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Phytochemistry and *in vitro* Antimicrobial, Antioxydant Activities of *Entandrophragma candollei* H.

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ABSTRACT

The research work was conducted to investigate the *in vitro* antimicrobial and antioxidant activity of extract of *E. candollei* (Family-Meliaceae). Phytochemical screening was done. Disc diffusion technique was used for *in vitro* antibacterial and antifungal screening. Minimal inhibitory concentration of promising extracts was determined by broth microdilution method. The phytochemical screening showed the presence of flavonoids compounds, tannins and saponins. The inhibition diameters were ranging from 7 to 17 mm and Minimum Inhibitory Concentrations (MIC) from 156.25 to 2500 µg/mL. The ethyl acetate and ethanolic extract revealed a high radical scavenging activity with IC_{50} ranging from 9.1026 to 11.8298 µg/mL. The Ferric reducing power activity was obtained with active concentration ranging from 12.5 to 200 µg/mL. These results suggest that extracts from *E. candollei* possess antibacterial and antioxidant activities and therefore justifies their usage in traditional medicine for the treatment of various diseases.

INTRODUCTION

The increasing incidence of infectious diseases in the world particularly in developing country has stimulated the search for new antimicrobial agents. Plants are used as drugs in many countries for the treatment of many diseases (Mahesh and Satish, 2008). A rich heritage of knowledge to preventive and curative medicines was available in ancient scholastic works included in the Atharvaveda, Charaka, Sushruta etc.

Over 50 % of all modern clinical drugs are of natural product origin (Stuffness *et al.,* 1982) and natural products play an important role in drug development program in the pharmaceutical industry (Baker *et al.*, 1995). Plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds (Hill, 1952).

E. candollei a large tropical forest tree belonging to the Meliaceae family (Notizbl, 1896) is used in Cameroon for the treatment of infectious diseases including malaria, bacterial and fungal infections (Dibong *et al.,* 2011; Din *et al.,* 2011).

Previous investigations of this plant have yielded *β*-sitosterol, methylangolensate, candollein, atomasin A, atomasin B, epoxiprieurianin and odoratone, prieuranin (Gerard *et al.*, 1997), epoxiprieurianin and prieurianin (Tchouankeu *et al.*, 1989). The present investigation is directed at to evaluate antimicrobial and antioxidant activities of rootbark, leaves and stem backs of *E. candollei*.

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MATERIALS AND METHODS

Collection of plant material

E. candollei was harvested in Yaoundé-Cameroon, (October 2014) and identified at the Cameroon National Herbarium (HNC), where a voucher specimens are deposited (1722/SRFK). Then, leaves stem and root bark were collected, cut into small pieces, dried at room temperature and powdered.

Extraction of plant material

500g of powdered leaves, root bark and stem bark were successively macerated in n-Hexane (n-Hex, 3 L), Ethyl acetate (AcOEt, 3 L), Ethanol-water (7:3, v/v) (, 3 L) and Distilled water (3 L) respectively at room temperature for 48 h. The macerate was filtered and evaporated under reduced pressure to obtain crude extracts labeled ECF1 (18 g); ECF2 (52 g), ECF3 (229 g), ECET1 (18 g), ECET2 (22 g), ECET3 (106 g), ECETT0 (23 g), ECET0 (22 g), ECER1 (13 g), ECER2 (40 g), ECER3 (121 g),ECERT0 (44 g) and ECER0 (18 g) respectively.

Phytochemical Screening

The extracts were subjected to phytochemical screening to detect the presence of alkaloids, tannins, saponins, flavonoids, glycosids, sterols, terpenoids, anthraquinone, phenols, cardiac glycosids, lipids, resins coumarins and polyphenols using protocols described by Harbone (1973).

Test bacteria

The microorganisms used in this study were strains and isolates respectively provided by American Type Culture Collection and Centre Pasteur Cameroon. They were: *Escherichia coli* ATCC 25922, *Escherichia coli* ATCC 11775, *Klebsiella pneumonia*e ATCC 700603, *Neisseria gonorrhoeae* ATCC 49226, *Pseudomonas aeruginosa* ATCC 10145, *Pseudomonas aeruginosa* ATCC 9027, *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC BAA 977, *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 51299, *Aerococcus viridans* ATCC 11563, *Salmonella enterica* and *Proteus mirabilis*.

Stock solutions and disc preparation

For the antimicrobial activity, stock solutions of plant extracts were prepared at 150 mg/mL in DMSO 10 %. Ciprofluxacin and Nyistatin were prepared in the same conditions. For disc preparation, 15 µL of each stock solution was dropped onto sterilized paper disks (6 mm diameter) and dried at room temperature for a final concentration of 1500 µg / disc.

In vitro antimicrobial screening

In vitro antimicrobial activity was screened by disc diffusion method using Mueller Hinton Agar (MHA) obtained from Mast Group Ltd. The MHA plates were prepared by pouring 15 mL of molten media into sterile plates (90 mm). The plates were allowed to solidify for 5 min and 0.1 mL of inoculum suspension was swabbed uniformly and the inoculum allowed drying for 5 minutes. The different extracts and referents drugs loaded at 1.500 µg /discs were placed on the surface of the medium and allowed to diffuse for 5 min.

The plates were incubated at 35 °C for 24 hours for bacteria and for 48 hours for yeast. Negative control was prepared using 10 % DMSO. Ciprofluxacin, and miconazol were used as positive control. At the end of incubation, inhibition zones formed around the disc were measured with a Vernier Calliper in millimeter.

Each experiment was performed in triplicate (Bauer *et al.*, 1966). The activity of extracts was classified as follows: < 8 mm no activity, 9 - 14 mm moderate activity; 15 – 19 mm good activity and > 20 mm high activity (Ponce *et al.*, 2003).

Determination of minimal inhibitory concentrations (MICs) and minimal bactericidal concentrations (MBCs)

The MIC was determined by microdilution method according to Clinical laboratory Standards Institute (CLSI) M27-A3 for yeast and M38 for bacteria, using (12 x 8 wells) microtitre plates. In the well of the plate, 100 µL of Mueller Hinton Broth for bacteria and Sabouraud Dextrose Broth for yeast were introduced. Later on, 100 µL of stock solution of extracts/fractions at 80mg/mL were added to the first well and mixed thoroughly before transferring 100 µL of the resultant mixture to the well of the second line. Serial two-fold dilutions of the test samples were made and 100 µL of inoculum standardized at 0.5 Macfarland for bacteria and at 2.5×10^3Cells/mL for yeast were introduced in the entire well containing the test substances except the column of blank which constitute the sterility control.

The concentrations ranged from 20 mg/ml to 0.15625 mg/mL for extracts and from 5 µg/mL to 0.195 µg/mL for nystatin and Ciprofloxacin. After the incubation period at 37°C for 48 hours for yeast and 24 hours for bacteria, turbidity was observed as indication of growth. Thus, the lowest concentration without turbidity was considered as the MIC. The MFC or MBC were determined by transferring 50µL aliquots of the clear wells into 150 µL of freshly prepared broth medium and incubated at 37°C for 48 and 24 hours.

The MFC and MBC were regarded as the lowest concentration of test sample which did not produce turbidity, indicating no microbial growth. All tests were performed in triplicates. The bactericidal and fungicidal effects were determined by calculating the CMB or MFC / MIC ratio (CLSI, 2008).The classification of extracts of plant material on the basis of CMI is as follows: - strong inhibition: MIC<500 mg/mL; - Moderate inhibition: MIC from 500 mg/mL to 1500 mg / mL; - Weak inhibition: MIC>1500 mg/mL (Aligiannis *et al.*, 2001).

Antioxidant activity

Free radical scavenging activity: DPPH test

Antioxidant activity of extracts was studied using 1,1-diphenyl-2-picrylhydrazyl free radical (DPPH) as described by (Mensor *et al.*, 2001). Briefly, 100 µL of extract/fraction prepared at 2000µg/mL were serially diluted and mixed with 900 µL of 0.3

mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) , to give five concentrations range from 12.5 - 200 µg/mL (12.5, 25, 50, 100 and 200 µg/mL). After an incubation period of 30 min at 25°C, the absorbance at 517 nm (the wavelength of maximum absorbance of DPPH) were recorded as $A_{(sample)}$. A blank experiment was also carried out applying the same procedure to a solution without thetest material and the absorbance was recorded as $A_{(blank)}$. The free radical-scavenging activity of each solution was then calculated as percent inhibition according tothe following equation:

RSA = [(Absorbance of DPPH - Absorbance of sample)/Absorbance of DPPH] x 100.

The radical scavenging percentages were plotted against the logarithmic values of the concentration of test samples and a linear regression curve was established in order to calculate the RSA_{50}, which is the amount of sample necessary to inhibit by 50% the free radical DPPH. Antioxidant activity of the extracts was expressed as RSA_{50} defined as the concentration of the test material required to cause a 50% decrease in initial DPPHconcentration. Ascorbic acid was used as a standard. All measurements were performed in triplicate.

Ferric reducing/antioxidant power (FRAP) assay

The ferric reducing power was determined as reported by (Padmaja *et al.*, 2011). Briefly, 400, 200, 100, 50, and 25 µL of solution of methanolic extracts (2000 µg/mL) were mixed with 500 µL of phosphate buffer (pH 6.6) and 500 µL of 1% potassium ferricyanide and incubated at 50°C for 20 min.

Then 500 µL of 10% trichloroacetic acid was added to the mixture and centrifuged at 3000 rpm for 10 min. Supernatant (500 µL) was diluted with 500 µL of water and shaken with 100 µL of freshly prepared 0.1% ferric chloride. The absorbance was measured at 700 nm. Vitamin C and BHT were used as a positive control.

Quantitative determination of total phenolic compounds
Phenol content

The total phenolic compounds were determined as described by (Ramde-Tiendrebeogo *et al.,* 2012) with slight modification. The reaction mixture consisted of 0.02 mL of extracts and fractions (2 µg/mL), 0.02 ml of 2N FCR (Folin Ciocalteu Reagent) and 0.4 mL of a 20% sodium carbonate solution. After 20min of incubation at room temperature the absorbance was measured at 760 nm. Distilled water was used as control. A standard curve was plotted using Gallic acid (0-0.2 µg/mL). All measurements were performed in triplicate.

Statistical Analysis

Data were statistically analyzed using the software SPSS 12.0 for windows and variance analysis by ANOVA coupled with Waller-Duncan test where $P<0.05$ was considered as statistically significant.

RESULTS AND DISCUSSION

Phytochemical content of plant extracts

The qualitative phytochemical screening of crude extracts showed the results presented in table 1. From these results, a number of metabolites classes were identified in all the plant extracts among which tannins, flavonoïds, alkaloids, saponins, terpenoïds, steroids and phenols.

***In vitro* antibacterial activities**

Inhibition Zone Diameters of extracts and reference antibiotic

The inhibition zone diameter ranged from 7-17 mm depending to the plants extracts and microorganisms (table 2). Extracts ECF2, ECF3 and ECER3exhibited antibacterial activity against four strains (*S. aureus ATCC* 25923, S. *aureus ATCC BAA* 977, A. *viridans ATCC 11563*, and *P. mirabilis*) with inhibition diameters ranging from 12-17 mm.

Extracts (ECF2, ECF3 and ECERT0) portrayed inhibition zone diameters ranging from 12-17 mm on *S. aureus ATCC* 25923, S. *aureus ATCC BAA* 977, *S. aureus ATCC 29213* and *E. coli ATCC 25922*.ExtractsECF2 and ECF3were active on seven strains including *S. aureus ATCC* 25923, S. *aureus ATCC BAA* 977, *S. aureus ATCC 29213*, *A. viridans ATCC 11563*, *S. entérica*, *P. mirabilis* and *E. coli ATCC 25922* with inhibition zone diameters ranging from 13 -17 mm. No extract inhibit the growth of *Candida albicans*.

Minimum Inhibitory Concentration (MIC) and Minimum Bacterial Concentration (MBC)

The MIC determination of selected extracts showed the results range from 156.25 to 2500µg/mL (Table 3). The most sensible strain was *Staphylococcus aureus* ATCC BAA 977 where the MIC values were ranging from 156.25 to 312.50µg/mL.This activity of extract was less than that of standard antibiotic e.g. ciprofloxacin and miconazole.

Antioxidant Activities

Total phenolic

The quantitative estimation of the phenolic compounds present in extracts of *E. candollei* are summarize in figure 1 below.

From these results, the quantity of phenol compounds is highly variable depending to extracts and plant part. Hydro-ethanol extracts of leaves is highly riche in phenol compounds (4.605mgEAG/mg) while the ethyl acetate extract of stem bark have the less concentration of phenol compounds (2.0529 mgEAG/mg).

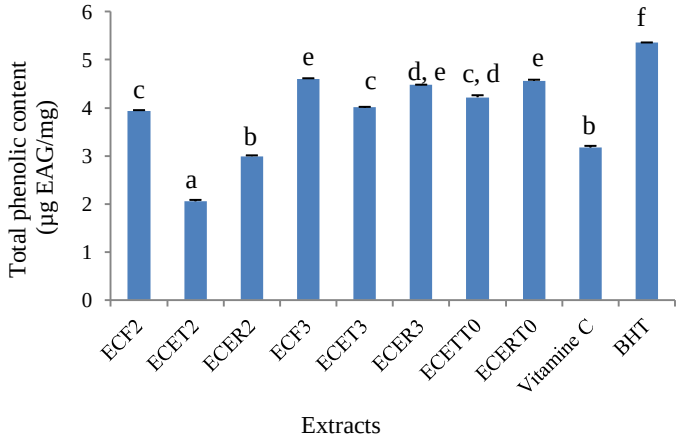

Fig. 1: Total phenolic content of extracts of *E. candollei*. leaves, stem and root bark. Values with the same letters are not significantly different (P > 0.05).

DPPH radical scavenging activity

The results for DPPH radical scavenging activity are presented in figure 2 and table 2. All the extracts showed scavenging effects at various levels, depending to extracts and plants parts. At the tested concentration, the radical-scavenging activity (RSA) of these extracts is higher than the BHT except ethyl acetate extract of stem bark. From table 4, the IC_{50} were range from 9.1026± 0.1635 to 11.8298±0.2077µg/mL. Ethyl acetate extract of stem bark was the most active while ethyl acetate extract of leaves was the less active.

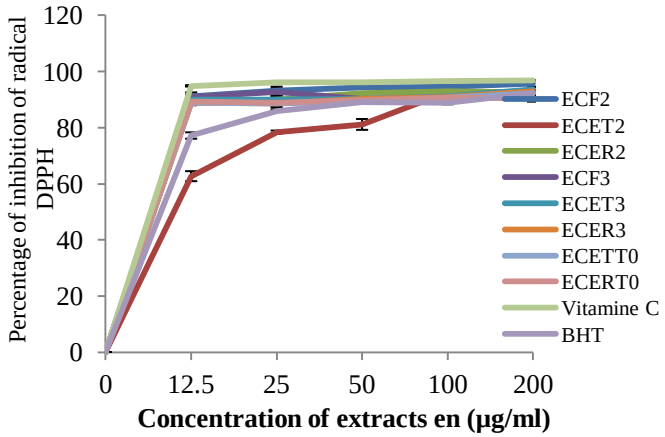

Fig. 2: DPPH radical scavenging activity of extracts.

Ferric reducing/antioxidant power (FRAP)

Concerning the Ferric Reducing Antioxidant Power activity, all the plant extracts interfered with the reduction of Fe^{3+} to Fe^{2+}, suggesting that they possess chelating activity (figure 3).The reducing power was ranging from 12.5 to 200 µg/mL. The highest reducing power was observed with ethyl acetate of leaves, while the ethyl acetate extract of stem bark was lowest. The phytochemical screening reveals the presence many classes of secondary metabolites whose members have already been shown to exhibit antimicrobial activities. These secondary metabolites obtained exert antimicrobial activity through different mechanisms. Tannins exert its antimicrobial activity by binding with proteins and adhesins, inhibiting enzymes, complexation with the cell wall and metal ions, or disruption of the plasmatic membrane (Cowan, 1999). Saponins have ability to cause leakage of proteins and certain enzymes from the cell (Okwu, 2001).

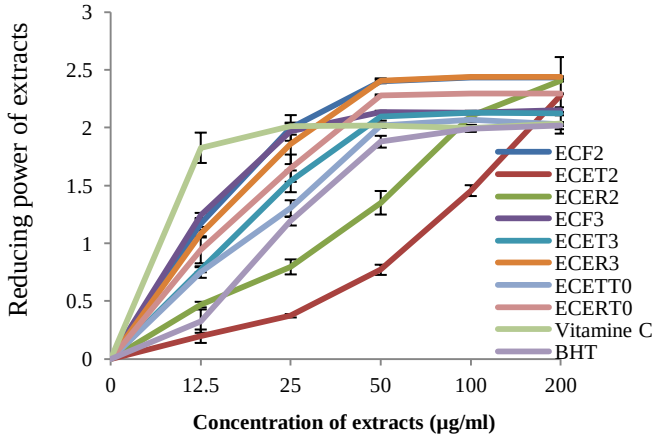

Fig. 3: Reducing power activities of the crude extracts of *E.candollei*. leaves, root and bark as well as vitamin C and BHT.

Flavonoïds have the ability to complex with proteins and bacterial cells forming irreversible complexes mainly with nucleophilic amino acids. This complex often leads to inactivation of the protein and loss of its function (Burkill, 1988; Shimada, 2006). Some flavonoids have shown several pharmacological activities including antibacterial and antifungal (Tittikpina *et al.*, 2013). These can explain the wide antibacterial spectrum of this plant. The different antibacterial activity of extracts from plant part of *E. Candollei* could be explained by the quantity of bioactive group of secondary metabolites in each extract and then, highlight the problem of standardization of therapeutic dose of plant extract used in the treatment of various infectious diseases.

The study of antioxidant activity extracts of *E. candollei* is a starting point to find the new antioxidant agent that could be used to fight against oxidative stress associated to many infectious diseases. The results showed that *E. candollei* extracts possess high anti-oxidant activities. In this study, the high reducing power of ethyl acetate of leaves and ethanolic and water extracts of root bark suggested that the phytochemical constituents with higher redox potential were more extractable with these solvents. These data suggest that leaves and root barks of *E. candollei* may contain several antioxidants with different polarities. The high DPPH radical scavenging activities of the various extracts which were comparable to the standard antioxidant, vitamin C and BHT, suggested that the extracts have some compounds with high proton donating ability and could therefore serve as free radical inhibitors. The results of antioxidants activities of these extracts suggest that they are rich sources of phytochemicals with powerful free radical scavenging phytochemical that could be used to fight against free radical upsurge, oxidative stress and consequently might help in the treatment of oxidative stress-associated metabolic disorders.

Table 1: Phytochemical composition of extracts of *E. candollei.*

Secondary metabolites	Extracts of stem bark					Extracts of root bark					Extracts of leaves		
	ECET1	ECET2	ECET3	ECETT0	ECET0	ECER1	ECER2	ECER3	ECERT0	ECER0	ECF1	ECF2	ECF3
Terpenoïds	+	+	-	+	+	-	+	-	+	+	-	-	+
Steroïds	+	-	-	-	-	+	-	-	-	-	+	+	-
Alkaloïds	-	+	-	+	-	-	+	-	+	-	-	+	-
Flavonoïds	+	+	+	+	+	+	+	+	+	+	+	+	+
Phenols	-	+	+	+	+	-	-	+	+	+	-	+	+
Coumarins	-	-	-	-	-	-	-	-	-	-	+	+	-
Reducteurssugas	-	-	+	+	-	+	-	-	-	+	-	-	-
Tanins	-	-	+	+	-	-	-	+	+	+	-	+	+
Saponins	+	-	+	+	+	+	+	+	+	+	+	-	+
Resins	-	-	-	-	-	-	-	-	-	-	-	-	-
Anthocyans	-	-	+	+	+	-	-	+	+	+	-	-	+
Cardiac glycosids	-	-	-	-	-	-	-	-	-	-	-	-	-
Lipids	-	+	-	-	-	-	+	-	-	-	-	-	-

Leaves: ECF1 = hexan extract ; ECF2 = Ethyl acetate extract; ECF3 = EtOH-H_2O residual; root bark: ECER1 = hexan extract ; ECER2 = Ethyl acetate extract; ECER3 = Extract EtOH-H_2O residual; ECERT0 = Extract EtOH-H_2O total; ECER0 = aqueous extract ; stem bark : ECET1 = hexan extract ; ECET2 = Ethyl acetate extract; ECET3 = Extract EtOH-H_2O residual; ECETT0 = Extract EtOH-H_2O total; ECET0 = aqueous extract. + = present ; - = absent.

Table 2: Inhibition Zone Diameters of extracts and reference antibiotic.

Microorganisms tested	Inhibition zone diameters (mm)														
	ECF1	ECF2	ECF3	ECER1	ECER2	ECER3	ECERT0	ECER0	ECET1	ECET2	ECET3	ECETT0	ECET0		C	M
S. aureus ATCC 25923	-	17	15,2	10	9	13	12	12	-	10	-	13,1	11			
S. aureus ATCC 29213	8	15	14	9	8	8	12	11	8	8,1	13,33	15	10			
S. aureus ATCC BAA 977	-	14	16	8	8	12	12	12	-	8	12	11	8			
E. coli ATCC 25922	10	13	14	-	11	12	12	-	-	10	-	11	10			
E. coli ATCC 11775	13	-	-	-	-	14	-	-	-	-	-	-	10			
K. pneumoniae ATCC 700603	12	-	-	-	-	13	-	-	-	-	-	-	10			
P.aeruginosa ATCC 10145	-	12	-	-	-	9	-	7	-	-	-	-	7			
P.aeruginosa ATCC 9027	-	12	8	-	-	8	-	-	-	-	-	-	-			
N. gonorrhoeae ATCC 49226	-	13	-	-	-	11	-	-	-	-	-	-	-			
A. viridans ATCC 11563	-	15	16	-	-	14	11	12	-	12	-	15	11			
E. faecalis ATCC 51299	-	12	9	-	-	11	9,1	8	-	-	9	-	-			
S. entérica	-	15	15	-	-	12	13	-	-	7	-	10	8	28		
P. mirabilis	-	15	12	-	-	17	11	9	-	7	-	10	10		28	
Candida. albicans	-	-	-	-	-	-	-	-	-	-	-	-	-	31		

Table 3: MICs, MBCs, MBCs/MICs of different extracts of *E. candollei*. Leaves, stem and root bark on isolates and strain of bacterial.

Microorganismstested	Parameters (µg/ml)	Extraitsreference			
		ECF2	ECER3	ECF3	Cipro
S. aureus ATCC 25923	CMI	625±00	1250±00	625±00	
	CMB	1250±00	2500±00	1250±00	
	CMB/CMI	2	2	2	
S. aureus ATCC 29213	CMI	1250±00	nd	1250±00	
	CMB	2500±00	nd	2500±00	
	CMB/CMI	2	nd	2	
S. aureus ATCC BAA 977	CMI	312,5±00	625±00	156,25±00	
	CMB	625±00	625±00	156,25±00	
	CMB/CMI	2	1	1	
A. viridans ATCC 11563	CMI	2500±00	2500±00	2500±00	
	CMB	5000±00	5000±00	5000±00	
	CMB/CMI	2	2	2	
E. faecalis ATCC 51299	CMI	2500±00	1250±00	2500±00	
	CMB	5000±00	2500±00	5000±00	
	CMB/CMI	2	2	2	
S. choleraesuis	CMI	625±00	1250±00	625±00	0,195±00
	CMB	1250±00	2500±00	1250±00	0,39±00
	CMB/CMI	2	2	2	2

Leaves: ECF2 = Ethyl acetate extract; ECF3 = EtOH-H_2O residual; root bark: ECER3 = Extract EtOH-H_2O residual; Reference (positive control): Cipro = Ciprofloxacine ; nd : no determined

Table 4: IC50 values of extracts of *E. candollei* leaves stem and root barks.

Extracts	IC_{50} (µg/mL)
ECF2	$9{,}1026\pm 0{,}1635^{b}$
ECET2	$11{,}8298\pm 0{,}2077^{g}$
ECER2	$9{,}6532\pm 0{,}0713^{c}$
ECF3	$9{,}7299\pm 0{,}0840^{c,d}$
ECET3	$9{,}7390\pm 0{,}1037^{c,d}$
ECER3	$9{,}9141\pm 0{,}0815^{d,e}$
ECETT0	$9{,}9953\pm 0{,}0122^{e}$
ECERT0	$10{,}0860\pm 0{,}2926^{e}$
Vitamin C	$8{,}6696\pm 0{,}0210^{a}$
BHT	$10{,}6456\pm 0{,}1015^{f}$

Values with the same superscripts are not significantly different. Waller Duncan (P > 0.05).
Leaves: ECF2 = Ethyl acetate extract; ECF3 = EtOH-H_2O residual; root bark: ECER2 = Ethyl acetate extract; ECER3 = Extract EtOH-H_2O residual; ECERT0 = Extract EtOH-H_2O total; stem bark: ECET2 = Ethyl acetate extract; ECET3 = Extract EtOH-H_2O residual; ECETT0 = Extract EtOH-H_2O total; BHT: Butylhydroxytoluene

CONCLUSION

It may be concluded from this study that the extracts from different organs of *E. candollei* are active against the tested microorganisms and also have antioxidant effects. In addition, the results confirm the use of the plant in traditional medicine. Now our study will be directed to explore the lead compound responsible for aforementioned activity from this plant.

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REFERENCES

Adejuwon, A.O., Agbaje, E.O., Idika, N. Antifungal and antibacterial activities of aqueous and methanolic root extracts of Carica papaya Linn. (Caricaceae). International Research Journal of Microbiology. 2011; 2 (8):270-277.

Aligiannis, N., Kalpotzakis, E., Mitaku S., Chinou, I.B. Composition and antimicrobial activity of the essential oils of two Origanum species. Journal of Agricultural and Food Chemistry. 2001; 40: 4168-4170.

Baker J.T., R.P., Borris, and B.,Carte , 1995. "Natural product drug discovery and development: New perspective on international collaboration" Journal of Natural Product58, pp. 1325-1357.

Bauer, A.W., Kirby, W.M.M., Sherries, J.C., Tuck, M. Antibiotic susceptibility testing by a standardized disc diffusion method. J. Am. Clin. Pathol. 1966; 45:493-496.

Bolou, G.E.K., Attioua, B., N'guessan, A.C., Coulibaly, A., N'guessan, J.D., Djaman A.J.1. Évaluation in vitro de l'activité antibactérienne des extraits de Terminalia glaucescens planch. sur Salmonella typhi et Salmonella typhimurium. Bulletin de la Société Royale des Sciences de Liège. 2011; 80: 772 – 790.

Burkill, H.M. The useful plants of West Tropical Africa. 2ndEdition, Royal BotanicalGarden, Kew. 1988; 5: 237-238.

Cowan, M.M. Plant products as antimicrobial agents. Clin. Microbiol. Rev. 1999; 12:564-582.

Dibong, S.D., Mpondo, M.E., Ngoye, A., Kwin, M.F., Betti, J.L. Ethnobotany and phytomedecine of médivinal plants sold in Douala markets (Cameroon). J APP Biosci. 2011; 37: 2496-2507.

Din, N., Dibong, S.D., Mpondo, M.E., Priso, R.J., Kwin, M.F., Ngoye, A. Inventory and identification of plants used in the treatment of diabets in Douala town (Cameroun). Eur J Med. Plant. 2011; 1(3): 60-73.

Favier, A. Le stress oxydant intérêt conceptuel et expérimental dans la compréhension des mécanismes des maladies et potentiel thérapeutique. L'actu. Chimique Novembre –Décembre 2003.

Gatsing, D., Adoga, G.I. Antisalmonella activity and screening of the various parts of Cassia petersianabolle (Caesalpiniaceae). Research Journal of Microbiology.2007;2(11): 876-880.

George, F., Zohar, K., Harinder, P.S., Makkar, Klaus, B. The biological action of saponins in animal systems: a review. British Journal of Nutrition. 2002; 88:587–605.

Gérard, A., Edi Kouassi, C., Daigremont, P., Détienne, D., Fouquet, M., Vernay. Synthèse sur les caractéristiques technologiques de référence des principaux bois commerciaux africains. CNRA Abidjan, CIRAD-Forêt. 1997. p. 142-158.

Gulcin, I., Beydemir, H.A., Alici, H.A., Elmastas, M., Buyukokuroglu, M.E. *In vitro*antioxidant properties of morphine. Pharmacological Research. 2004; 49:59-66.

Harbone, J. B. Phytochemical Methods: a Guide to Modern Techniques of Plants Analysis. London: Chapman and Hall Ltd. 1973:50-116.

Hill, A.F. 1952. Economic Botany. A textbook of useful plants and plant products. 2nd edn. McGarw-Hill Book Company Inc, New York.

Huang, D., Ou, B., Prior, R.L. The chemistry behind antioxidant capacity assays. Journal of Agricultural and Food Chemistry. 2005; 53(6):1841-56.

Mahesh, B., Satish, S. Antimicrobial activity of some important medicinal plant against plant and human pathogens. World J. Agric. Sci. 2008; 4: 839 – 843.

Mensor, L.L., Menezes, F.S., Leitao, G.G.., Reis, A.S.0., Dos Santos, T.C., Coube, C.S., Leitao, S.G. Screening of Brazilian plant extracts for antioxidant activity by the used DPPH free radical method. Phytotherapy Research. 2001;15:127-130.

Notizbl. Königl. 1896. Botanique. Bot. Gart. Berlin. 1: 181.

Okwu, D.E. Flavouring properties of spices on cassava Fufu. Afr. J. Roots Tuber Crops. 1999; 3(2): 19-21.

Okwu, D.E. Evaluation of the chemical composition of indigenous spices and flavouring Agents. Global J. Pure Appl. Sci. 2001;7(3): 455-459.

Oussou, K.R., Yolou, S., Boti, J.B., Guessennd, K.N., Kanko, C., Ahibo, C., et al. Étude chimique et activité antidiarrheique des huiles essentielles de deux plantes aromatiques de la pharmacopée ivoirienne.European Journal of ScientificResearch. 2008; 24 (1): 94-103).

Padmaja, M., Sravanthi, M., Hemalatha, K.P.J. Evaluation of Antioxidant Activity of Two Indian Medicinal Plants. Journal of Phytology. 2011;3(3):86-91.

Parekh, J., Chanda, S. In viro antimicrobial activities of extracts of LauneaprocumbensRoxd. (Labiateae), Vitisvinifera L. (Vitaceae) and Cyperusrotundus L. (Cyperaceae). Afr. J . Biomed. Res. 2006; 9: 89-93.

Ponce, A.G., Fritz, R., del Valle, C.E., Roura, S.I. Antimicrobial activity of essential oils on the native microflora of organic Swiss chard.Lebensmittel-Wissenschaft und –Technologie. 2003; 36: 679-684.

Ramde-Tiendrebeogo, A., Tibiri, A.,Hilou, A., Lompo, M., Millogo-Kone, H., Nacoulma, O.G., Guissou, I.P. Antioxidative and antibacterial activities of phenolic compounds from Ficus sue Forssk. International Journal of Biological and Chemical Sciences. 2012; 6(1):328-336.

Shimada, T. Salivary proteins as a defense against dietary tannins. J. Chem. Ecol. 2006; 32(6):1149-1163.

Stuffness M. and J. Douros, 1982. Current status of the NCI plant and animal productprogram. Journal of Natural product45, pp. 1-14.

Tchouankeu, J.C., Tsamo, E., Sondengam, B.L., Connolly, J.D. Phytochemistry.1989; 28: 2855-2857.

Tittikpina, N.K., Agban, A., Gbogbo, K.A., Hoekou, Y.P., Pereki, H., Batawila, K., et al. Évaluation des propriétés antimicrobiennes de PterocarpuserinaceusPoir (Faboïdeae) etDanielliaoliveri (Rolfe) Hutch. etDalz (Caesalpinoïdeae), utilisées en médecine traditionnelle au Togo. International Journal of Biological andChemical Sciences. 2013; 7(4):1586-1594.

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