QE/g of fraction) and that of n-butanol (16.704 mg QE/g of fraction). n-hexane fraction have presented the lowest level of total flavonoids (0.648±0.247 mg QE/g of fraction). Compared to *C. gynandra*, methanol and n-butanol have been the best solvents to extract total phenolics in the sample of *Maerua angolensis*, with levels of 128.043 and 107.553 mg GAE/g of fraction, respectively (Table 2). The ACNF presented the lowest contents of total phenolics (3.750 mg GAE/g of fraction). The same trend is obtained in flavonoids quantification. One can noticed that n-BuOH and MeOH have mainly extracted flavonoids. However the lowest content of total flavonoid is obtained by AE fraction (0.936 Mg QE/g of fraction).

Relationship between antioxidants activities, total phenolic and flavonoids contents

To know the implication of polyphenolic compounds (phenolic and flavonoids) in the antioxidant activities measured, we evaluated the correlations. Table 3 gives the correlative values obtained between FRAP, ABTS, DPPH and the phenolic and flavonoids contents. These correlations varied from 0.1235 (anti-ABTS and total flavonoids of *M. angolensis*) to 0.8677 (anti-DPPH and total phenolic of *C. gynandra*). With the exception of the correlation between ABTS and total phenolic of *C. gynandra* (0.3642), all the correlative values related with the total phenolic are higher than 0.5. On the other hand the strongest correlative value with the total flavonoids is $R^2 = 0.356$ (ABTS and *M. angolensis*). Altogether the best antioxidant activity is obtained between DPPH with *C. gynandra* ($R^2 = 0.8677$) and total phenolic, followed of that of ABTS with *M. angolensis* ($R^2 = 0.801$). The weak correlations are held by *M. angolensis* ($R^2 = 0.1235$ = ABTS and total flavonoids), followed of that of FRAP and total flavonoids ($R^2 = 0.1641$: *C. gynandra*). Correlative values between FRAP and the total phenolic on the one hand, FRAP and total flavonoids on the other hand are in the proportion ½. However that the report of the averages between total flavonoids and total phenolic is in the order of 1/10. That enables us to admit that the flavonoids took part in this precise case in the antioxidant activity. This rise of activity could be due to the contribution of the fraction n-hexane (8.454 mg QE/g of fraction) which is very weak in the case of *C. gynandra* (0.648 mg QE/g of fraction). It emerges from these investigations that total phenolic are implied in the antioxidant activities. That was brought back by several authors (Meda et al., 2010; Bangou et al., 2011). In the same way, it generally arises a controversy as for the implication of flavonoids in the antioxidant activities (Bangou et al., 2012).

Sometimes there is a correlation (Bala et al., 2011; Mishra et al., 2011; Bangou et al., 2012), often not as in a similar situation. Bangou et al., (2012) showed that this controversy would be due to several possibilities: (i) either the compounds existing in the extracts have large molecular weights or are heterosidic, (ii) or the majority of the flavonoids are not antioxidants, (iii) or there is under estimate of the flavonoids by the method of $AlCl_3$.

Several studies were made on the extracts of *C. gynandra* in connection with the antioxidant activities, but especially related to the ethanolic extracts and decoctions (Mishra et al., 2011). To our knowledge, only Moyo et al., (2013) studied the anti-DPPH

Table 3: Relationship between antioxidant activities, total phenolic and flavonoid content.

R^2	FRAP		ABTS		DPPH	
Species	*C. gynandra*	*M. angolensis*	*C. gynandra*	*M. angolensis*	*C. gynandra*	*M. angolensis*
T. phenolic	0.5816	0.6281	0.3642	0.801	0.8677	0.6487
T. flavonoids	0.1641	0.356	0.2132	0.1235	0.2614	0.2589

activity of the methanolic extract (50%) of *C. gynandra*, but they didn't measure the IC_{50}. Studies also showed that *C. gynandra* possessed an antioxidizing activity directed to the reactive oxygenated species (Uzilday et al., 2012). Kumar et al. (2012) showed that *C. gynandra* contains in particular the kaempferol and the hexacosanol. Of the same Moyo et al. (2013) highlighted gallic acid, gallotannins, saponins and iridoid. Several studies support that these above-mentioned compounds are implied in the antioxidant activities (Tiffany, 2008; Meda et al., 2010; Tatsimo et al., 2012; Bhouri et al., 2011).

According to Tiffany (2008), kaempferol is one of the most important flavonoids that inhibit heart, spinal cord and brain disease. Because monocyte chemo-attractant protein (MPC-1) is an important molecule in the initial step of atherosclerotic plaque formation, involve in cardiovascular and neurodegenerative disorder. Effect of kaempferol on catalase, super oxide dismutase, glutathione and reduced glutathione in liver and colon were conduct by Nirmala & Ramanathan (2011). They found that its action is comparable to that of irinotecan at the dose of 200mg/kg, the first line drug used in the management of colorectal cancer. Abdelwahed et al. (2007) were also showed that gallic acid has antimutagen activity directly influencing the activity of DNA repair enzymes through modulating their gene expression. Others authors showed that gallic acid were antidiabetic (Punithavathi et al., 2011), anticancer and anti-atherosclerose (Meda et al., 2010). Liu & Wang (2011) were concluded in their studies that iridoid glycosides could scavenge oxidative-free radicals, reduce the accumulation of leukocytes, and down-regulate multiple pro-inflammatory cytokines through blocking the activation of upstream target NF-κβp65 in the cascade of inflammation.

The inhibition of ROS avoids the oxidation of the lipids membrane which leads to the production of the arachidonic acid. Indeed several former studies showed that the plants extracts (fruits, bark, root, leaves) are endowed with capacity antioxidant (Lamien-Meda et al., 2008; Meda et al., 2010; Compaore et al., 2011). Antioxidant compounds importance consists in neutralizing the toxicity of free radicals. Thus they protect biological molecules in particular DNA, RNA, proteins and lipidic acids (Tiwari, 2004) whose multiple oxidations are in the beginning of many pathologies such as inflammatory diseases (Compaore et al., 2011). The ethanolic extract of *C. gynandra* leaf might exert antiinflammatory activity by modifying the lysosomal membrane in such a way that it is capable of fusing with the plasma membrane and thereby preventing the release of lysosomal enzymes, and could retard complications and spread of the inflammatory process by reducing the destruction of TNF-α during rheumatism (Narendhirakannan et al., 2007).

Others searchers are showed that ethanolic of *C. gynandra* have stimulatory effect on both humoral immunity as well as cellmediated immunity by stimulating phagocytosis, increases in delayed type hypersensitivity response from 50/kg (Kumar et al., 2012).

CONCLUSION

All 3 methods showed that butanolic and methanolic fractions have an interesting antioxidant capacity. *C. gynandra* which is an edible plant and which has beyond its antioxidant properties, an inhibiting capacity against the reactive oxygenated species and anticancerigenic, must be to encourage in consumption. The antioxidant activities shown in this present study can explain the traditional uses of these plants, in particular in the treatments of the inflammatory diseases, cardiovascular and diabetes. Our next study aims: (1) to determine the sets of the polyphenolic compounds of *C. gynandra*, (2) to isolate and identify the compounds responsible for the antioxidant activities.

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