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Antioxidative properties of *Mallotus oppositifolium* decoction leaves extract using *in vitro* models

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ABSTRACT

In an attempt to explain the scientific basis for the medicinal benefits of *Mallotus oppositifolium* (Euphorbiaceae), the phytochemical constituent and the antioxidative properties of the aqueous decoction leaves extract of the plant versus standard Vitamin C were assessed. The phytochemical analysis (screening and quantitative assay), the 1,1-Diphenyl-2-picrylhydrazyl (DPPH), Nitric oxide, and hydroxyl radical-scavenging, the reducing power, ferrous reducing antioxidant power (FRAP) and the β carotene bleaching of the extract were performed using colorimetric or spectrophotometric methods. Qualitative screening of the extract showed the presence of alkaloids, flavonoids, steroids, phenolic compounds, tannins, phlobatannins, saponins, lipids, carbohydrates, mucilages, and proteins. The phytochemical quantitative assay of the decoction extract revealed that the plant depicted flavonols (38.278 mg.g⁻¹ catechin equivalent), tannins (23.13 mg.g⁻¹ tannic acid equivalent), phenolics (13.73 mg.g⁻¹ tannic acid content), flavonoids (10.62 mg.g⁻¹ quercetin equivalent), proanthocyanidins (2.53 mg.g⁻¹ quercetin equivalent) contents. Alkaloids and saponins content were 200 mg.g⁻¹ and 285 mg.g⁻¹, respectively. The ferric reducing antioxidant power (FRAP) of Mo extract and the IC₅₀ of vitamin C were 228±0.4 mg.mL⁻¹ and 592.0±0.8 mg.mL⁻¹, respectively. The DPPH radical-scavenging properties of IC₅₀ of vitamin C was 480.0±0.8mg.mL⁻¹ and that of Mo extract was 360.0±0.8 mg.mL⁻¹. The IC₅₀ of Mo's reduce abilities was 39.2±0.8 mg.mL⁻¹ and that of vitamin C was 320.0±0.8 mg.mL⁻¹. The IC₅₀ of the hydroxyl radical scavenging activity of Mo was 75.2±0,8 mg.mL⁻¹ whereas that of IC₅₀ of vitaminC was not defined. The nitric oxide radical scavenging activity of IC₅₀ of Mo was 121.6±0.8 mg.mL⁻¹ and that of the vitamin C was 130.4±0.8 mg.mL⁻¹, respectively. The β carotene bleaching gives the IC₅₀ of *M. oppositifolium* was 12,0±0,0 mg.mL⁻¹ and for the vitamin C was 16.0±0,0 mg.mL⁻¹. The decoction leaves of *Mallotus oppositifolium* (Mo) exhibited powerful antioxidative properties as vitamin C. The plant can be a source of antioxidative materials.

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Keywords: Traditional medicine, phytochemical analysis, antioxidant.

INTRODUCTION

The World Health Organization (WHO) estimates that up to 80% of the population of developing countries relies on

traditional remedies such as herbs, sources of their medicine, especially for the treatment of poverty related diseases (Tripathi and Tripathi, 2003). So far, many of these

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medicinal plants are not officially recognized because the research on quality, safety and efficacy are not sufficient (Nguyen Hoai, 2009). That is the case with *Mallotus oppositifolium* (Euphorbiaceae), found throughout the equatorial and tropical part of Africa. Different parts of the plants are traditionally used in the southern countries for the treatment of diverse ailments like helminthiasis, headaches and lumbago (Zintchem et al., 2009). In Cameroon, *Mallotus oppositifolium* (Mo) leaves are used against gastrointestinal disorder such as dysentery and acute diarrhoea (Kamgang et al., 2001). The present study was undertaken for the phytochemical analysis and the antioxidative properties of the aqueous decoction extract of Mo.

MATERIALS AND METHODS

Plant material and preparation of extract

Mallotus oppositifolium (Mo) leaves were collected from the Gouife-Bep village (Bafia- Cameroon) on July 2012. The species was identified and authenticated in the national herbarium of Cameroon where a voucher specimen was deposited under the number HNC 16619. The leaves were air-dried at room temperature, powdered and used for extraction. 100 g of the sample powder in 1L of distilled water was heated on water bath for 15 min. The decoction was first filtered through cotton to remove the debris. The resulting solution was then filtered using Whatman N° 1 filter paper and concentrated *in vacuum*. The semi solid extract obtained was stored in a refrigerator for further use.

Chemicals, reagents and solvents

The following chemical were used for the various experiments: 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,4,6-tris (2-pyridyl)-S-triazine (TPTZ), aluminium chloride (AlCl_3), potassium acetate ($\text{CH}_3\text{CO}_2\text{K}$), ferric chloride (FeCl_2), ascorbic acid, Folin-Ciocalteu reagent, sodium carbonate (Na_2CO_3), phosphate buffer, potassium ferricyanide [$\text{K}_3\text{Fe}(\text{CN})_6$], trichloroacetic acid

(TCA), glacial acetic acid (CH_3COOH), H_2O_2 , H_2SO_4 , sodium nitroprusside ($\text{Na}_2[\text{Fe}(\text{CN})_5\text{NO}]2\text{H}_2\text{O}$), 10-phenanthroline.

Phytochemical analysis

Phytochemical screening

The aqueous decoction extract or the powder of Mo leaves was screened using standard protocols to identify the constituents as described by Anyasor et al. (2010).

Alkaloids test (Mayer's, Wagner's and Picric acid test): 5 mL of filtrate was added in test tubes, respectively to 1 mL of Mayer's, Wagner's reagents and to 2 mL of picric acid solution. A cream brown colored precipitate and orange coloration were respectively observed.

Proteins test: 2 mL of the extract solution was treated with 2 mL of sodium hydroxide solution (10%) in a test tube and heated for 10 minutes. A drop of 7% copper sulfate solution was added in the above mixture. Formation of purple colour indicated the presence of proteins.

Glycosides test: 5 mL of dilute sulfuric acid was added to 1 mL of the extract solution in a test tube and boiled for 15 minutes, cooled and neutralized with NaOH (10%) then 1 mL of Fehling solution A and B was added. A brick red precipitate of reducing sugar indicated the presence of glycosides.

Reducing sugars test: 5mL of filtrate was boiled with drops of Fehling's solution A and B for 5 minutes. An orange red precipitate indicated the presence of reducing sugars.

Saponins (Froth) test: Increased volumes of extract (1 to 10 mL) were added to different tubes containing decreased volumes (9 to 0 mL) of distilled water. The tubes were vigorously shook for about 30 seconds, then allowed to stand in a vertical position and observed over 30 minutes. Honey comb froth above the surface of liquids persisted after 30 minutes showing the presence of saponins.

Fixed oils (Spot) test: Few drops of the extract were pressed between two filter papers and the appearance of oil stain on the paper indicated the presence of fixed oil. Some

drops of alcoholic potassium hydroxide (0.5 N) were added to a small quantity of the extract along with a drop of phenolphthalein. The mixture was heated on a water bath for 1-2 hours. Formation of soap or partial neutralization of alkali indicated the presence of fixed oils and fats.

Volatile oils test: 2 mL of Mo extract solution was shook with 0.1 mL of sodium hydroxide (1M) and some drops of hydrochloric acid (1M) were added. A white precipitate was formed with volatile oils.

Phenol (Ferric chloride and Lead acetate) test: 2 mL of filtrate was added respectively to 2 mL of ferric chloride solution (1%) and to 1 mL of lead acetate (10%) in test tubes. A deep bluish green solution and a white precipitate indicated the presence of phenols.

Flavonoids (Aqueous sodium hydroxide, Sulfuric acid, Schinoda's) test: 2 mL of the extract solution were added respectively to 2 mL of aqueous sodium hydroxide (1N) solution and few drop of concentrated sulfuric acid. The same quantity of filtrate was treated with a piece of magnesium turnings followed by a few drops of concentrated hydrochloric acid and heated slightly in a test tube. Yellow-orange, orange and dark pink coloration were respectively observed.

Steroids (Salkowski's, Lieberman's) test: 0.5 g extracts were dissolved respectively in 2 mL of chloroform and in 2 mL of acetic anhydride cooled in ice-bath test tubes. 3 mL of concentrated sulfuric acid was added on the wall of the first test tube to form layer. Concentrated sulfuric acid was then carefully added to the second tube. A reddish brown color at the interface and the color changing from purple to blue-green indicated the presence of a steroid nucleus (i.e. aglycone portion of cardiac glycosides).

Tannins test: Few drops of ferric chloride solution (10%) were added to 5 mL of the extract. A blue or green color indicated the presence of tannins.

Phlobatannins test: 5 mL of the extract was boiled with hydrochloric acid solution (10%). A red precipitate showed the presence of phlobatannins.

Mucilages test: 5mL of the extract was boiled with absolute alcohol solution. A white precipitate showed the presence of mucilages.

Phytochemical quantitative assays

Total phenolic assay: The total phenol content in the Mo extract was determined as described by Saha et al. (2008). 2.5 mL of Folin-Ciocalteu reagent (10%) and 2 mL of Na_2CO_3 (2%) were added to 0.5 mL of plant solution ($1\text{mg}\cdot\text{mL}^{-1}$). The resulting mixture was incubated at 45 °C with 15 min shaking. The absorbance of the samples was measured at 765 nm using UV/visible light (Spectrophotometer reading). Total phenolic content was expressed as $\text{mg}\cdot\text{g}^{-1}$ tannic acid equivalent using the following equation from the calibration curve: $Y = 0.1216x$, $R = 0.936512$, where Y was the absorbance and x was the tannic acid equivalent ($\text{mg}\cdot\text{g}^{-1}$). The assay was conducted in triplicates and the results were reported as mean \pm SD.

Total flavonoids assay: Total flavonoids content in the extracts were determined by aluminium colorimetric assay, following the procedure elaborated by Ebrahimzadeh et al. (2006) and Nabavi et al. (2008). 0.5 mL of the extract solution was mixed with 1.5 mL of methanol, 0.1 mL of aluminium chloride (10%), 0.1 mL of potassium acetate (1M) and 2.8 mL of distilled water. The mixture was incubated at room temperature for 30 min and the absorbance of the reaction was measured at 420 nm with UV/light spectrophotometer. All determinations were done in triplicates and values were calculated from calibration curve obtained from equations: $Y = 0,0255x$, $R = 0.9812$, where Y was the absorbance and x the quercetin equivalent ($\text{mg}\cdot\text{g}^{-1}$).

Total flavonols assay: Total flavonols content was determined by the method described by Kumaran and Karunakaran (2007). The reaction mixture consists of 0.1 mL of the sample and 0.1 mL of AlCl_3

prepared in ethanol with 1.5mL of sodium acetate solution (50g.L⁻¹). The absorbance at 440 nm was measured after 2.5 h at 20 °C. Total flavonol content was calculated as mg.g⁻¹ of quercetin equivalent from the calibration curve using the equation $Y = 0.0255x$, $R = 0.9812$, where Y was the absorbance and x the quercetin equivalent (mg.g⁻¹).

Total proanthocyanidins assay: The butanol-hydrochloride acid method described by Sanchez al. (2010), was used to measure proanthocyanidins. Reaction mixtures consisted of 0.5 mL of plant extract (1mg.mL⁻¹), 3.0 mL of concentrated hydrochloride(5%) in 1-Butanol (5:95) and 0.1 mL of NH₄(SO₄)₂12H₂O (2%) in Hydrochloric acid (2N). Plant extracts were diluted when necessary to ensure that proanthocyanidin concentrations fell within the range of the standard curve. The mixtures were heated at 95 °C for 50 min in covered test tubes and then cooled with ice water. Absorbance at 550 nm was recorded.

Tannins determination: Tannins were estimated by the method described by Oyedemi et al. (2010). To 0.2g of the sample was added 20 mL of methanol (50%). This was shook thoroughly and placed in a water bath at 80 °C for 1 h to ensure uniform mixing. The extract was filtered into a 100mL volumetric flask, followed by the addition of 20 mL of distilled water, 2.5 mL of Folin-Denis reagent, 10 mL of aqueous Na₂CO₃ (17%) and was thoroughly mixed. The mixture was made up to 100 mL with distilled water, mixed and allowed to stand for 20 min. The bluish-green colour developed at the end of the reaction mixture of different concentrations ranges from 0 to 10 ppm. The absorbance of the tannic acid standard solutions as well as sample was measured after colour development at 760 nm. Results were expressed as mg.g⁻¹ of tannic acid equivalent using the calibration curve: $Y = 0.0593x - 0.0485$, $R^2 = 0.9826$, where x is the absorbance and Y is the tannic acid equivalent.

Alkaloids determination: Alkaloids was quantitatively estimated by using the

Harborne's method modified by Khan et al. (2012). 200 mL of acetic acid (10%) in ethanol was added to 5 g of powdered plant sample, covered and stand for 4 h. The filtrate was then concentrated on a water bath to 1/4 of its original volume. Concentrated ammonium hydroxide drop was added to the extract until the precipitation was completed and the whole solution was settled. The collected precipitates were washed with dilute ammonium hydroxide and then filtered. The residue was dried and weighed. The alkaloid content was determined using the formula: % alkaloid = (final weight of the sample / initial weight of the extract) x 100.

Saponins determination: Quantitative determination of saponins was based on the method described by Kim et al. (2003). The powdered sample (20 g) was added to 100 mL of aqueous ethanol (20%) and kept in a shaker for 30 min. The samples were heated over a water bath for 4 h at 55 °C. The mixture was then filtered and the residue re-extracted with another 200 mL of the same ethanol. The combined extracts were reduced to approximately 40 mL over the water bath at 90 °C, transferred into a 250 mL flask decant and extracted twice with 20 mL of diethyl ether. The ether layer was discarded while the aqueous layer was retained and to which 60 ml *n*-butanol was added. The *n*-butanol extracts were washed twice with 10 mL of aqueous sodium chloride (5%). The remaining solution was heated on a water bath. After evaporation, the samples were dried in the oven at 40 °C to a constant weight. The saponins content was calculated using the formula: % saponins = (final weight of sample/initial weight of extracts) x100.

In vitro antioxidant assays

The antioxidant activities of *Mallotus oppositifolium* decoction leaves extracts were determined using DPPH, ferric reducing power, nitric oxide and hydroxyl peroxide, The Ferrous reducing antioxidant power (FRAP) and the β-carotene bleaching inhibition assay.

DPPH radical scavenging assay

The plant's extracts ability to scavenge the DPPH was evaluated by the method described by Liyana-Pathiranan and Shahidi (2005). The reaction mixture containing 1 mL of 1, 1-diphenyl-2-picryl-hydrazyl (0.1 mM) prepared in methanol and various concentrations of the Mo extract (20, 40, 80, 160, 320 and 640 $\mu\text{g}\cdot\text{mL}^{-1}$) were made up to 3 mL with water. Then the tubes were incubated for 30 minutes. Once the chromophore blue colour was formed, the absorbance of this solution was measured at 517 nm, against reagent blank containing water in place of Mo extract. Vitamin C was used as the standard for the comparison. The ability to scavenge the DPPH radical in terms of percentage of inhibition was calculated according to the following equation: % inhibition = $\{(A_0 - A_1) / A_0 \times 100\}$ where A_0 is the absorbance of the control (without extract) and A_1 is the absorbance in the presence of the extract.

Determination of reducing power of the extracts

The reducing power of Mo extract was evaluated according to the method described by (Aiyegoro and Okoh, 2010). The mixture containing 2.5 mL of phosphate buffer (0.2M, pH 6.6) and 2.5 mL of $\text{K}_3\text{Fe}(\text{CN})_6$ (1% w/v) was added to 1.0 mL of the extracts and standards (20–640 $\mu\text{g}\cdot\text{mL}^{-1}$) prepared in distilled water. The resulting mixture was incubated for 20 min at 50 °C, followed by the addition of 2.5 mL of TCA (10% w/v), which was then centrifuged at 3000 rpm for 10 min. 2.5 mL of the supernatant was mixed with 2.5 mL of distilled water and 0.5 mL of FeCl_3 (0.1% w/v). The absorbance of the reaction mixture indicating the reducing power of the extract was then measured at 700 nm against blank sample. Vitamin C was used as positive control.

Nitric oxide scavenging activity

The Mo's extracts properties to scavenge nitric oxide radicals was evaluated by the method described by Oyedemi et al. (2010). 2 mL of sodium nitroprusside (10

mM) prepared in phosphate buffered saline (0.5 mM, pH 7.4) was mixed with 0.5 mL of plant extracts and vitamin C respectively at 20–640 $\mu\text{g}\cdot\text{mL}^{-1}$ concentrations. The mixture was incubated at 25 °C for 150 min. 0.5 mL of the incubated solution was mixed with 0.5 mL of Griess reagent [1.0 mL sulfanilic acid reagent (0.33% prepared in 20% glacial acetic) acid at room temperature for 5 min with 1 mL of naphthylenediamine dichloride (0.1% w/v)]. The mixture was incubated at room temperature for 30 min, followed by the measurement of the absorbance at 540 nm. The amount of nitric oxide radicals inhibited by the extract was calculated using the following equation: NO radical scavenging activity (%) = $[(\text{Abs control} - \text{Abs sample}) / (\text{Abs control})] \times 100$, where Abs control is the absorbance of NO radicals + methanol and Abs sample is the absorbance of NO radical + extract or standard.

Hydroxyl radical scavenging assay

Hydroxyl radical inhibitory properties were performed by the method described by Yu et al., (2004). To the reaction mixture [containing 10-phenanthroline (1 mM, 90 μL), FeCl_3 (1 mM, 60 μL), and hydrogen peroxide (0, 17 M, 150 μL) in 2.4 mL phosphate buffer (20 mM, pH 7.4)], 0.2 mL of various concentrations of the extract (20, 40, 80, 160, 320 and 640 $\mu\text{g}\cdot\text{mL}^{-1}$) or standard in distilled water were added to give a total volume of 1.1 mL. The solutions were then incubated for 5 min in room temperature. The absorbance was measured at 560 nm with blank reagent containing water in the place of extract and Vitamin C as the standard for the comparison. The percentage of inhibition was calculated using the formula given before.

Ferrous reducing antioxidant power assay (total antioxidant activity)

The Ferrous reducing antioxidant power (FRAP) of Mo extracts was evaluated with the method described by Aiyegoro and Okoh (2010). The stock solution included 300 mM acetate buffer (pH 3.6), 10 mM of 2,4,6-tris (2-pyridyl)-S-tri-azine (TPTZ) in 40mM HCl, and 20 mM FeCl_3 solution. The fresh solution was prepared by mixing 25 mL of

acetate buffer, 2.5 mL of TPTZ and 2.5 mL of FeCl_3 . The temperature of the solution was raised to 37 °C before using. Plants extract (1.5 mL) were allowed to react with 2.85 mL of the FRAP for 30 min in the dark condition. Readings of colored product (ferrous tripydyltriazine complex) were taken at 593 nm, with Vitamin C as positive control.

β -carotene bleaching inhibition assay

This method evaluate the capacity *M. oppositifolium* decoction leaves extract to reduce the oxidative loss of β -carotene in a β -carotene linoleic acid emulsion (Kabouche et al., 2007) in three replicates test. β -carotene (0.5 mg) in 1 mL of chloroform was added to 25 μL of linoleic acid, and 200 mg of tween-80 emulsified mixture. Chloroform was evaporated at 40 °C with a rotary evaporator. 100 mL of distilled water saturated with was slowly added to the residue and the solution was vigorously shaken until a stable emulsion was formed. 4mL of this mixture was added to different test tubes containing 200 μL of plant extract (20, 40, 80, 160, 320, 640 $\mu\text{g}\cdot\text{mL}^{-1}$) and the absorbance was immediately measured at 470 nm. The tubes were then incubated for 2 hours at 50 °C and the absorbance was measured in the second time. Ascorbic acid was used as standard and the antioxidant activity was calculated as percentage of inhibition (I%) relative to the absorbance control (Ac) as follow: $I\% = [1 - (\text{As}_0 - \text{As}_{120}) / (\text{Ac}_0 - \text{Ac}_{120})]$, where As_0 was initial absorbance and As_{120} the sample absorbance at 120 min, Ac_0 was the initial absorbance of negative control and Ac_{120} , the absorbance of negative control at 120 min.

Statistical analysis

Where applicable, data were expressed as mean \pm standard deviation (SD) of triplicate determination. The IC_{50} was carried out using Stat graphics plus v50. The data were compared by Student's t-test. Values of $p < 0.05$ are considered statistically significant.

RESULTS

Phytochemical investigation of *Mallotus oppositifolium* (Mo) decoction leaves

Phytochemical screening

The screening of Mo metabolites using different standard test as shown in Table 1

revealed that Mo leaf was observed the presence of alkaloids, proteins, flavonoids, saponins, phenols, tannins, anthraquinones, phlobatannins, hydrocarbons and lipids.

Phytochemical assays

The result of total phenolics, flavonoids, flavonols, protoanthocyanidins and tannins contents in decoction leaves extract of *Mallotus oppositifolium* are shown in Figure 1. Phytochemical assays revealed Mo depicted high flavonols contents (38.278 $\text{mg}\cdot\text{g}^{-1}$ catechin equivalent), followed by tannins (23.13 $\text{mg}\cdot\text{g}^{-1}$ tannic acid equivalent), phenolics content (13.73 $\text{mg}\cdot\text{g}^{-1}$ tannic acid content), flavonoids (10.62 $\text{mg}\cdot\text{g}^{-1}$ quercetin equivalent), proanthocyanidins (2.53 $\text{mg}\cdot\text{g}^{-1}$ quercetin equivalent). Quantitative estimation indicated that the alkaloid content was 200 $\text{mg}\cdot\text{g}^{-1}$ and saponin 285 $\text{mg}\cdot\text{g}^{-1}$. These compounds have been reported to possess strong antioxidant potentials and may contribute significantly to the radical scavenging activities and reducing power of the extract as observed in this study.

Study of the reducing capacity of Mo as illustrated in Figure 2 indicated the reducing capacity of the extract and reference drugs at various concentrations. The transformation of Fe^{2+} to Fe^{3+} in the presence of the plant extract was used to measure its antioxidant activity. The reducing power of the extract and vitamin C were concentration-dependant. The reductive capability by the percentage inhibition of FRAP radical was shown in the following order: Vitamin C was greater than Mo aqueous extract.

DPPH scavenging potential of the extract by measuring the percentage of DPPH radical at different concentrations used in this study was determined together with reference drugs (Vitamin C). The plant showed concentration-response curves comparable with that of vitamin C. This activity was increased with increasing concentrations of both extract and reference drug. The IC_{50} of vitamin C was $480 \pm 0.8 \text{ mg}\cdot\text{mL}^{-1}$ and *M. oppositifolium* was $360.0 \pm 0.8 \text{ mg}\cdot\text{mL}^{-1}$ (Figure 3).

Inhibition potential of Mo decoction leaves was evaluated measuring absorbance at 700 nm at different concentration as shown in Figure 4, comparing with standard vitamin C.

Mo extract absorbance was less than that of the reference and was concentration dependent. The IC_{50} of vitamin C was $39.2 \pm 0.8 \text{ mg.mL}^{-1}$ whereas that of *M. oppositifolium* was $320.0 \pm 0.8 \text{ mg.mL}^{-1}$ (Figure 4).

Studies on the hydroxyl radical scavenging activity measuring the percentage radical hydroxyl of *M. oppositifolium* concentrations of up to 700 mg.mL^{-1} ,

standard vitamin C showed that the hydroxyl radical scavenging of the aqueous extract was higher than that of the reference with the IC_{50} of *M. oppositifolium* was $75.2 \pm 0.8 \text{ mg.mL}^{-1}$. The IC_{50} of vitamin C was not defined (Figure 5).

The result of nitric oxide antiradical activity of the extract was increased with

increasing concentration. Vitamin C was used as reference compound. There was no significant difference between the two compound with the IC_{50} of *M. oppositifolium* recorded as $121.6 \pm 0.8 \text{ mg.mL}^{-1}$ and for the vitamin C was 130.4 mg.mL^{-1} (Figure 6).

The effects of various concentrations of Mo decoction extract ($25\text{-}400 \text{ }\mu\text{g.mL}^{-1}$) against β carotene are shown in Figure 7. Compared to vitamin C, Mo extract showed dose dependant bleaching inhibition on β carotene. The IC_{50} of *M. oppositifolium* was $12,0 \pm 0,0 \text{ mg.mL}^{-1}$ and for the vitamin C was $16,0 \pm 0,0 \text{ mg.mL}^{-1}$. β carotene inhibition level was low for the Mo extract compared to vitamin C.

Table 1: Phytochemical constituents of Mo using different standard tests.

Mo metabolites and tests	Observations
Alkaloids	
Mayer's test	+
Wagner's test	+
Picric acid test	+
Phenols	
Ferric chloride test	+
Lead acetate test	+
Flavonoids	
Aqueous sodium hydroxide test	+
Sulfuric acid test	+
Schinodo's test	+
Steroidal compounds	
Salkowski's test:	+
Lieberman's test	+
Proteins	+
Saponins	+
Tannins	+
Anthraquinones	+
Phlobatannins	+
Glucosides	+
Reducing sugar	+
Volatile oils	+
Fixed oils	+
Mucilages	+

+ =Present

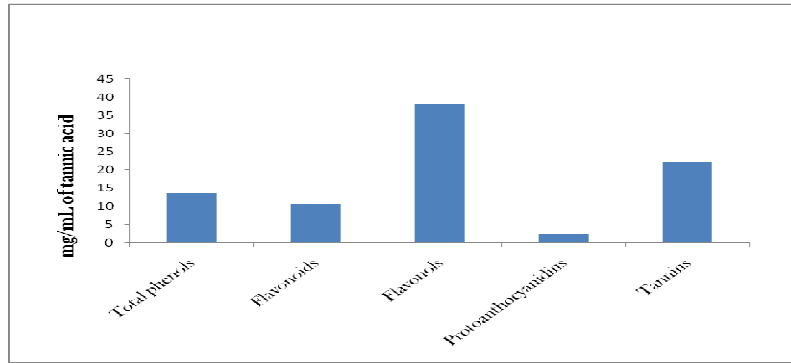


Figure 1: Total polyphenol contents identified in Mo decoction leaves.

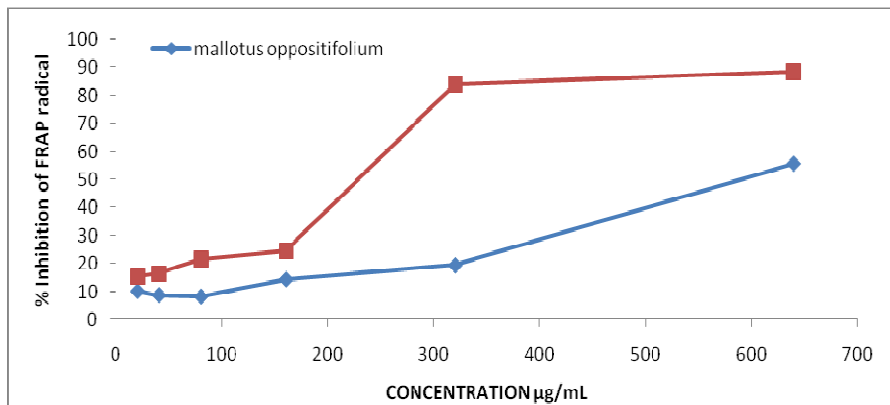


Figure 2: The FRAP radical scavenging activity of *M. oppositifolium* and the standard vitamin C. The data represent the percentage FRAP inhibition. Each value represents means±S.D (n = 3). The IC_{50} of vitamin C is $228.0 \pm 0.4 \text{ mg.mL}^{-1}$ and *M. oppositifolium* is $592.0 \pm 0.8 \text{ mg.mL}^{-1}$.

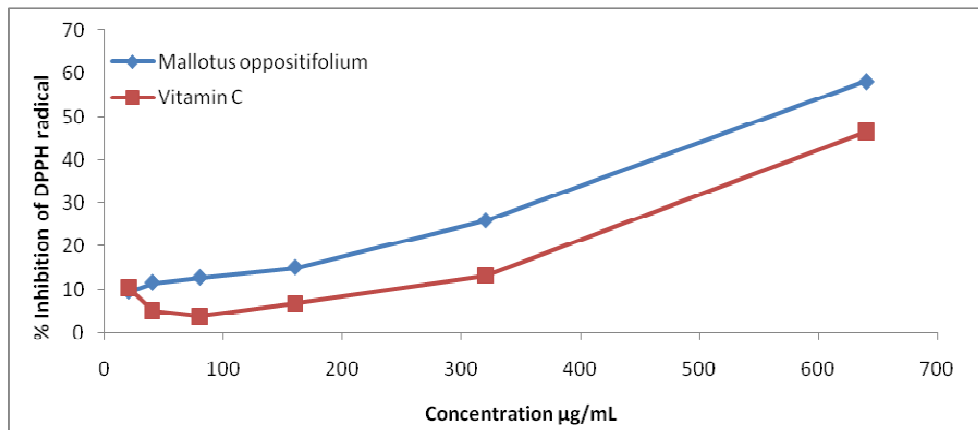


Figure 3: The DPPH radical scavenging activity of *M. oppositifolium* and the standard vitamin C. The data represent the percentage DPPH inhibition. Each value represents means±S.D (n = 3). The IC_{50} of vitamin C was $480.0 \pm 0.8 \text{ mg.mL}^{-1}$ and *M. oppositifolium* was $360.0 \pm 0.8 \text{ mg.mL}^{-1}$.

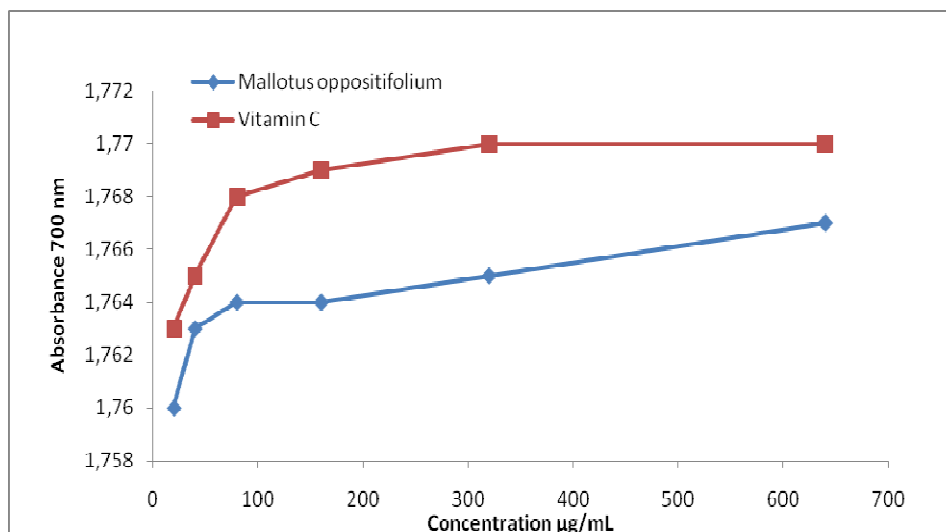


Figure 4: The reduce abilities of aqueous leaves extract of *M. oppositifolium* and vitamin C. The data represent the absorbance reduced abilities. Each value represents means±S.D (n = 3). The IC₅₀ of vitamin C was 39.2±0.8 mg.mL⁻¹ and *M. oppositifolium* was 320.0±0.8 mg.mL⁻¹.

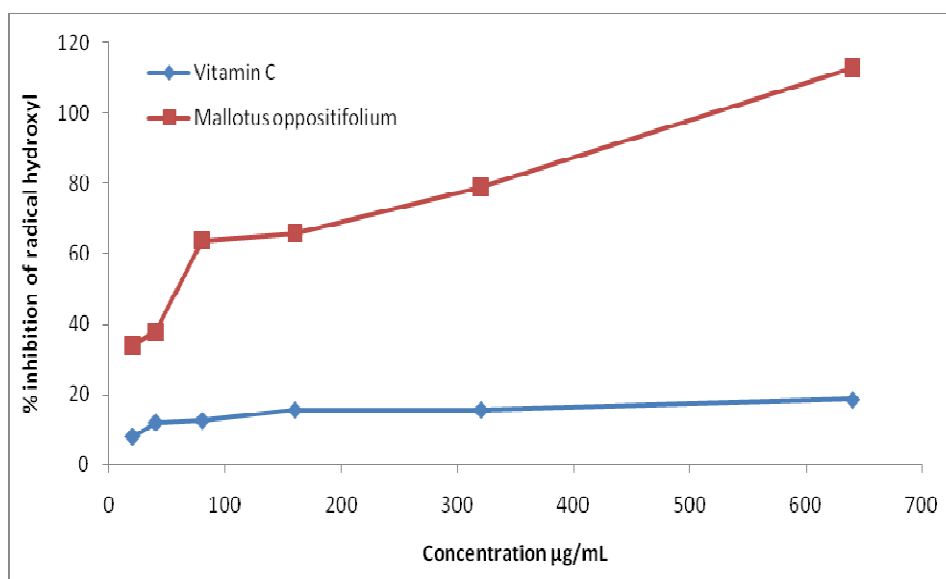


Figure 5: The hydroxyl radical scavenging activity of *M. oppositifolium* and the standard vitamin C. The data represent the percentage of radical inhibition. Each value represents means±S.D (n = 3). The IC₅₀ of *M. oppositifolium* is 75.2±0.8 mg.mL⁻¹. The IC₅₀ of vitaminC is not defined.

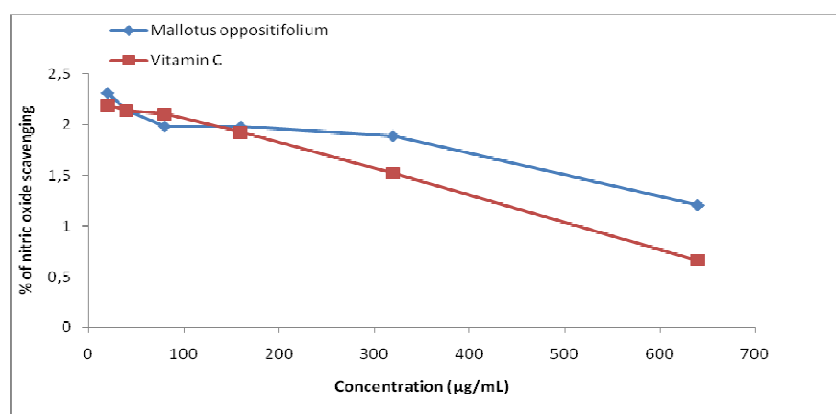


Figure 6: The nitric oxide radical scavenging activity of *M. oppositifolium* and the standard vitamin C. The data represent the percentage of nitric oxide inhibition. Each value represents means±S.D (n = 3). The IC₅₀ of *M. oppositifolium* was 121.6±0.8 mg.mL⁻¹ and for the vitamin C was 130.4mg.mL⁻¹.

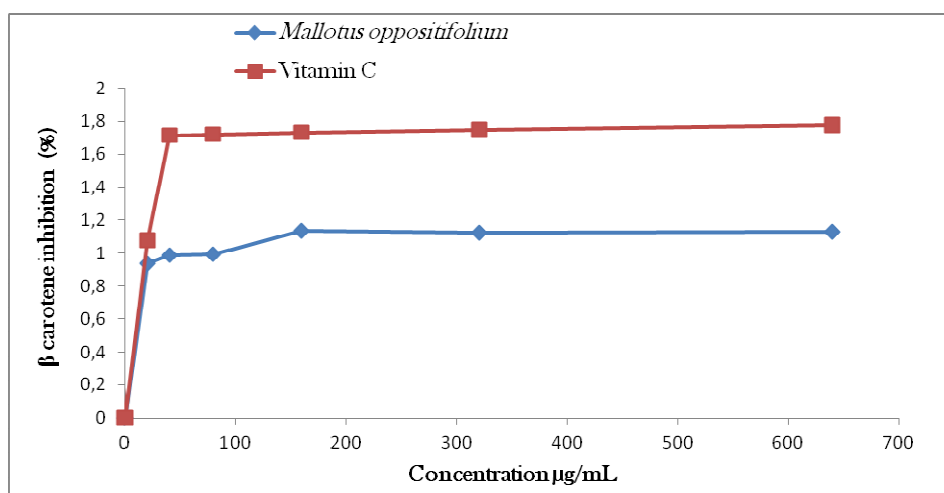


Figure 7: beta carotene bleaching inhibition of *M. oppositifolium* and the standard Ascorbic acid. The data represent the percentage of beta carotene inhibition. Each value represents means±S.D (n = 3). The IC₅₀ of *M. oppositifolium* was 12.0±0.0 mg.mL⁻¹ and for the vitamin C was 16.0±0.0 mg.mL⁻¹.

DISCUSSION

A variety of herbs and herbal extracts contain different phytochemicals with biological activity that can be of valuable therapeutic index. Much of the protective effects of fruits and vegetables have been attributed by phytochemicals, which are the non-nutrient plant compound. Different

phytochemicals have been found to possess a wide range of activities, which may help in protection against chronic diseases. For example glycosides, flavonoids, tannins and alkaloids have hypoglycemic activities; steroids and saponin are responsible for central nervous system activities (Argal and Pathak, 2006). Antidiarrheal and

antidysenteric properties were found to be due to presence of tannins, alkaloids, saponins, flavonoids, steroids, and/or terpenoids in some plants extracts (Oben et al., 2006; Teke et al., 2011). Even though, this is only a preliminary study of the occurrence of certain properties of *Mallotus oppositifolium* leaves an in-depth study will provide a good concrete base of all the phytochemicals function mention above.

Phenolic compounds are known to exhibit strong antioxidant activity, which have direct antioxidant properties due to the presence of hydroxyl groups, which act as hydrogen donor (Ozgen et al., 2010). Flavonoids are hydroxylated phenolics and are potent water-soluble antioxidants which help in radical scavenging and prevention of oxidative cell damage. They have been reported to possess strong antioxidant activities.

Proanthocyanidins are group of polyphenolic bioflavonoids which have a protective effect in eliminating hydroxyl radicals. Several phenolic compounds including tannins are inhibitors of many hydrolytic enzymes such as proteolytic macerating enzymes used by plant against pathogens (Dash et al., 2008).

Tannins are known to be useful for the prevention of cancer as well as treatment of inflamed or ulcerated tissues. Alkaloids have been associated with medicinal purpose for centuries as agents possessing analgesic, antimalarial, antiseptic and bactericidal activities, but could be toxic to cells Saponins in medicinal plants are responsible for most biological effects related to cell growth and division in humans and have inhibitory effect on inflammation (Okwu and Elenike, 2006).

The ability of a substance to act as an antioxidant depends on its strength to reduce RONS (reactive oxygen and nitrogen species) by donating hydrogen atom for instance. The reducing capacity of a compound generally depends on the presence of reductants which exhibit antioxidative potential by breaking the free radical chain and donating hydrogen atom (Adedapo et al., 2008). It has been found that

the transformation of Fe^{3+} to Fe^{2+} occurred in the presence of Mo extract showed the reductive capabilities when compared to ascorbic acid.

DPPH is a relatively stable free radical scavenger which converts the unpaired electrons to paired ones by hydrogen proton donation. Scavenging of DPPH radical in this study indicates the potency of the plant extracts in donating hydrogen proton to the lone pair electron of the radicals (Mondal et al., 2005). Because the inhibition was much higher than that of the positive control, it could be suggested that the plant extracts contain compounds capable of donating protons to the free radicals. The methods have proven the effectiveness of the extracts in a concentration-dependent manner.

Nitric oxide is an important chemical mediator generated by endothelial cells, macrophages and neurons and is involved in the regulation of various physiological processes. Excess concentration of nitric oxide is associated with several diseases. Oxygen reacts with the excess nitric oxide to generate nitrite and peroxy nitrite anions, which act as free radicals (Ara and Nur, 2009). In the present study, the extract showed a great activity by competing with oxygen to react with nitric oxide and thus inhibits the generation of the anions.

Hydroxyl radicals have a short half-life and are the most reactive and damaging oxygen species causing lipid peroxidation and cellular damage (Hazra et al., 2008; Veerapur et al., (2009); Lobo et al., 2010), the plant extract showed a good scavenging activity compared to the positive control.

The β -carotene bleaching assay is a commonly used model to analyze the antioxidant activity of the plant extracts because β -carotene is extremely sensitive to free radical mediated oxidation of linoleic acid. In this assay, oxidation of linoleic acid, an unsaturated fatty acid occurs due to the production of reactive oxygen species formed from halogenated water. The reactive oxygen species will initiate β -carotene oxidation leading to discolouration (Gutierrez et al., 2006). The plant extract inhibited β -carotene oxidation, suggesting that the antioxidant activity could be related to high level of phenolic compounds.

Conclusion

In this study, we have found that most of the biologically active phytochemicals were present in *Mallotus oppositifolium* decoction leaves. The medical properties of Mo leaves extract may be due to the presence of the above mentioned identified phytochemicals. Further studies are needed to isolate the active components and test the safety of the decoction *in vitro* on cell lines and *in vivo* on animal models. The study for new chemical entities from the herbal medicinal plants of Cameroon is a step in building a potential national data base for natural product phytochemical library. There is the need to exploit the medicinal plant cocktail within our rich Congo Basin dense tropical forest which has a huge undocumented biodiversity of medicinal plants of pharmaceutical importance.

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