European Journal of Medicinal Plants

19(1): 1-14, 2017; Article no.EJMP.33343
ISSN: 2231-0894, NLM ID: 101583475

Antimicrobial, Antioxidant and Protective Effect of Methanol Extract of *Trichilia emetica* (Meliaceae) Stem and Root Bark against Free Radical-induced Oxidative Haemolysis

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Authors' contributions

This work was carried out in collaboration between all authors. Author KMLB designed the study, wrote the protocol and wrote the first draft of the manuscript. Author TTF managed the analyses of the study. Author TE performed the chemical analysis and managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJMP/2017/33343
Editor(s):
(1) Patrizia Diana, Department of Molecular and Biomolecular Sciences and Technologies, University of Palermo, Palermo, Italy.
(2) Marcello Iriti, Professor of Plant Biology and Pathology, Department of Agricultural and Environmental Sciences, Milan State University, Italy.
Reviewers:
(1) Cheikh Sall, University of Thies, Senegal.
(2) Mosad Ahmed Ghareeb, Theodor Bilharz Research Institute (TBRI), Egypt.
Complete Peer review History: http://www.sciencedomain.org/review-history/19228

Original Research Article

Received 11th April 2017
Accepted 19th May 2017
Published 29th May 2017

ABSTRACT

Background: The objectives of this study are to investigate the antimicrobial, antioxidant and antiradical properties of methanol extract of the stem and root bark of *Trichilia emetica* on a model of free radical-induced membrane damage.
Methodology: p-Iodonitrotetrazolium chloride colorometric assay was used to determined bacterial and yeast susceptibility to plant samples. The antioxidant activity was evaluated using DPPH radical, hydroxyl radical, nitric oxide scavenging and reducing power assays. The cellular oxidative stress was evaluated through haematolysis and haematoprotective assays.

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Results: The stem bark extract revealed a great antibacterial activity against 62.5% of bacteria strains tested with minimal inhibitory concentrations ranging from 32 to 64 µg/ml. The IC_{50} value the stem and root bark extracts were 9.68 ± 0.75 and 10.05 ± 0.86 µg/ml, 9.98 ± 0.52 and 200.49 ± 3.40 µg/ml, 45.92 ± 2.45 and 4081 ± 212 µg/ml, respectively for DPPH radical, hydroxyl radical and reducing ability assays. Both extracts exhibited concentration dependent haemolysis of sheep red blood cells (RBCs). Stem and root bark extracts (25 µg/ml) diminish respectively by 95.9 and 85.68% hydrogen peroxide-mediated cytotoxicity.
Conclusion: Our data reveals that antibacterial and antioxidant activities of the methanol extract of *Trichilia emetica* stem bark were better than that of root bark.

Keywords: Antimicrobial; antioxidant; hematoprotective effect; Trichilia emetica.

1. INTRODUCTION

Drug resistance to pathogenic bacteria is a public health problem all over the world [1]. The abusive and indiscriminate use of antimicrobial compounds over many years is the main factor responsible for the appearance of the phenomenon of bacterial resistance to such compounds [2]. With increased incidence of resistance to antibiotics, natural products from plants could be an interesting alternative [3,4]. Plants with their complex chemical storehouse of biodynamic compounds can serve as sources of natural antibacterial agents [5,6]. A number of studies have been conducted in different part of the world to demonstrate such efficacy [7-9]. Free radicals are the main cause of many chronic and degenerative diseases such as atherosclerosis, neurodegenerative diseases, cancer, diabetes, inflammatory diseases, and aging processes [10,11]. Oxidative stress is defined as an imbalance between production of free radicals and reactive metabolites (oxidants), and their elimination by protective mechanisms (antioxidative systems). This imbalance leads to damage of biomolecules and organs with potential impact on the whole organism. Antioxidants can delay, inhibit or prevent the oxidation of oxidizable materials by scavenging free radicals and diminishing oxidative stress [12,13]. The literature is rich on natural antioxidant studies. The purpose of all those studies is to find compounds which can protect against oxidative related diseases. To date, many plants have been claimed to pose beneficial health effects such as antioxidant properties [14,15]. Antioxidant activity of plants might be due to their phenolic compounds [16]. Flavonoids are a group of polyphenolic compounds with known properties which include free-radical scavenging, inhibition of hydrolytic and oxidative enzymes, and anti-inflammatory actions [17,18]. The antioxidant activities of polyphenols were attributed to their redox properties, which allow them to act as reducing agents, hydrogen donators and singlet oxygen quenchers, as well as their metal chelating abilities [19].

Trichilia emetica, a plant native to Africa, is used in traditional medicine to treat various ailments such as malaria, cough, gastrointestinal disorder, skin disease, gastritis, asthma, dysmenorhea, cirrhosis [20]. Several biological activities of this species have been explored, including anti-inflammatory, antischistosomal, antiplasmodial, anticonvulsant, antitrypanosomal, antitussive, antimutagenic and hepatoprotective properties [21]. The anti-oxidant properties of flavonoid rich fractions from the leaves of these plants in models using rats have been reported [22]. Study carry out on aqueous extract and ethyl ether fraction of the aqueous extract from *Trichilia emetica* root against clinical isolated bacterial strains, which are commonly responsible for respiratory infections have been reported [23]. The antioxidant activity of free phenolic acids extracted from the same part of the plant using two *in vitro* assays: autooxidation of methyl linoleate (MeLo) and ascorbate/Fe^{2+}-mediated lipid peroxidation in rat microsomes have been also reported [24]. It has been recognized that the antioxidant components differ from a plant material to another and often it is difficult to compare the results for the antioxidant properties even for the same plant. It is also clear that the effectiveness of the extracts largely depends on the type of solvent used. The organic extracts provided a more powerful antimicrobial activity, as compared to the aqueous extracts [25]. Hence, this study was therefore aimed to assess the possible antimicrobial activity of the methanolic extract of the stem and root bark of *Trichilia emetica*, against gastroenteritic bacteria and yeasts instead of bacteria causing respiratory tract diseases done elsewhere [23]. Moreover, the antioxidant and antiradical properties of those extracts on a model of free

radical-induced membrane damage were also performed.

2. MATERIALS AND METHODS

2.1 Plant Materials

The stem bark and root bark of *Trichilia emetica* (Meliaceae) were collected in the north region of Cameroon in January 2014. Identification of the plant was done at the National Herbarium Yaoundé (voucher specimen: N° 20886/SRF/Cam). The plant materials were then air dried at room temperature. The dried plant materials were ground into a fine powder.

2.2 Preparation of Plant-Extract

Plant extracts were prepared by maceration. Powder plant part (500 g) was mixed with 2500 ml of methanol in a Soxhlet apparatus for 18 h [26]. The extract was filtered and concentrated using a rotary vacuum evaporator. The complete evaporation of solvent residue was done in an oven at 30°C and the extract was stored at -20°C until used. The yield of each extract was determined.

2.3 Preliminary Phytochemical Screening of Extracts

A portion of each extract was submitted to the following identification reactions in order to look for the presence of major secondary metabolites. The protocols described by Matos was used [27]: gelatin reactions for tannins characterization, Liebermann-Burchard reagent for triterpenoids and sterols, Dragendorff and Mayer reagents for alkaloids, reactions using magnesium chip and concentrated hydrochloric acid for flavonoids, the formation of foam for saponins, and the formation of blue and green precipitate using $FeCl_3$ and $K_3Fe(CN)_6$ for polyphenols and phenols.

2.4 Antimicrobial Assay

2.4.1 Chemicals

Ciprofloxacin, amoxicilin and nystatine (Sigma-Aldrich, St. Quentin Fallavier, France) were used as reference antibiotics and p-iodonitrotetrazolium chloride (INT) (Sigma-Aldrich) as microbial growth indicator.

2.4.2 Microbial strains

Bacterial strains tested were *Staphylococcus aureus* (ATCC25922), *Enterococcus faecalis* (ATCC10541), *Escherichia coli* (ATCC11775), *Pseudomonas aeruginosa* (ATCC27853), *Salmonella typhi* (ATCC6539), *Klebsiella pneumonia* (ATCC2513883), two clinical isolated bacterial strains (*Proteus mirabilis* and *Shigella flexneri)* and the yeasts *Candida albicans* (CPC2091), *Candida glabrata* (CIPA35), *Candida tropicalis* (ATCC750), *Candida guilliermondi, Candida krusei* (ATCC6258), *Candida lusitaneae* (ATCC200950), *Candida parapsilosis* (ATCC22019) and *Cryptococcus neoformans* (IP95026) from the Pasteur Institute (IP, Paris-France). Each microbial strain was precultured overnight on Mueller Hinton Agar (bacterial) and Sabouraud Dextrose Agar (yeast), prior to any assay. Mueller-Hinton broth (MHB) and Sabouraud dextrose broth (SDB) were used for susceptibility tests for microbial strains [28].

2.4.3 Bacterial and yeast susceptibility determinations

The minimal inhibitory concentrations value of the two plant extracts and reference drugs were evaluated using a rapid INT colorimetric assay [29]. Briefly, the test extracts were first dissolved in dimethyl sulfoxide (DMSO)-medium 2.5% and serially diluted two fold (in a 96-well microplate) in MHB or SDB. One hundred microliters of inoculums (1.5×10^5 CFU/ml) prepared in the same medium was then added. The plates were covered with a sterile plate sealer and then agitated with a shaker to mix the contents of the wells and incubated at 37°C for 18 h. Wells containing MHB or SDB, 100 µl of inoculum and DMSO 2.5% served as the negative control. The minimal inhibitory concentrations of samples were detected after 18 h of incubation at 37°C, by adding (40 µl) of 0.2 mg/ml INT followed by an incubation at 37°C for 30 min. INT is used for the measurement of oxidation-reduction reaction, when it is added to a sample containing bacteria, it is reduced and its color changes from yellow dye to pink which can be measure visually. The minimal inhibitory concentration was the lowest sample concentration that prevented this color change, indicative of the complete inhibition of microbial growth. Each assay was repeated three different times.

2.5 Chemical Antioxidant Study

2.5.1 Chemicals

Gallic acid, Folin-Ciocalteu reagent, rutin, ascorbic acid and 1,1-diphenyl-2-picryl-hydrazyl (DPPH) were purchased from Sigma–Aldrich

(Steinheim, Germany). Hydrogen peroxyde (H_2O_2, 30% v/v, 8.9 M) and copper sulfate pentahydrate ($CuSO_4$, $5H_2O$) were purchased from Merck (Darmstadt, Germany). All other chemicals and solvents used were of analytical grade and purchased from Merck.

2.5.2 Red blood cells suspension preparation

Blood was obtained by veinpuncture from healthy sheep, and collected into EDTA containing tubes. Red blood cells (RBCs) were then obtained from blood by centrifugation at 3000 g for 15 min, and washed three times with phosphate buffer saline (PBS; 100 mM pH 7.4). The packed RBCs were finally suspended in PBS so as to produce a RBC suspension of 10% hematocrit which is the equivalent of 10^8 cellules/ml.

2.5.3 Estimation of total phenolic content

Folin–Ciocalteu colorimetric method was used to measure the total phenolic content of plant extract [30]. 0.5 ml of the extract samples (1 mg/ml) mixed with 2.5 ml of 0.2 N Folin-Ciocalteau reagent was allowed to stand for 5 min at room temperature. Then, 2.0 ml of 75 g/l sodium carbonate were added to the mixture and the absorbance of the reaction was measured at 760 nm after 2 h of incubation at the same temperature. Solutions of gallic acid in methanol/water (1:1, v/v) ranging from 25 to 500 µg/ml were used to prepare the standard curve. All determinations were carried out in triplicate. Total phenol values were expressed in terms of gallic acid equivalent (GAE) mg/g of dry mass sample.

2.5.4 Estimation of total flavonoid content

Total flavonoid content of extracts was estimated using aluminium chloride colorimetric method [31]. Each plant extract (0.5 ml, 1 mg/ml) was successively mixed with methanol (1.5 ml), aluminum chloride (0.1 ml, 10%), potassium acetate (0.1 ml, 1 M), and distilled water (2.8 ml), and kept for 30 min at room temperature. The absorbance of the reaction mixture was measured at 415 nm with a spectrophotometer (Thermo scientific BioMatesTM 3S). Solutions of rutin in methanol (25 to 500 µg/ml) were used to prepare the standard curve. Total flavonoid content was expressed in terms of rutin equivalent (RE) mg/g of dry mass sample. All determinations were carried out in triplicate.

2.5.5 DPPH radical scavenging assay

The free radical-scavenging capacity of the extracts was determined using DPPH [32]. Various concentrations of plant extracts (1.62 to 200 µg/ml) were added to DPPH methanol (1ml, 0.004%) and mixed vigorously with vortex. The tubes were then incubated in dark for 30 minutes at room temperature, and the absorbance was taken at 517 nm. Ascorbic acid and gallic acid were taken as known free-radical scavengers. All tests were performed in triplicate. The percentage of inhibition activity was scored using the following formula, % Inhibition = $[1 - (A_1) / A_0] \times 100$, Where A_0 was the absorbance of the control (without extract) and A_1 was the absorbance of the extract or standard. IC_{50} (inhibitory concentration), which was defined as the concentration (µg/ml) of sample required to inhibit the formation of DPPH radicals by 50%, was determined.

2.5.6 Determination of reducing power

Adesegun et al. [33], method was used to determine the reducing power ability of the extracts. Extracts solutions (2.5 ml, 1.62 to 200 µg/ml) were successively mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6), potassium ferricyanide (2.5 ml, 1%) and incubated for 30 min at 50°C. Trichloroacetic acid (2.5 ml, 10%) was added to the mixture and centrifuged (2000 rpm for 10 min). The supernatant (2.5 ml) was mixed with equal volumes of distilled water and ferric chloride (0.5 ml, 0.1%).The absorbance was measured at 700 nm against a blank reagent (the same mixture of reagent without plant-extract). Ascorbic acid and gallic acid were used as standards materials. All tests were performed in triplicate. A higher absorbance indicated a higher reducing power. The effective concentration (EC_{50}) at which the absorbance was 0.5 for reducing power was calculated.

2.5.7 Nitrogen oxide (NO) scavenging assay

Estimation of Nitric oxide radical inhibition was done using Griess Illosvoy reaction [34]. At physiological pH, sodium nitroprusside spontaneously generates nitric oxide in aqueous solution. This ended product interacts with oxygen to produce nitrite ions, which can be measured at 550 nm in the presence of Griess reagent [34]. In this study, we used naphthyl ethylene diamine dihydrochloride (0.1% w/v) instead of using 1-naphthylamine (5%) as Griess Illosvoy reagent. The reaction mixture (3 ml)

containing sodium nitroprusside (10 mM, 2 ml), phosphate buffer saline (0.5 ml) and the extract (1.62 to 200 µg/ml) were incubated at 25°C for 150 minutes. From 0.5 ml of the later reaction mixture, 1 ml of sulfanilic acid reagent (0.33% in 20% glacial acetic acid) was added and allowed to stand for 5 min for completion of the reaction process of diazotization. After diazotization, 1 ml of naphthyl ethylene diamine dihydrochoride was added, mixed and was allowed to stand for 30 min at 25°C. The absorbance was taken at 550 nm. Gallic acid and ascorbic acid at the same dilution (1.62 to 200 µg/ml) were used as standards. All tests were performed in triplicate. Inhibition of nitrite formation by the plant extracts and standards were calculated relative to the control (% Inhibition = $[1 - (A_1) / A_0] \times 100$, Where A_0 was the absorbance of the control (without extract) and A_1 was the absorbance of the extract or standard). IC_{50} of the nitric oxide formation was determined.

2.5.8 Hydroxyl radical scavenging assay

Fenton reaction was used to measure the scavenging activity for hydroxyl radicals [35]. Reaction mixture contained $FeCl_2$ (60 µl, 1.0 mM), 1,10-phenanthroline (90 µl, 1 mM), phosphate buffer (2.4 ml, 0.2 M pH 7.8), H_2O_2 (150 µl, 0.17 M), and extract (1.0 ml, 1.62 to 200 µg/ml) was prepared and incubated at room temperature for 5 min. The reaction started when H_2O_2 was added. After incubation, the absorbance of the mixture was measured at 560 nm with spectrophotometer (Thermo scientific BioMatesTM 3S). Gallic acid and ascorbic acid (1.62 to 200 µg/ml) were used as a standard. All tests were performed in triplicate. The percentage inhibition of hydroxyl scavenging activity was calculated using the following formula; % Inhibition = $[1 - (A_1) / A_0] \times 100$, Where A_0 was the absorbance of the control (without extract) and A_1 was the absorbance of the extract or standard.

2.6 Cellular Antioxidant Study

The cellular antioxidant activity was measured on red blood cells as described by Amzal H [36]. Due to the high content of polyunsaturated fatty acids in their cell membranes, erythrocytes are susceptible to oxidative damage and can be used as valuable diagnostic material [37]. The method measures the ability of the plant extracts to manage intracellular generation of hydroxyl radicals in response to $H_2O_2/CuSO_4$ mixture, used as a generator of hydroxyl radicals.

2.6.1 Haemolysis test

Cellular toxicity of the extract was determined through haemolysis test as previously described [38]. 500 µl of the plant-extracts (0.25 to 32 mg/ml) were incubated with an equal volume of 10% hematocrit sheep red blood cells in phosphate buffered saline (10mM PBS, pH 7.4) at 37°C for 3 h. Phosphate buffered saline alone was taking as non-hemolytic control and phosphate buffered saline containing 1% Triton X-100 as 100% hemolytic control. Cell damage was monitored by measuring the release of hemoglobin from red blood cells spectrophotometrically (Thermo scientific BioMatesTM 3S) at 540 nm. The assay was repeated three times independently.

2.6.2 Protective effect of plant-extracts against $CuSO_4/H_2O_2$ induced membrane damage

The results obtained from the haemolysis test above showed that, extracts at low concentration (≤ 0.25 mg/ml) have a similar effect as the non hemolytic control. Hence, the concentration of 25 µg/ml was considered as a non-haemolytic concentration and used for the study of the haematoprotective effect of extracts and standard against lysis solution on red blood cells. $CuSO_4/H_2O_2$ was used to initiate erythrocyte haemolysis. 10% hematocrit sheep erythrocyte suspension in phosphate buffer saline (PBS, 100 mM) were preincubated for 30 minutes with an equal volume of a non-haemolytic concentration of plant-extracts or standards (25 µg/ml). The same volume of the lysis solution $CuSO_4/H_2O_2$ (100 µM/25 µM in 100 mM PBS) was then added to the various mixtures. Under these conditions, hydroxyl radicals were generated from the H_2O_2-Cu^{2+} mixture. The different combinations of reaction mixtures were incubated at 37°C for 3 h with continuous shaking. Then, the reaction mixture was diluted with PBS (1/ 8 v/v) and centrifuged at 3000 g for 5 min. The optical density of the supernatant fraction was recorded at 540 nm before (0 hour) and after 1, 2, or 3 h of incubation in order to monitor the cell lysis through hemoglobin release. Standards were gallic acid, vitamin E and butylated hydroxytoluene (BHT). The absorbance of tested samples (RBCs + lysis solution + extracts or standards) was compared with controls (RBCs without extract, standard or lysis solution) and (RBCs + lysis solution). The results were express as percentage of RBC lysis reduction, the optical density of the lysis solution taking as 100% of

hemolysis. The assay was repeated three times independently.

2.7 Statistical Analysis

All the experiments on chemical and cellular antioxidant assay were conducted in triplicates and the mean ± standard deviation (SD) were determined. We used analysis of variance following by the Duncan's test to statistically evaluate our data. The differences between groups were considered significant at $p < 0.05$.

2.8 Ethics Statement

The study was conducted on blood sample from sheep brought to abattoir in Dschang – Cameroon. The protocol was approved by the Cameroon National Ethics Committee (Reg. No. FWA-IRB 00001954). All animals were handled in strict accordance with the internationally accepted principle guidelines of the European Union on Animal Care (CEE Council 86/609).

3. RESULTS

3.1 Phytochemical Screening

The results on phytochemical analysis showed that both extracts contain alkaloids, flavonoids, polyphenols, tannins and triterpenoids. Saponins were present only in stem bark extract (Table 1).

3.2 Antimicrobial Assay

Tables 2 and 3 summarize the results of the antimicrobial evaluation of the tested plant-extracts. The MIC values obtained were ranged from 1024 to 32 µg/ml. It is known that, plant extracts with MIC values below 100 µg/ml are very promising [39]. So, methanol extract of *Trichilia emetica* stem bark presented a very strong activity against *Shigella flexneri, Pseudomonas aeruginosa, Enterococcus faecalis, Staphylococcus aureus* and *Escherichia coli* with MIC value of 64, 32, 32, 64 and 64 µg/ml respectively. However this extract did not shown any activity on yeast strains tested. The activity of the stem bark extract (32 µg/ml) against *Pseudomonas aeruginosa* was higher than the activity obtained with amoxicillin (128 µg/ml), the standard drug. Root bark extract of this plant was not active on both bacterial and fungal strains tested.

3.3 Chemical Antioxidant Assay

3.3.1 Phenolic and flavonoid content

The phenolic content was found to be 13.23 ± 1.35 and 8.04 ± 1.1 mg gallic acid equivalents (GAE)/g dry weight, and the flavonoid content, 6.75 ± 1.28 and 3.51 ± 0.36 mg rutin equivalents (RE)/g dry weight respectively in the stem bark and root bark extract (Table 4).

Table 1. Classes of phytocompounds of *Trichilia emetica* (TE) extracts

Plant extract	Alc	Flav	Phe	Sap	Tan	Trit	Ster
TE stem bark	+	+	+	+	+	+	-
TE root bark	+	+	+	-	+	+	-

Alc : Alcaloïdes, Flav : Flavonoïdes, Phe : Phénols, Sap : Saponines, Tan : Tanins, Trit : Triterpènes, Ster : Stérol

Table 2. Antibacterial activity of *Trichilia emetica* (TE) extracts

Plant extract /standards	MIC (µg/ml)							
	Kp	Sf	St	Pa	Ef	Ec	Sa	Pm
TE (stem bark)	128	64	128	32	32	64	64	256
TE (root bark)	1024	1024	1024	512	512	512	512	512
Ciprofloxacin	0.5	0.032	0.5	0.5	0.125	0.032	0.25	0.25
Amoxicillin	16	32	16	128	8	8	8	8

Kp: Klebsiella pneumonia, Sf: Shigella flexneri, St: Salmonella typhi, Pa: Pseudomonas aeruginosa, Ef: Enterococcus faecalis, Sa: Staphylococcus aureus, Ec: Escherichia coli, Pm: Proteus mirabilis

Table 3. Antifungal activity of *Trichilia emetica* (TE) extracts

Plant extract /standards	MIC (µg/ml)							
	Ca	Ck	Ct	Cn	Cg	Cp	Cgl	C
TE (stem bark)	1024	512	512	512	256	>1024	>1024	>1024
TE (root bark)	>1024	>1024	1024	1024	512	512	>1024	>1024
Nystatin	0.125	1	0.062	0.062	0.062	0.031	0.031	0.062

Ca: Candida albicans, Cg: Candida glabrata, Ct: Candida tropicalis, Cgl: Candida guilliermondi, Ck: Candida krusei, Cn: Candida lusitaneae, Cp: Candida parapsilosis, C : Cryptococcus neoformans

Total phenolic and flavonoid content of the stem bark were significantly higher than that of the root bark.

3.3.2 Scavenging effect on 2, 2-diphenyl-1-picryl hydrazyl radical

The DPPH radical scavenging activity was concentration dependant. Both the two extracts show a similar activity compared to ascorbic acid as standard, but that activity was less compared to gallic acid as standard. The IC_{50} values of stem and root bark extract, ascorbic and gallic acid were 9.68 ± 0.75, 10.05 ± 0.86, 12.5 ± 0.99 and 5.49 ± 0.40 µg/ml respectively (Table 5).

3.3.3 Hydroxyl radical scavenging activity

Stem and root bark extracts exhibited an increase of scavenging activity against hydroxyl radical with the concentration. It was also found that the stem bark extract is significantly more effective than the root bark extract as far as hydroxyl radical scavenging activity is concerned. The IC_{50} value were found to be 9.98 ± 0.52 and 200.49 ± 3.40 µg/ml respectively for stem and root bark extract while IC_{50} value for ascobic and gallic acid were 10.61± 0.40 and 1.23 ± 0.1 µg/ml respectively (Table 5).

3.3.4 Nitric oxide scavenging activity

We noticed a significant decrease in the NO radical due to the scavenging ability of ascorbic acid or gallic acid, as standards compared with extracts. But, root extract was significantly active than that of stem as far as NO radical scavenging activity is concerned. The IC_{50} value for scavenging of nitric oxide for stem and root extract were 619.56 ± 25.1 and 565.61 ± 65.35 µg/ml, while the IC_{50} value for ascorbic acid and gallic acid were 74.37 ± 1.13 and 53.85 ± 1.42 µg/ml respectively (Table 5).

3.3.5 Reducing power activity

The reducing potential of the stem bark extract, ascorbic acid and gallic acid were significantly high compared to that of the root bark extract. The EC_{50} value of reducing power for stem and root extract were 45.92 ± 2.45 and 4081 ± 212 µg/ml, while the EC_{50} value for ascorbic acid and gallic acid were 12.27 ± 0.11 and 3.42 ± 0.25 µg/ml respectively (Table 5).

3.4 Cellular Antioxidant Activity

3.4.1 Haemolysis activity

Haemolytic activity of *Trichilia emetica* was evaluated as described in the experimental section above. The results obtained are shown on Fig. 1. From the figure, we noticed that, at concentration less than 0.5 mg/ml both extracts had similar haemolytic effect as non haemolytic control (10mM PBS, pH 7.4).

Table 4. Extraction yields, total phenolic and flavonoids content of *Trichilia emetica* (TE) extracts

Plant materials	Yield of extraction (%)	Total phenolic GAE (mg/g of dry mass)	Total Flavonoïds RE (mg/g of dry mass)
TE (stem bark)	5	13.23± 1.35[a]	6.75 ± 1.28[a]
TE (root bark)	22.6	8.04 ± 1.1[b]	3.51 ± 0.36[b]

Data are means ± standard deviation (n = 3 tests). Values in the same column with different superscript [(a, b)] are significantly different from each other (p < 0.05). GAE: gallic acid equivalent; RE: rutin equivalent

Table 5. Antioxidant activity of *Trichilia emetica* (TE) extracts

Plant extracts and standards	DPPH (IC_{50} µg/mL ± SD)	Reducing power (EC_{50} µg/mL ± SD)	Hydroxyl radical (IC_{50} µg/mL ± SD)	Nitric oxide (IC_{50} µg/mL ± SD)
TE (stem bark)	9.68 ± 0.75[a]	45.92 ± 2.45 [a]	9.98 ± 0.52 [b]	619.56 ± 25.1 [b]
TE (root bark)	10.05 ± 0.86 [a]	4081 ± 212[d]	200.49 ± 3.40 [d]	565.61 ± 65.35 [c]
Asc Ac	12.5 ± 0.99 [a]	12.27 ± 0.11 [a]	10.61± 0.40 [b]	74.37 ± 1.13 [a]
Gall Ac	5.49 ± 0.40[b]	3.42 ± 0.25 [a]	1.23 ± 0.1 [a]	53.85 ± 1.42 [a]

Data are means ± standard deviation (n = 3 tests). Values in the same column with different superscript [(a, b, c or d)] are significantly different compared with standards (p < 0.05)

3.4.2 Haematoprotective activity

Haemoglobin releasing from RBC was considerably increased with hydrogen peroxide treatment (Fig. 2) compared to the negative control (RBCs alone). Thus, indicating the RBC cytotoxicity effect of $CuSO_4$ and H_2O_2 combination. Incubation of RBCs with plant-extracts (25 µg/ml) before exposing them to $CuSO_4$ and H_2O_2 reduced the cytotoxicity of RBCs. As indicated in Fig. 2, the antihaemolytic activity of all the tested extracts decreases with time as compared to what was observed with the reference products used. Stem and root bark extracts of *Trichilia emetica* (25 µg/ml) diminished respectively by 95.9 and 85.68% hydrogen peroxide-mediated cytotoxicity of RBC after a 1-h incubation compared to the negative control (red blood cell + lysis solution) taking as 100% of cell lysis. At the same time of incubation, 25 µg/ml of standards (Vitamin E, gallic acid and butylated hydroxytoluene) diminished by 63.5, 57.19 and 64.88% respectively, hydrogen peroxide-mediated cytotoxicity of RBC (Fig. 2). The most potent antihaemolytic products after 1-h incubation were stem and root bark extracts of *Trichilia emetica,* butylated hydroxytoluene (BHT), vitamin E and gallic acid respectively.

4. DISCUSSION

Methanol extract of *Trichilia emetica* stem bark presented a very strong activity against *Shigella flexneri, Pseudomonas aeruginosa, Enterococcus faecalis, Staphylococcus aureus* and *Escherichia coli* with MIC ranging from 32 to 64 µg/ml. However this extract did not show any activity on yeast strains tested. *Shigella flexneri* and *Escherichia coli*, gram-negative bacteria, are significant cause of gastroenteritis in both developing and industrialized countries [40]. *P. aeruginosa* is the most common gram-negative bacterium found in nosocomial infections. *S. aureus* can cause septicaemia if it reaches the lymphatic channels or blood [41]. Unlike stem bark extract, root bark extract of this plant was not active on both bacterial and fungal strains tested. The difference of activity between the two extracts may be due to their chemical composition. Preliminary phytochemical analysis revealed the presence of flavonoids, tannins, alkaloids, phenolics and triterpenoids in the two extracts (Table 1). In addition to those chemical compounds, the stem bark extract contains saponins. Several molecules belonging to the detected classes of secondary metabolites were found active on pathogenic microorganisms [42-44]. Flavonoids exert their antimicrobial effect by enzymatic inhibition of DNA gyrase [45]. The antimicrobial action of tannins may be related to their ability to inactivate microbial adhesions, enzymes, cell envelope transport protein, etc. Some alkaloids are able to intercalate with DNA, this may be the mechanism of their antimicrobial activity [46]. Triterpenes and sterols increasing cell permeability and loss of cellular components [47]. Saponins are known to interact with

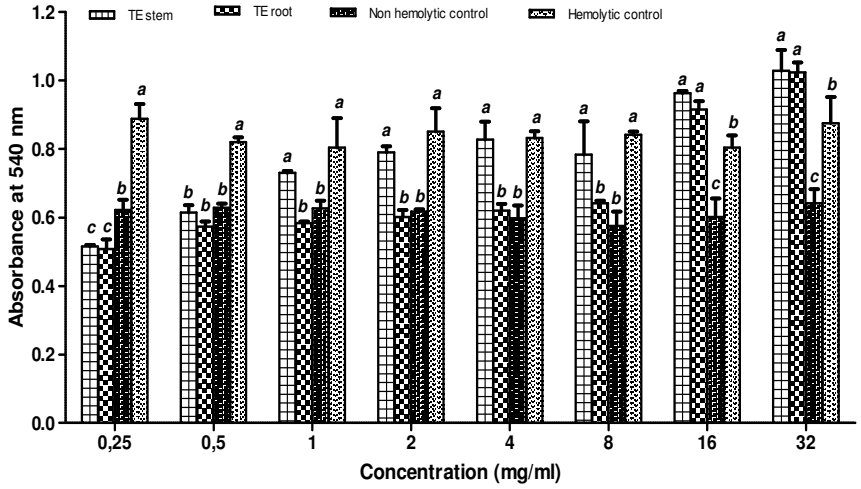

Fig. 1. Hemolytic effect of stem and root bark extracts of *Trichilia emetica* (TE) using hemoglobin (Hb) release (absorbance at 540 nm)

Non hemolytic control (10 mM PBS, pH 7.4), Hemolytic control (PBS + 1% Triton X-100). Each data column represents the mean ± SD (n = 3). Data column at the same concentration with different superscript [a, b or c] are significantly different (p < 0.05)

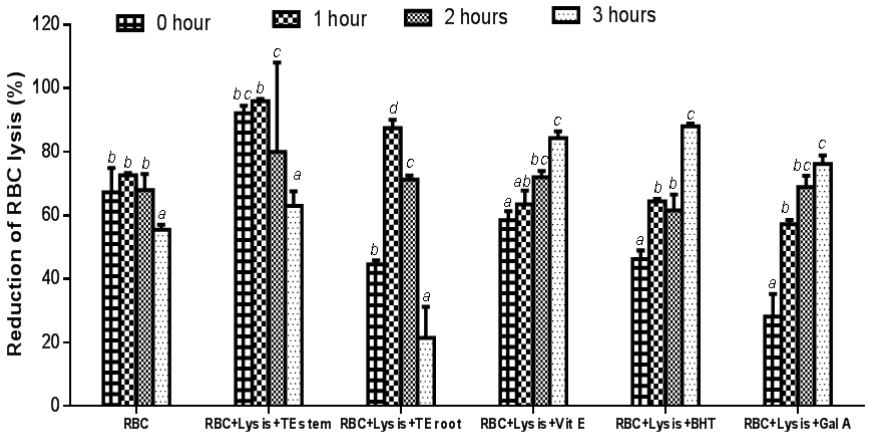

Fig. 2. Effect of various treatments (25 µg/ml) on hemolysis (% of RBC lysis reduction) at different incubation times (0, 1, 2 and 3 h)

Red blood cells (RBC), Trichilia emetica root bark (TE root), Trichilia emetica stem bark (TE stem), Vitamin E (Vit E), Butylated hydroxytoluene (BHT), Gallic acid (Gal A). Each data column represents the mean ± SD (n = 3). Data column of the same treatment with different superscript [a, b, c or d] are significantly different (p < 0.05)

cell membranes, increasing permeability and producing cell damage [48]. Thus, the difference in the chemical composition of the two plants extracts can provide a preliminary explanation on their different antibacterial activities. The antibacterial activity of a crude aqueous extract from *Trichilia emetica* root against clinical isolated bacterial strains responsible for respiratory infections have been reported [23] and the present study provides additional data on the ability of the stem back extract of this plant to fight gastrointestinal bacteria.

Antioxidants protect the body against reactive oxygen species (ROS) generated during normal cell aerobic respiration [49]. In the present study, the antioxidant activity of the stem and root bark extracts of *Trichilia emetica* was evaluated using four chemical assays. Their activity against cellular oxidative stress was measured by haematolysis and haematoprotective assays.

DPPH assay estimates the ability of sample to scavenge free radicals species capable of causing damage to natural macromolecules, such as nucleic acids, polysaccharides, and lipids [50]. We noticed that the antiradical activity of stem bark and root bark extract from *Trichilia emetica* was found to be comparable to that of ascorbic acid as far as the DPPH assay was concerned (Table 5). Kiessoun Konate et al. [22], using similar methods, reported an improved DPPH antiradical activity of flavonoid-rich fractions from *Trichilia emetica* leaves.

A reduicing property is the ability to break the free radical chain by donating a hydrogen atom [51]. In this study, stem bark extract of *Trichilia emetica* had a significant ($P < 0.05$) successful reducing ability for Fe (III) to Fe (II) than root bark extract (Table 5). The reducing ability of the stem bark extract of the tested plant was similar to that of vitamin E, BHT and gallic acid used here as reference antioxidant.

Hydroxyl radical is a highly reactive oxygen radical formed from the reaction of various hydroperoxides with transition metal ions. It attacks proteins, DNA, polyunsaturated fatty acid in membranes, and most biological molecules it contacts [52] and is known to be capable of abstracting hydrogen atoms from membrane lipids and bringing about peroxidic reaction of the lipids. We noticed that, stem bark extract of *Trichilia emetica* possessed marked scavenging activity against hydroxyl radicals generated in a Fenton reaction system than the root bark extract (Table 5). The presence of certain secondary metabolites may explain the difference in scavenging obtained.

Nitric oxide (NO) is an important chemical mediator generated by endothelial cells, macrophages, neurons, etc. and is involved in the regulation of various physiological processes [53]. Excess concentration of NO is associated with several diseases [54]. NO scavenging capacity is determined by the decrease in the absorbance at 550 nm, induced by antioxidants.

None of the plant-extract showed a remarkable activity against nitric oxide scavenging compared to standards used (Table 5).

Red blood cells are rich in antioxidant enzymes [55]. Tan and Yeung, [56] believe that a high concentration of oxygen and hemoglobin in erythrocytes makes them potential powerful promoters of the oxidative processes. As indicated in Fig. 2, stem and root bark extracts of *Trichilia emetica* (25 µg/ml) diminished respectively by 95.9 and 85.68%, hydrogen peroxide-mediated cytotoxicity of RBC after a 1-h incubation, but this antihaemolytic activity decreases with time.

Our data suggests that the methanol extract of *Trichilia emetica* stem bark exhibits a better antioxidant property than that of the root bark. The differences in chemical composition has probably caused such difference of antioxidant properties. The phytochemical tests indicated the presence of alkaloids, flavonoids, polyphenols, tannins and triterpenoids in both the two extracts (Table 1). Only stem bark extract showed the presence of saponins. Since saponins are recognized by their non hemolytic activity and foaming properties [57,58], the observed antiradical activity of *Trichilia emetica* stem bark against cellular membrane damage may be partially due to its content in saponins. This observation is in accordance with other studies reporting that saponins of Panax ginseng (ginsenosides) protect human erythrocytes from free radical-induced haemolysis [59].

Phenolics and flavonoids act as primary antioxidants [60], by reacting with hydroxyl radicals [61], superoxide anion radicals [62] and lipid peroxyradicals [63]. They are also known to protect DNA from oxidative damage, inhibit growth of tumor cells and possess anti-inflammatory and antimicrobial properties. In the present study, flavonoid and phenolic content of the stem bark extract of *Trichilia emetica* was significantly higher than that of the root bark (Table 4). The results obtained in this work using chemical and cellular assays showed that the extracts with a higher content in phenolic compound and flavonoids have a higher antioxidant capacity. Correlation between phenolic compounds and antioxidant activity has been demonstrated [56,64-66]. Our results are similar to the previous studies. Indeed, it has been demonstrated that free phenolic acids extracts from the root of this plant showed an antioxidant properties in two *in vitro* assays: autooxidation of methyl linoleate (MeLo) and ascorbate/Fe^{2+}-mediated lipid peroxidation in rat microsomes [24]. Studies on the chemical constituents on this plant have revealed that, it yields fats, resin, tannin, and bitter principle related to calicedrin [67]. A preliminary phytochemical analysis showed a high polyphenolic content in the aqueous extract and the presence of limonoids in the ethyl ether fraction of this plant [24]. Several representatives of the limonoids have been reported to exhibit anti-inflammatory effects [68]. Others studies have led to the isolation of a number limonoids [69,70] and kurubasch aldehyde, a sesquiterpenoid with an hydroxylated humulene skeleton from *Trichilia emetica* [71]. Hence, these compounds may be considered responsible for the bioactivity of the tested plant.

5. CONCLUSION

Our data reveals that the methanol extract of *Trichilia emetica* stem bark possesses a remarkable antibacterial activity. The activity of *Trichilia emetica* stem bark extract was better than that of root bark, as antioxidant activity is concerned. The overall activities of *Trichilia emetica* stem bark extract may be due to its phenolic compounds content as compared to that of the root back extract. The results of the present study may partially justify the traditional uses of this plant. Further, more detailed studies on specific chemical composition of those extracts, as well as in *vivo* assays are essential to characterize these crude extract as biological antimicrobial and antioxidant.

CONSENT

It is not applicable.

ETHICAL STATEMENT

Blood collection from sheep was conducted in accordance with the internationally accepted principle guidelines of the European Union on Animal Care (EEC Directive of 1986; 86/609).

The Cameroon National Ethical Committee approved the protocols of the study (Ref n° FWIRB 00001954).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. N'guessan JD, Dinzedi MR, Guessennd N, Coulibaly A, Dosso M, Djaman AJ, Guede-Guina F. Antibacterial activity of the aqueous extract of *Thonningia sanguinea* against extended-spectrum lactamases (ESBL) producing *Escherichia coli* and *Klebsiella pneumoniae* strains. Tropical Journal of Pharmaceutical Research. 2007;6(3):779-783.
2. Andremont A. The future control of bacterial resistance to antimicrobial agents. American Journal Infect Control. 2001;29(4):256-258.
3. Lu Y, Zhao YP, Wang ZC, Chen SY, Fu CX. Composition and antimicrobial activity of the essential oil of *Actinidia macrosperma* from China. Natural Product Research. 2007;3(21):227-233.
4. Mbwambo ZH, Moshi MJ, Masimba PJ, Kapingu MC, Nondo RS. Antimicrobial activity and brine shrimp toxicity of extracts of *Terminalia brownii* roots and stem. BMC Complementary and Alternative Medicine. 2007;9(7):1-5.
5. Abel C, Busia K. An explanatory ethnobotanical study of the practice of herbal medicine by the Akan peoples of Ghana. Altern Med Rev. 2005;10:112-22.
6. Roosita K, Kusharto CM, Sekiyama M, Fachrurozi Y, Ohtsuka R. Medicinal plants used by the villagers of a Sundanese community in West Java, Indonesia. J Ethnopharmacol. 2008;115:72-81.
7. Benoit-Vical F, Grellier P, Abdoulaye A, Moussa I, Ousmane A, Berry A. *In vitro* and *in vivo* antiplasmodial activity of *Momordica balsamina* alone or in a traditional mixture. Chemotherapy. 2006;52(6):288-292.
8. Senatore F, Rigano D, Formisano C, Grassia A, Basile A, Sorbo S. Phytogrowth-inhibitory and antibacterial activity of *Verbascum sinuatum*. Fitoterapia. 2007;78(3):244-247.
9. Singh G, Maurya S, De Lampasona MP, Catalan CA. A comparison of chemical, antioxidant and antimicrobial studies of cinnamon leaf and bark volatile oils, oleoresins and their constituents. Food and Chemical Toxicology. 2007;45(9):1650-1661.
10. Davies KJ. Oxidative stress, antioxidant defenses and damage removal, repair and replacement systems. IUBMB Life. 2000;50:279-289.
11. Fenkel T, Holbrook NJ. Oxidants, oxidative stress and the biology of aging. Nature. 2000;408:240-247.
12. Duracková Z. Some current insights into oxidative stress. Physiological Research. 2010;59(4):459-469.
13. Reuter S, Gupta SC, Chaturvedi MM, Aggarwal BB. Oxidative stress, inflammation, and cancer: How are they linked? Free Radical Biology & Medicine. 2010;49(11):1603-1616.
14. Kaur GJ, Arora DS. Antibacterial and phytochemical screening of *Anethum graveolens*, *Foeniculum vulgare* and *Trachyspermum ammi*. BMC Complementary and Alternative Medicine. 2009;9(30):1-10.
15. Newman DJ, Cragg GM. Natural products as sources of new drugs over the last 25 years. Journal of Natural Products. 2007;70(3):461-477.
16. Cook NC, Samman S. Flavonoids-chemistry, metabolism, cardioprotective effects, and dietary sources. Journal of Nutritional Biochemistry. 1996;7:66-76.
17. Wang L, YI C, Tu, Lian TW, Hung JT, Yen JH, Wu MJ. Distinctive antioxidant and antiinflammatory effects of flavonols. Journal of Agricultural and Food Chemistry. 2006;54:9798-9804.
18. Clavin M, Gorzalczany S, Macho. Anti-inflammatory activity of flavonoids from *Eupatorium arnottianum*. Journal of Ethnopharmacology. 2007;112:585-589.
19. Vladimir-Knezević S, Blazeković B, Stefan MB, Alegro A, Koszegi T, Petrik J. Antioxidant activities and polyphenolic contents of three selected *Micromeria* species from Croatia. Molecules. 2011;16:1454-1470.
20. Diallo. Ethnopharmocological survey of medical plants in Mali a phytochemical study of four of them: *Glinus oppositifolius* (Aizoaceae), *Diospyros abyssinica* (Ebenaceae), *Entada africana* (Mimosaceae), *Trichilia emetica* (Méliaceae). These de Doctorat. Lausanne. 2000;148-176.
21. Baatile MK, Eugene IO, Alvaro MV. *Trichilia emetica* (Meliaceae) – A review of traditional uses, biological activities and phytochemistry. Phytochemistry Letters. 2011;4:1-9.
22. Konatć K, Kassi Y, Oksana S, Patrice Z, Marian B, Van DP, Paul G, Nicolas B. Free radicals scavenging capacity, antidiabetic and antihypertensive activities of flavonoid-

rich fractions from leaves of *Trichilia emetica* and *Opilia amentacea* in an animal model of type 2 diabetes mellitus. Evidence-Based Complementary and Alternative Medicine. 2014;13. Article ID 867075.
23. Germanò MP, D'Angelo V, Sanogo R, Catania S, Alma R, De Pasquale R, Bisignano G. Hepatoprotective and antibacterial effects of extracts from *Trichilia emetica* Vahl. (Meliaceae). Journal of Ethnopharmacol. 2005;96:227-232.
24. Germano MP, D'Angelo V, Biasini T, Sanogo R, De Pasquale R, Catania S. Evaluation of the antioxidant properties and bioavailability of free and bound phenolic acids from *Trichilia emetica* Vahl. Journal of Ethnopharmacol. 2006;105: 368-373.
25. Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev. 1999;12:564-82.
26. Thakurta P, Bhowmik P, Mukherjee S, Hajra TK, Patra A, Bag PK. Antibacterial, antisecretory and antihemorrhagic activity of *Azadirachta indica* used to treat cholera and diarrhea in India. J Ethnopharmacol. 2007;111:607-612.
27. Matos FJA. Introdução à Fitoquímica Experimental. EUFC, Fortaleza, Brazil ISBN. 1997;85-7282-026-4.
28. Eloff JN. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Med. 1998;64:711-713.
29. Kuete V, Mbaveng TA, Tsafack M, Beng PV, Etoa FX, Nkengfack AE, Marion Meyer JJ, Lall N. Antitumor, antioxidant and antimicrobial activities of *Bersama engleriana* (Melianthaceae). J. Ethno-pharmacol. 2008;115:494-501.
30. Ebrahimzadeh MA, Pourmorad F, Hafezi S. Antioxidant activities of Iranian corn silk. Turk J Biol. 2008;32:43-49.
31. Chang C, Yang M, Wen H, Chern J. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Anal. 2002;10:178-182.
32. Hasan MS, Ahmed MI, Mondal S, Uddin SJ, Masud MM, Sadhu SK. Antioxidant, antinociceptive activity and general toxicity study of *Dendrophthoe falcata* and isolation of quercitrin as the major component. OPEM. 2006;6:355-560.
33. Adesegun SA, Elechi NA, Coker HAB. Antioxidant activities of methanolic extract of *Sapium elliticum*. Pak J Biol Sci. 2008;11:453-457.
34. Hyoung LS. Antioxidant activity of browing reaction products isolated from storage-ged orange juice. J Agric Food Chem. 1992;40:550-552.
35. Yu W, Zhao Y, Shu B. The radical scavenging activities of radix puerariae isoflavonoids: A chemiluminescence study. Food Chem. 2004;86:525.
36. Amzal H. Étude de l'activité antioxydante des saponines du tourteau de l'arganier. PhD Thesis. Mohammed V Agdal University, Faculté des Sciences Rabat. French; 2010.
37. Situ H, Bobek LA. *In vitro* assessment of antifungal therapeutic potential of salivary histatin-5, two variants of histatin-5, and salivary mucin (MUC7) domain 1. Antimicrob Agents Chemother. 2000;44: 1485-1493.
38. Ordonez F, Rosety-Plaza M, Rosety-Rodriguez M. Glucose-6-phosphate-dehydrogenase is also increased in erythrocytes from adolescents with Down syndrome. Down Syndrome Research and Practice. 2006;11:84-87.
39. Cos P, Vlietinck AJ, Berghe DV, Maes L. Anti-infective potential of natural products: how to develop a stronger in *vitro* 'proof-of-concept'. Journal of Ethnopharmacology. 2006;106(3):290-302.
40. Boumghar-Bourtchai LB, Kurkdjian PM, Bingen E, Filliol I, Dhalluin A, Ifrane SA, Weill FX, Leclercq R. Macrolide-resistant *Shigella sonnei*. Emerging Infectious Diseases. 2008;14(8):1297-1299.
41. Harris LG, Foster SJ, Richards RG. An introduction to *Staphylococcus aureus* and techniques for identifying and quantifying *S. aureus* adhesins in relation to adhesion to biomaterials. Review. European Cells & Materials Journal. 2002;4(1):39-60.
42. Awouafack MD, Tane P, Kuete V, Eloff JN. 2-sesquiterpenes from the medicinal plants of Africa. In: Kuete, V. (Ed.), Medicinal Plant Research in Africa. Elsevier, Oxford. 2013;33-103.
43. Ndhlala AR, Amoo SO, Ncube B, Moyo M, Nair JJ, Van Staden JL. 16-antibacterial, antifungal, and antiviral activities of African medicinal plants. In: Kuete, V. (Ed.), Medicinal Plant Research in Africa. Elsevier, Oxford. 2013;621-659.
44. Tsopmo A, Awah FM, Kuete V. 12-Lignans and Stilbenes from African medicinal plants. In: Kuete, V. (Ed.), Medicinal Plant

Research in Africa. Elsevier, Oxford. 2013;435-478.
45. Cushnie TPT, Lamb AJ. Antimicrobial activity of flavonoids. International Journal of Antimicrobial Agents. 2005;26(5):343-356.
46. Phillipson JD, O'Neil MJ. New leads to the treatment of protozoal infections based on natural product molecules. Acta Pharmaceutica Nordica. 1989;1(1):131-144.
47. Bagamboula CF, Uyttendaele M, Debevere J. Antimicrobial and antioxidative activities of the essencial oils and methanol extracts of *Salvia cryptanha* (Montbret et Aucher ex Benth.) and *Salvia multicaulis* (Vahl.). Food Chemistry. 2004;84(4):519-525.
48. Francis G, Kerem Z, Makkar HPS, Becker K. The biological action of saponins in animal systems: A review. British Journal of Nutrition. 2002;88(6):587-605.
49. Gutteridge JMC. Free radicals in disease processes: A compilation of cause and consequence. Free Radical Research Communications. 1993;19:141-158.
50. Issa AY, Volate SR, Wargovich MJ. The role of phytochemicals in inhibition of cancer and inflammation: New directions and perspectives. Journal of Food Composition and Analysis. 2006;19:405-419.
51. Schimada VL, Fujikawa K, Yahara K, Nakamura TJ. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. Agric Food Chem. 1992;50:945-948.
52. Nabavi SM. *In vitro* antioxidant and free radical scavenging activity of *Diospyros lotus* and *Pyrus boissieriana* growing in Iran. Pharmacognosy Magazine. 2009; 4(18):123-127.
53. Lata H, Ahuja GK. Role of free radicals in health and disease. Ind J Physiol Allied Sci. 2003;57:124-28.
54. Ialenti S, Moncada M, Di R. Modulation of adjuvant arthritis by endogenous nitric oxide. Br J Pharmacol. 1993;110:701-705.
55. Richards RS, Roberts TK, Dunstan RH. Erythrocyte antioxidant systems protect cultured endothelial cells against oxidant damage. Biochem Mol Biol Int. 1998;46: 857-865.
56. Tan W, Yeung ES. Simultaneous determination of enzyme activity and enzyme quantity in single human erythrocytes. Anal Biochem. 1995;226:74-79.
57. Bachran C, Sutherland M, Heisler I, Hebestreit P, Melzig MF, Fuchs H. The saponin-mediated enhanced uptake of targeted saporin-based drugs is strongly dependent on the saponin structure. Exp Biol Med (Maywood). 2006;231:412-420,
58. Vincken JP, Lynn H, Aede de G, Harry G. Saponins, classification and occurrence in the kingdom. Phytochemistry. 2007;68(3): 275-297.
59. Liu Z, Luo QXY, Sun YX, Chen YP, Wang ZC. Can ginsenosides protect human erythrocytes against free-radical-induced hemolysis? Biochim Biophys Acta. 2002;1572:58-66.
60. Adesegun SA, Fajana A, Orabueze CI, Coker HAB. Evaluation of antioxidant properties of *Phaulopsis fascisepala* C.B.Cl. (Acanthaceae). Evidence Based Complementary and Alternative Medicine. 2009;6:227-231.
61. Husain SR, Cillard J, Cillard P. Hydroxyl radical scavenging activity of flavonoids. Phytochemistry. 1987;26:2489-2491.
62. Afanaslev IB, Dorozhko AI, Bordskii AV. Chelating and free radical scavenging mechanisms of inhibitory action of rutin and quercetin in lipid peroxidation. Biochemical Pharmacology. 1989;38:1763-1769.
63. Torel J, Cillard J, Cillard P. Antioxidant activity of flavonoids and reactivity with peroxy radical. Phytochemistry. 1986;25: 383-385.
64. Yen GC, Hsieh CL. Antioxidant activity of extracts from Du-Zhong (*Eucoma ulmoides*) toward various lipid peroxidation models *in vitro.* J Agric Food Chem. 1998;46:3952-3957.
65. Chao GR. Antioxidant properties and polysaccharide composition analysis of ear mushrooms. Master's Thesis. National Chung-Hsing University, Taichung, Taiwan; 2001.
66. Gülçin I, Oktay M, Kıreçci E, Küfrevio Il. Screening of antioxidant and antimicrobial activities of anise (*Pimpella anisum* L.) seed extracts. Food Chem. 2003;83:371-382.
67. Iwu MM. Handbook of African medicinal plants. CRC Press, INC. 1993;252–253.
68. Bohlin L. Structure-activity studies of natural products with anti-inflammatory effects. In: Hostettmann K, editor. Phytochemistry of Plants Used in Traditional e. Oxford: Clarendon Press; 1995.

69. Nakatani M, Iwashita T, Naoki H, Hase T, Structure of limonoid antifeedant from *Trichilia emetic*. Phytochemistry. 1985;24: 195-196.
70. Nakatani M, James JC, Nakanishi K. Isolation and structures of trichilins, antifeedants against the Southern Army Worm. Journal of American Chemical Society. 1981;103:1228-1230.
71. Traore M, Zhai L, Chen M, Olsen CE, Odile N, Pierre GI, Bosco OJ, Robert GT, Christensen SB. Cytotoxic kurubasch aldehyde from *Trichilia emetic*. Nat Prod Res. 2007;21(1):13-17.

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