Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2015; 7(6); 1047-1053

ISSN: 0975-4873

Research Article

Phytochemical Composition, Antioxidant and Antibacterial Activities of Root of *Uvaria chamae* P. Beauv. (Annonaceae) Used in Treatment of Dysentery in North of Côte d'Ivoire.

Kone Monon[1], Toure Abdoulaye[2*], Ouattara Karamoko[1], Coulibaly Adama[1].

[1]*Laboratoire de pharmacodynamie-Biochimique. UFR Biosciences, Université Félix HOUPHOUET-BOIGNY de Cocody Abidjan 22 BP 582 Abidjan 22, Côte d'Ivoire.*
[2]*Laboratoire de Biochimie et Sciences des Aliments. UFR Sciences Biologiques, Université Peleforo GON COULIBALY de Korhogo. BP 1328 Korhogo, Côte d'Ivoire.*

Available Online :4th October, 2015

ABSTRACT
In the present work phytochemical composition, antioxidant and antibacterial properties of aqueous and ethanol extracts of root of *Uvaria chamae* were studied. Antibacterial activity of extracts of plant was evaluated on four bacterial strains (*E. coli* sensitive, *E. coli* ESBL, *S. flexneri* ESBL and *Shigella* sp) by agar both dilution and agar plate methods. For antioxidant property, free radicals scavenging capacity of the two extracts were assessed *in vitro* by 1,1 diphenyl-2-picrylhydrazyl (DPPH) radical scavenging and ferric reducing antioxidant power (FRAP) assays, while inhibition of free radicals generation was assessed by ability of the extracts to inhibit lipid peroxides formation. Phytoconstituants of two extracts of *Uvaria chamae* (tannins, flavonoids, and phenols) were also assayed by colorimetric method. Ethanolic extract of *Uvaria chamae* presented bactericidal effect against four strains tested (MBC/MIC = 2). Antibacterial parameters revealed that among the four bacteria tested, aqueous extract of *Uvaria chamae* has bacteriostatic power against ESBL strains (MBC/MIC = 16). The two extracts of plant showed significant ($p<0.05$) reducing, chelating and free radical scavenging activities with concentrations required for 50% inhibition (IC_{50}) of DPPH varying from 3.52±0.38 µg/ml to 14.35±4.86 µg/ml. Ethanolic extract of *Uvaria chamae* showed significant ($p<0.05$) high antioxidant activity (4.02 ± 0.50 µg/ml) than aqueous extract (12.59 ± 2.77 µg/ml). Phytoconstituents analysis revealed that the two extracts contain significantly ($p<0.05$) high quantity of total phenols, followed by flavonoids and tannins. The high presence of phenolic compounds in ethanolic extract could be responsible of it antioxidant and antibacterial potentiality. The results of these investigations could justify traditional used of *Uvaria chamae* in treatment of bacterial dysentery.

Keywords: *Uvaria chamae*, antibacterial, antioxydant, phenolic compound, dysentery, Côte d'Ivoire.

INTRODUCTION

Oxidative stress results from an imbalance between the oxidant production and a biological system's ability to readily detoxify the reactive intermediates or easily repair the resulting damage[1]. Increased oxidative stress is associated with many of the risk factors implicated in the pathophysiology of atherosclerosis, including diabetes, hypercholesterimia, renal failure, aging, hypertension and smoking[2]. Reactive oxygen species (ROS) such as superoxides, peroxides and hydroxyl radicals are known to play an important role and have been identified as major contributors to all cell and tissue damage in many disease conditions[3]. The mains endogenous sources of most of the oxidants produced by cells appear to be normal aerobic respiration stimulates polymorphonuclear leukocytes and peroxisomes[4]. Exogenous sources of reactive oxygen species include environnemental pollutants, radiations, antibiotic resistance in pathogenic organisms and the persistence of pathogens in immune compromised individuals[5,6]. To protect the cells and organ systems of the body against reactive oxygen species, the human body has evolved a highly complex antioxidant protection system. Most synthetic or naturally occurring antioxidants have phenolic hydroxyl groups in their structures and the antioxidant properties are attributed in part to the ability of these natural compounds to scavenge free radicals[7]. Several synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisol (BHA) are widely used as feed additives to prevent spoilage. Natural or synthetic antioxidants adverse effects on human health are still worrying. In order to find molecules most effective natural antioxidants and negligible side effects, scientific research has focused in recent years, its investigations into new agents of plant origin. In this context, through an ethno pharmacological investigation, Koné *et al.*, 2013 showed that *Uvaria chamae*, used by the people of northern Côte d'Ivoire in treatment of dysentery is rich in various metabolites including polyphenols. *Uvaria chamae*, commonly known as finger root or bush banana belongs to the family Annonaceae. *U.chamae* is an evergreen plant which grows to a height of 3.6-4.5 m. Leaves are stipulate, leaf apex 2

**Author for Correspondence*

cuminate and the leaf vestiture is glabrous[8]. It is a small tree native to the tropical rain forest of West and Central Africa where it grows in wet and coastal shrublands[9]. All parts of the plant are fragrant, the roots and roots-bark have a widely spread reputation[10]. The root (pounded or pulverised) is used for the treatment of nose bleeding, heart diseases (bronchi, lungs etc.), and blood in urine, pile and fever[11,12] . These natural substances are widely distributed in plant species and are recognized through their beneficial health effects[13]. Their natural antibacterial and antioxidant roles have more and more interest in the prevention and treatment of cancer, inflammatory and cardiovascular diseases[14]. The secondary metabolites of medicinal plant could be responsible for the therapeutic activity attributed to *Uvaria chamae*. In order to check the therapeutic benefits given to this plant, the present study aims to investigate the *in vitro* antibacterial activity and antioxidant property of *Uvaria chamae*.

MATERIALS AND METHODS

Chemicals and reagents

Folin-Ciocalteu reagent, sodium carbonate, methanol, aluminum chloride, potassium acetate, 2,2-diphenyl-1-picryl hydrazyl (DPPH), ethanol, sulfuric acid, potassium dihydrogen phosphate, potassium ferricyanide, trichoroacetic acid, ferrosine, iron II chloride, quercetin and gallic acid were purchased from Ryca-Pharma and Chemical Laboratory Equipment (CLE), Côte d'Ivoire. All reagents and chemicals used in this study are analytical grade.

Collection of plant materials

Roots of *Uvaria chamae* P. Beauv. (Annonaceae) were colleted from Boundiali Northen Côte d'Ivoire in September 2010. The plant was identified and authentificated by Professor Ake-Assi of National Floristique Center of University Felix Houphouët Boigny, Abidjan, Côte d'Ivoire.

Preparation of plant extracts

The roots of plant *Uvaria chamae* were whased thoroughly with distillated to remove dirt. After which root bark were detached and air-dried at room temperature for five weeks. The dried root bark were ground well in mechanical grinder; then passed through the mesh to get uniformly coarse powder (40 mesh size). This fine powder was stored in an air tight container. The ethanolic and aqueous extracts were prepared from 100g powdered plant material with respectively 400 ml of 70% ethanol (v/v) and 400 ml of distilled water kept for maceration for 72 hrs at room temperature. After which, each extract was filtered twice through cotton wool, then through Whatman filter paper N°1 and concentrated by rotary vacuum evaporator at 45˚C. The two extracts (ethanolic and aqueous) obtained were dispersed into sterile containers and stored in a refrigerator for future use.

Qualitative phytochemical analysis

Qualitative phytochemical analysis of ethanolic and aqueous extracts of *Uvaria chamae* was carried out to identify the secondary metabolites by following the standard procedures[15]. Phenolics, flavonoids and tannins were quantified by the following methods.

Determination of total phenolics

Different concentrations (20, 40, 60, 80,100 µg/ml) of Gallic acid standard and two concentrations 50mg/ml and 100 mg/ml of plant extracts were prepared; 1 ml of each sample and standard were placed in different test tubes. Then 2.5 ml of a 10% Folin-Ciocalteu reagent and 2 ml of 2% sodium carbonate were added and the test tubes were covered with aluminum foil. This mixture was incubated for 30 minutes at room temperature and the absorbance of standard gallic acid and the plant extract were measured spectrophotometrically at 765 nm. The total phenolic content of plant sample was calculated from the Gallic acid standard curve. The results were expressed as mg/g of Gallic acid equivalents[16].

Determination of total flavonoids

The content of total flavonoids was done by aluminum chloride method. In this method the calibration curve was drawn from different concentrations of standard quercetin 2 to 10µg/ml and each extracts of root bark of *Uvaria chamae* were prepared. 1 ml of each sample was mixed with 4 ml of distilled water in a volumetric flask and 300 µl of 5% sodium nitrate was added and incubated for 5 minutes at room temperature. Then 300 µl of 10% aluminum chloride was added to flask and 2 ml of 1 % sodium hydroxide was added immediately. The volume is then made up to 10 ml with distilled water. The absorbance was taken at 510 nm using UV-visible spectrophotometer. The results are expressed as mg/g of quercetin equivalent[17].

Determination of total tannins

Tannins contents in each extract were determined using method described by Baindridge *et al.,* 1996[22]. 1 ml of each sample was mixed with 5 ml of vanillin reagent (1%) in a test tube. Then the tube was incubated for 20 minutes in the dark and the absorbance was taken at 500 nm using UV-visible spectrophotometer. The results are expressed as mg/g of tannic acid (2 mg/ml) equivalent.

Antibacterial assay

Antibacterial activity of extracts of *Uvaria chamae* were performed by broth dilution agar method coupled with seeding on agar plate. Four strains (*E. coli* sensible, *E. coli* ESBL, *S. flexneri* ESBL and *Shigella* sp) obtained from Bacteriology Laboratory of Pasteur Institut of Côte d'Ivoire were tested. Each test compound was incorporated into growth medium in tubes and Petri dishes to give serial two fold dilutions. The resulting concentrations ranged from 3.125 to 100 mg/ml. A tube and Petri dishe containing nutrient broth only, seeded with test organism was served as growth control. Bacterial cell suspensions were inoculated on the tubes and plates using a bacterial planter (10 µl). All the inoculated tubes and plates were then incubated at 37°C±2°C for 18 h. The lowest concentration of the plate, which did not show any visible growth after incubation, was considered as minimal inhibitory concentration (MIC). To determine the minimal bactericidal concentration (MBC) for each set of test tubes in the MIC determination, a loopful of broth was collected from those tubes which did not show any growth and inoculated on sterile nutrient agar by streaking. Plates inoculated with bacteria were then incubated at 37°C for

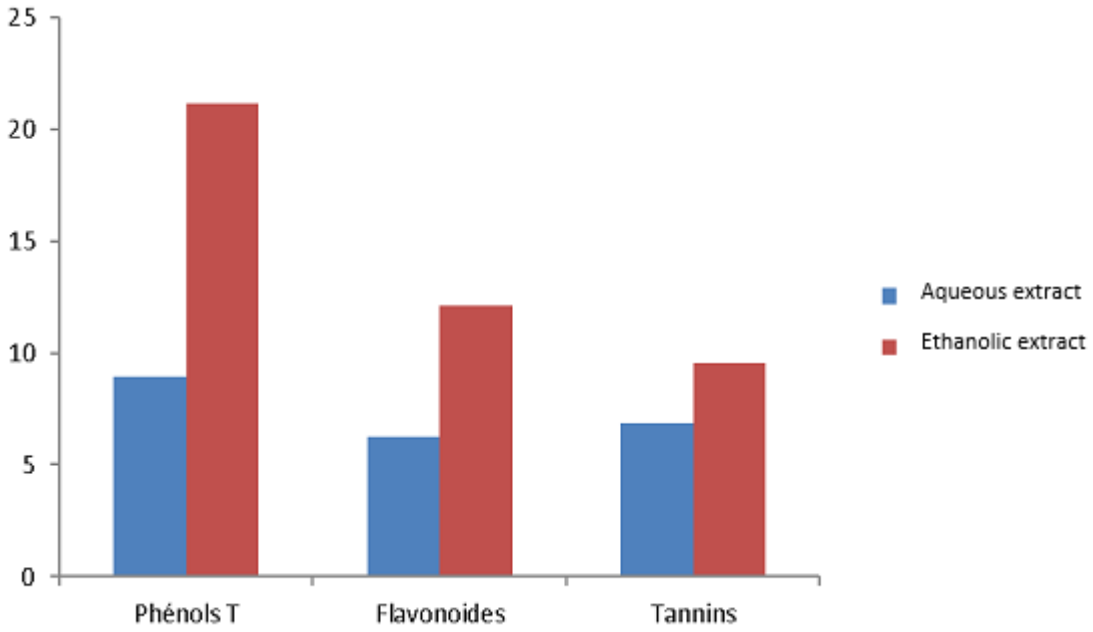

Figure 1: Comparison of phytochemical test constituants of ethanolic and aqueous extracts Antibacterial activity

Table 1 : Composition of phytochemical test constituants of ethanolic and aqueous extracts of *Uvaria chamae*

Extracts	Total polyphenols (mg GAE/ g)	Tannins (mg TAE/ g)	Flavonoids (mg QE/ g)
Ethanolic extract	21.14±0.51	9.519±0.96	12.13±1.68
Aqueous extract	8.917±0.92	6.253±1.10	6.833±0.01

24 hours. After incubation the concentration at which no visible growth was noted as MBC[17].

DPPH radical scavenging actvity

Antioxidant activity of the extracts of *Uvaria chamae* was assessed through DPPH scavenging potential with ascorbic acid as the standard[18]. Twenty microliters of various concentrations of extracts in methanol were added to 1 ml of 0.004% methanol solution of DPPH. The reaction tubes were wrapped in aluminum foils and incubated at room temperature for 1 to 5 min in the dark then, the absorbance was read at 517 nm. All readings were recorded in dim light 30. The percent (%) inhibition of free radical (DPPH) was calculated using the formula:

Inhibition (%) of DPPH = [(Ac-As)/Ac] × 100

where, Ac is the absorbance of the control (containing all reagents except the test sample/standard) and As is the absorbance of the test sample. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the standard graph plotted using inhibition percentage against concentration.

Ferric reducing antioxidant power (FRAP)

The FRAP assay used antioxydants in the redox linked colorimetric method with absorbance measured with a spectrophotometer[19]. A 300 nmol/l acetate buffer of pH 3.6 (3.1 g of sodium acetate + 16 ml of glacial acetic acid made up to one litre with distilled water, 10 nmol/L 1,2,4,6-tri 2-pyridyl 1,3,5-triazine, 98%, 3.1 mg/ml in 40 nmol/l HCl) and 20 mmol/l of ferric chloride were mixed together in the ratio of 10:1:1, respectively to give the FRAP working reagent. 1 ml of extract or standard (vitamin C) was added to 2.5 ml of $KH_2PO_4^-$ KOH buffer (0.2 mM, pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50°C for min. 2.5 ml of 10% trichloroacetic acid was added to each test tube before centrifuged at 3000 trs/min for 10 min. Supernatant was separated and 2.5 ml of this were mixed with 0.5 ml of 0.1 % iron II chloride. The mixture was incubated for 10 min at room temperature and absorbance was measured at 700 nm against a control in same conditions. Extracts concentrations providing 50% absorbance (EC_{50}) was calculated from the standard (vitamin C).

Chelating activity assay

Chelating power of extracts of *Uvaria chamae* was measured by spectrophotometric method based on the dosage of the complex formed by the ferrous ion (Fe2+) and ferrosine. To 3.7 ml of methanol, 0.1 ml of iron II chloride (2 mM) was added. After 5 min of incubation, 0.2 ml of ferrosine (5 mM) was added. Mixture was homogenized and keeps at room temperature for 10 min before measuring the absorbance of complexe Fe^{2+}-ferrosine at 562 nm against a control. Different concentrations of EDTA were used as standard control. Chelating power of samples was determined by following formula:

Chelating power (%) = $[(A_{control} - A_{sample})/A_{control}] \times 100$

Extracts concentrations providing 50% absorbance (IC_{50}) was calculated from the standard (EDTA).

Statistical analysis

Data were expressed presented and were presented as mean values ± SD (standard deviations). All the data were

Table 2: Determination of antibacterial parameters of ethanol extracts of roots of *Uvaria chamae*

Antibacterial parameters of ethanol extracts				
Strains	MIC (mg/ml)	MBC (mg/ml)	MBC/MIC	Interpretation
Escherichia coli sensible	6.25	12.5	2	Bactericidal
Escherichia coli ESBL	50	100	2	Bactericidal
Shigella sp.	6.25	12.5	2	Bactericidal
Shigella flexineri ESBL	3.125	6.25	2	Bactericidal

Table 3: Determination of antibacterial parameters of aqueous extracts extracts of roots of *Uvaria chamae*

Antibacterial Parameters of aqueous extracts				
Strains	MIC (mg/ml)	MBC (mg/ml)	MBC/MIC	Interpretation
Escherichia coli sensible	50	> 100	> 100	-
Escherichia coli ESBL	6.25	100	16	Bacteriostatic
Shigella sp.	100	> 100	> 100	-
Shigella flexineri ESBL	6.25	100	16	Bacteriostatic

Table 4: Antioxidant activity of extracts of *Uvaria chamae* and vitamin C.

	Anti-radical activity	Chelating activity	Reducing power
Extracts	IC_{50} (µg/ml)	IC_{50} (µg/ml)	EC_{50} (µg/ml)
Aqueous extract	12.59±2.77	11.00±0.01	14.35±4.86
Ethanolic extract	4.02±0.50	6.11±0.55	9.00±0.66
Vitamin C	3.52±0.38	4.30±0.78	6.11±0.90

analyzed by one-way ANOVA and differences between the means were assessed with Dunnet/Turkey's multiple comparison tests. Differences were considered significant at $p < 0.05$. All analyses were carried out using Graph Pad software, version 5.01 (USA).

RESULTS AND DISCUSSION

Phytochemical analysis

Table 1 presented composition of total phenols, flavonoids and tannins of aqueous and ethanolic extracts of *Uvaria chamae*. Figure 1 showed comparison of composition of these phytochemical compounds of the two extracts. These

results revealed that botn extracts are rich in these phytochemicals. However high total phenols content is noted in relation to flavonoids and tannins. In addition ethanolic extract has phytochemical test compounds content (9.51±0.96 µg to 21.14±0.51µg) than the aqueous extracts (6.253±1.10 µg to 8.917±0.92 µg.). The results of this study differ from those obtained by Donatus et *al.*, 2009[22]. For this author, the contents of total phenols, flavonoids and tannins of extracts of roots of *Uvaria chamae* were respectively 0.10±0.30 µg, 5.70±0.03 µg and 0.40±0.03 µg. This concentration difference can be explained by several factors including the method used[18]. Recent studies have shown the intrinsic factors that may cause this difference. These are geographical, climatic and genetic factors but also the degree of maturation of the plant[19].

The MIC values of ethanolic and aqueous extracts of *Uvaria chamae* against test strains of bacteria were shown in Tables 2 and 3. Ethanolic extract had significantly ($p<0.05$) stronger activity against *Shigella flexneri* ESBL with values of MIC (3.125 mg/ml) and MBC (6.25 mg/ml). This extract had lower level of activity against *E. coli* ESBL at MIC of 50 mg/ml and MBC of 100 mg/ml. MIC and MBC of ethanolic extract against *E. coli* and *Shigella* sp were identical with respectively values of 6.25 mg/ml and 12.25 mg/ml. For all strains tested, ethanolic extract have shown bactericidal activity according values of MBC per MIC equal to 2. Aqueous extract had bacteriostatic activity against the two ESBL strains with MIC of 6.25 mg/ml and MBC of 100 mg/ml. Antibacterial parameters of this extract against *E. coli* sensible and *Shigella* sp could not be determined at concentration of 100 mg/ml. However, antibacterial activity of ethanolic extract of *Uvaria chamae* was much higher than the aqueous extract. The results of this study also indicate that ethanol is a better solvent than water in the extraction of the active principles of this plant. This corroborates the reports of Ogbulie *et al.*, 2007[20] and Oguede *et al.*, 2006[21] that ethanol is the best solvent for the extraction of most plant active principles of medicinal importance. The high antibacterial activity of ethanolic extract can be attributed to best concentration of phenolic compounds by this extract. Moreover the work of Scalbert, 1991 indicate that more phenolic compounds are oxidized the more they are inhibitors of microorganisms.

Antioxidant activity

Hydroxyl radical scavening activity

Figure 2 show the inhibition curves for the DPPH radical by aqueous and ethanolic extracts of roots of *U. chamae* and vitamin C. Values of IC_{50} obtained from these curves (Table 4) show that extracts of *U. chamae* have significant ($p<0.05$) anti-radical power. Anti-radical activity of ethanolic extract (IC_{50} = 4.02±0.50 µg/ml) and that of reference molecule (vitamin C: IC_{50} = 3.52±0.38 µg/ml) are almost equal. Ethanolic extract presented also the best antioxidant activity among the two extracts of *U. chamae.* These results agree with those obtained by Yéo et *al.*,2014[22]. These authors in determining the antioxidant

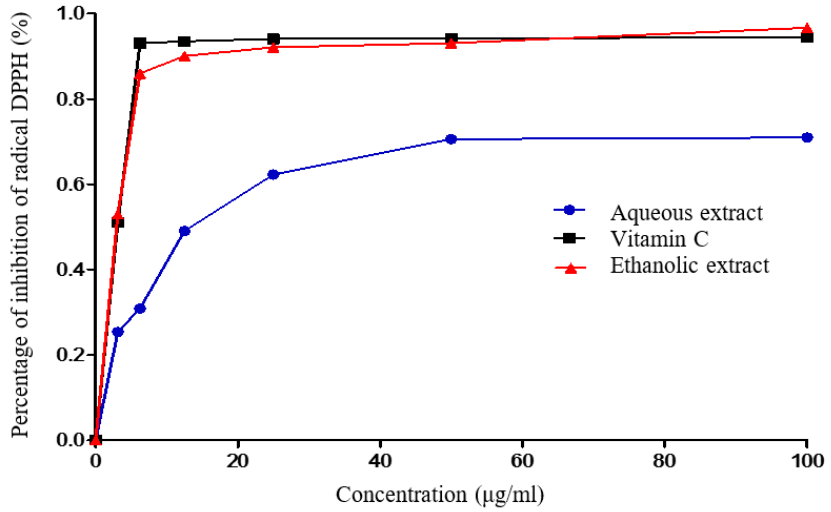

Figure 2: Inhibition of radical DPPH (%) by extracts of *U. chamae* and vitamin C.

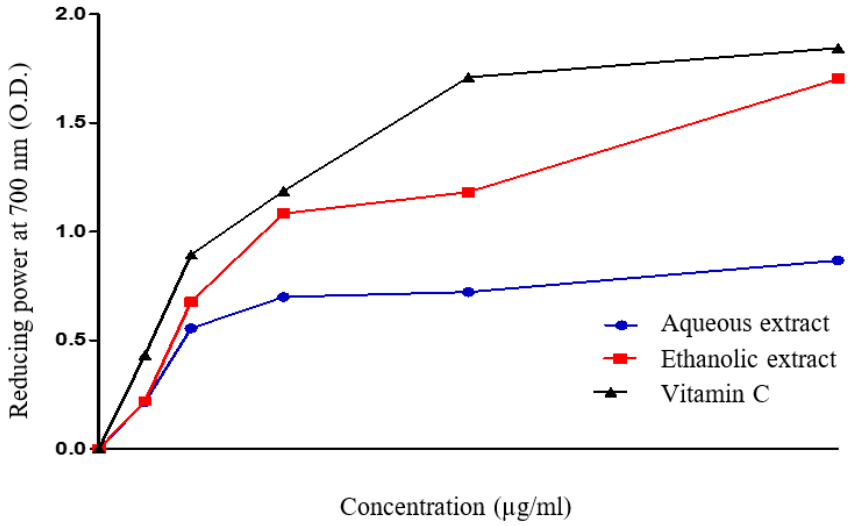

Figure 3: Reducing power of iron by extracts of *U. chamae* and vitamin C.

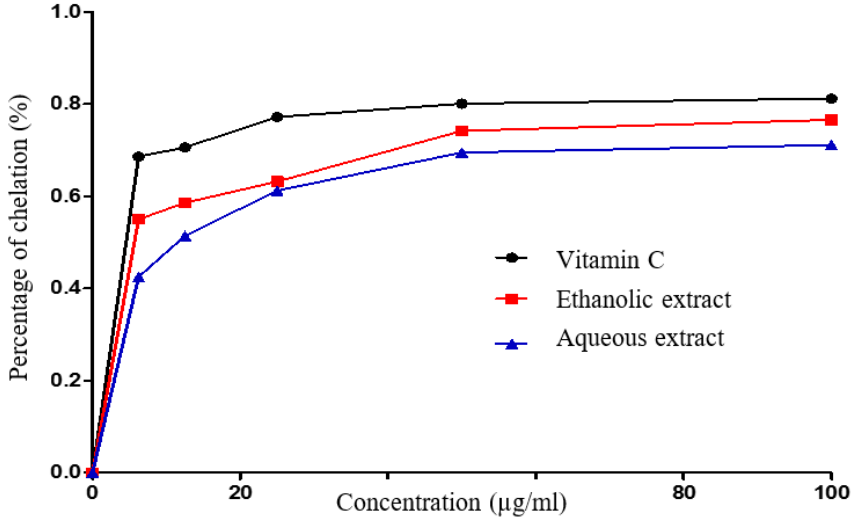

Figure 4: Chelating activity of extracts of *U. chamae* and vitamin C.

activity of several extracts of *Cochlospermum planchonii* with ethanolic and aqueous extrats reached similar conclusions. Also, the IC50 of ethanol extract (4.02 ± 0.50 g/ml) is substantially equal to that of the methanolic extract of *Chrysophyllum perpulchrum* (IC_{50} = 4.00 ± 0.288 µg/ml) obtained by Bidié *et al.*,2011[23]. But greater than

that of *Combretum sp* (6.00 ± 0.45 µg/ml) obtained by par N'guessan et *al.*, 2007[24].

This interesting activity of ethanoic extract may be due to the richness of this extract at various secondary metabolites. According to work of N'guessan et *al.*, 2007[24], there is a correlation between polyphenol content of extract and anti-radical activity. This justifies the best anti-radical activity of ethanolic extract of *U. chamae* that is rich in total phenols. In addition phenolic compounds are recognizing as capable of reacting with free radicals. However, a synergistic activity between total polyphenols and flavonoids could also contribute to this activity[25].

Reducing power

The curves in figure 3 shows evolution of reducing power of ethanolic and aqueous extracts of roots of *U. chamae* and reference molecule (vitamin C). At concentration of 50 mg/ml, the reducing powers of extracts of *U. chamae* are significantly ($p<0.05$) below that of vitamin C. However, based on EC_{50} (Table 4) the reducing power of the ethanol extract (9.00± 0.66 µg/ml) is near to that of the reference molecule (6.11 ± 0.90 µg/ml). Furthermore, EC_{50} value of aqueous extract (14.35 ± 4.86 µg/ml) is almost double of that of vitamin C (6.11± 0.90 µg/ml). The reducing power of *U. chamae* is probably due to presence of hydroxyl group in phenolic compounds that can be used as an electron donor. Indeed antioxidants are considered as reducing and oxidizing inactivators[26]. Some previous studies have also shown that reducing power of a compound is a significant indicator of evaluation of its potential antioxidant activity[27,28].

Chelating activity

The results of study of chelating power of ethanolic and aqueous extracts of *U. chamae* are presented in figure 4. Values of IC_{50} in Table 4 indicate that the ethanolic extract (6.113±0.55 µg/ml) have significantly the high chelating activity comparatively to aqueous extract (11.0±0.1 µg/ml). This activity is near that of vitamin C (4.307 ± 0.78µg/ml) and probably due to synergic interaction of phenolic compounds. Among many study of ferric chelating action of plants extracts that of Le *et al.*, 2007 show that value of IC_{50} of ethanolic extract of seed of *Lycium barbarum* (10mg/ml) is very superior comparatively to ethanolic extract of *U. chamae*. The chelating capacity of this extract is very important because of reducing of concentration of transition metals catalysts of lipid peroxidation. Indeed, iron can stimuli lipid oxidation by Fenton reaction and also accelerates the oxidation decomposing hydroperoxides peroxyl radicals and alkoxyl wich in turn can sustain the chain reaction.

CONCLUSION

The present study shows that *Uvaria chamae* has high content of phenolic compounds and high antioxydant activity. Therefore they can be used to treat several diseases in which there is an increase in free radical production. This study justified the traditional use of the roots of *Uvaria chamae* for management of dysentery in West Africa. However, further studies are needed to identify which phytogenic phenols are responsible for the antioxidant activity of the roots of plant, and assess the way in which these substances contribute to this activity. In addition to that *in vivo* antioxidant and antibacterial assays are needed to confirm the potential use of this plant in the treatment of some diseases as dysentery.

REFERENCES

1. De Diego-Otero Y., Romero-Zerbo Y., el Bekay R., Decara J., Sanchez L., Rodriguez-de F. & del Arco-Herrera I. (2009) : "Alpha-tocopherol protects against oxidative stress in the fragile X knockout mouse: An experimental therapeutic approach for the FMR 1 deficiency. *Neuropsychopharmacol*., 34(4): 1011-1026.
2. Stephens J.W., Bain S.C., Khanolkar M.P. (2009): Atheroclerosis, 202, 321-329.
3. Chu W.L., Lim Y.W., Radhakrishnan A.K. & Lim P.E. (2010): Protective effect of aqueous extract from Spirulina platensis against cell death induced by free radicals. *BMC Complementary and Alternative Medicine*, 10 (53), 2-8.
4. Kumari A. & Kakkar P. (2008) : Screening of antioxidant potential of selected barks of Indian medicinal plants by multiple *in vitro* assays. *Biomedical and environmental sciences*, 21, 24-29.
5. Ansari K. N. (1997) : The free radicals-the hidden culprits-an update. *Indian Journal of Medical Sciences*, 51, 319-336.
6. Song J.H. (2008): What's new on the antimicrobial horizon? *International Journal of Antimicrobial Agents*, 32 (4), 207-213.
7. Bartosz G. (2003) : Generation of reactive oxygen species in biological systems. *Comments on Toxicology*, *9*, 5-21.
8. Bongers F., Parren P.M.E. & Traore D. (2005): Forest climbing plants of West Africa*: Diversity, Ecology and Management*, Zelhi: CAB International, pp. 342 – 348.
9. Arbonnier M. (2004): Trees, shrubs and lianas of West African dry zones. Germany: CIRAD Press Ltd. pp. 243 - 248.
10. Iwu MM (1993): Handbook of Africa medicinal plants. Ogadijo: *CRC Press*. pp. 18 – 22.
11. Adams M.R. & Moss M.O. (1999): Food Microbiology. Cambridge: *The Royal Society of Chemistry*, pp. 471 – 476.
12. Etukudo I (2003): Ethnobotany, conventional and traditional uses of plants, Uyo: *The Verdict Press*. p.191.
13. Koechlin-Ramonatxo C. (2006) : Oxygen, oxidative stress and antioxidant supplementation, or another way for nutrition in respiratory diseases. *Nutrition Clinique et Métabolique*. 20, 165- 177.
14. Vârban D.I., Duda M., Vârban R., & Muntean S. (2009): Research Concerning the Organic Technology for Satureja Hortensis L. *Culture Bulletin UASVM Agriculture*. 66(2), 225- 229.
15. Trease GE, Evans WC. Pharmacognosy: a physician's guide to herbal medicine, 13th ed. London: Bailliere Tindall; 1989.
16. Aiyegroro, O.A., Okoh, A.I. 2010. Preliminary phytochemical screening and in vitro antioxidant

activities of aqueous extract of Helichrysum longifolium DC. *BMC compl. And Alt. Med*., 10:21

17. Ouattara K., Toure A., Ouattara L., Ouattara A. & Coulibaly A. (2014) : *In vitro* evaluation of antibacterial activity of flavonoids of *Thonningia sanguinea* Vahl. (Balanophoraceae). *J. Microbiol. Biotech. Res.*, 4 (6):38-41
18. Ayoola G.A., Sofidiya T., Odukoya O. & Coker H.A.B. (2006) : Phytochemical screening and free radical scavenging activity of some Nigerian medicinal plants. *J. Pharm. Sci. & Pharm. Pract.* 8: 133-136.
19. Benzie I.F.F. & Strain J.J. (1999) : Ferric reducing ability of plasma (FRAP) as a measurement of antioxidant power. The FRAP assay: *Analytical Biochem*., 239: 70-76.
20. Ogbulie J.N., Ogueke C.C. & Nwanebu F.C. 2007. Antibacterial properties of *Uvaria chamae, Congromena latifolium, Garcinia kola, Vemonia amygdalina* and *Afromomium melegueta*. *Afr. J. Biotechnol.* 6(13) : 1549-1553.
21. Ogueke C.C., Ogbulie J.N. & Njoku H.O. 2006. Antimicrobial properties and preliminary phytochemical analysis of ethanolic extracts of Alstonia bonnie. *Niger. J. Microbiol.* 20(2): 896-899.
22. Yeo S. O., Guessennd K. N., Meité S., Ouattara K., Bahi G. A., N'Guessan J. D. & Coulibaly A. (2014) : *In vitro* antioxidant activity of extracts of the root *Cochlospermum planchonii* Hook. f. ex. Planch (Cochlospermaceae). *Journal of Pharmacognosy and Phytochemistry*. 3(4): 164-170.
23. Bidié A.P., Banga B., N'Guessan Yapo A.F., N'Guessan J.D. & Djaman A.J. (2011) : Activités antioxydantes de dix plantes médicinales de la pharmacopée ivoirienne. *Sci Nat*; 1:1-11.
24. N'Guessan J.D., Zirihi G.N., Kra A.K.M., Kouakou K., Djaman A.J. & Guede-Guina F. (2007) : Free radical scavenging activity, flavonoid and phenolic contents of selected Ivoirian plants. *International Journal of Natural and Applied Sciences* 4:425-429.
25. Aurand L.W., Boonnmen N.H. & Gidding G.G. (1977): Superoxide and singlet oxygen in milk lipid peroxidation. *J. Diar Sci*, 363-369.
26. Siddhuraju P. & Becker K. (2007) : The antioxidant and free radical scavenging activities of processed cowpea (Vigna unguiculata (L.) Walp.) seed extracts. *Food Chemistry*. 101(1) 10-19.
27. Jeong S.M., Kim S.Y., Kim D.R., Jo S.C., Nam K.C., Ahn D.U., & Lee S.C. (2004) : Effects of heat treatment on the antioxidant activity of extracts from citrus peels. *Journal of Agriculture and Food Chemistry*. 52, 3389–3393.
28. Kumaran, A. & Karunakaran, R.J. (2007) : *In vitro* antioxidant activities of methanol extracts of five Phyllanthus species from India. *Lebensmittel-Wissenschaft und Technologie*. 40, 344–352