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REVIEW ARTICLE

PHYTOCHEMICAL STUDY AND ANTI-RADICAL ACTIVITY OF CLEOME VISCOSA LINN OF THE TOGOLESE FLORA

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ABSTRACT

Cleome viscosa L. is a plant widely used to treat various diseases and grows on degraded or marginal agricultural lands. This study is aimed to investigate the phytochemical screening and anti-radical activity of *Cleome viscosa*. An ethnobotanical survey was conducted. Alkaloids were checked using BOUCHARDAT reagent and flavonoids were detected using SHINODA reagent. The 1,1-Diphenyl-2-Pyridyl Hydrazil (DPPH) free radical scavenging and the Ferric-Reducing Antioxidant Power (FRAP) assays were used to highlight the antioxidant activities. The ethnobotanical investigations conducted show that women (57%) uses more *Cleome viscosa* than men (47%) to fight diseases. Phytochemical screening revealed that *Cleome viscosa* contains alkaloids, flavonoids, saponins and tannins. The DPPH free radical value is 16.18 ± 0.024 mg of quercetin per gram of plant material and the FRAP value is 986.88 ± 0.012 $\mu\text{mol/L}$ equivalent in Fe^{2+} per gram of plant material. Our result shows that *Cleome viscosa* possesses phytochemical compounds and anti-radical activity.

INTRODUCTION

Health problems in Africa today are still very worrying. More than 80% of the African population use medicinal plants (OMS, 2012). These medicinal plants are used for the treatment of many pathologies. Active drugs are discovered from isolated medicinal plant and microorganisms. On the planet 300,000 species of medicinal plants are listed. In tropical countries of Africa 200,000 species of medicinal plants are counted (Kolling, 2010). Togo, a subtropical country has these medicinal plants. *Cleome Viscosa* L., family (Capparidaceae) is a widely distributed herb with yellow flowers and long slender pods containing seeds (figure1). The whole plant is sticky in nature and has a strong odour resembling asafoetida. *Cleome viscosa* L., is a sticky annual herb found as a common weed in plains of Pakistan, India, China and in Togo (Akobundu et al., 1987). The species are found in woods, sandy soils, meadows, roadside and rocky soils (Packialakshmi, 2014). It is called "somboessou" in "ewe" language of Togo. In traditional medicine, leaves, seeds, roots and stem of *Cleome viscosa* are used to fight malaria, hemorrhoids, fever, headaches, abscesses, vomiting, sinusitis and wounds.

The whole herb is used in the treatment of inflammation, middle ear, wounds and ulcers (Devi, 2002; Devi, 2003; Ahouansinkpo, 2016). The decoction is used as an expectorant and digestive stimulant (Lakshmi, 2011; Lazzeri, 2004) The vapour from a steaming decoction of the whole plant is inhaled to treat headache (Shveta Saroop And Veenu Kaul, 2015; Lev, 2002). Phytochemicals are bioactive compounds obtained from the plants and are applied in traditional medicine. In plant 2 types of metabolites are produced: Primary metabolites and Secondary metabolites (Pragadheeswari, R., & Sangeetha, 2016). Primary metabolites participated in the nutrition and reproduction of the plant. Secondary metabolites plays a role in the interaction of the organism with its environment. Secondary metabolites are synthesized in all parts of the plant like bark, leaves, stem, root, flower, fruits, seeds, etc. Oxidative stress is commonly defined as a disturbance in the balance between the production of free radicals and antioxidant defenses. Antioxidant property is shown by a compounds such as flavonoids, tannins isoflavones, and phenolic (Fattouch, 2007; Zheng, 2001). Moreover, the benefits of phenolic antioxidant compounds from plants in prevention of chronic diseases have been reported (Sies, 2012; Watson, 2011). In view of its traditional claim in literatures, researches have focused on its biological and pharmacological properties.

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This study was aimed to investigate the phytochemical screening and anti-radical activity of *Cleome viscosa*.

Classification



Kingdom : Plantae
 Class : Equisetopsida
 Order : Brassicales
 Family : Capparidaceae
 Kind : Cleome
 Species : Cleome viscosa

Figure 1. *Cleome viscosa* plant (17)

MATERIALS AND METHODS

Ethnobotanical survey

Situation of the study area: The survey was carried out in 9 localities belonging to 5 Cities of Togo namely: Golfe City (Bè market, Nukafu market and Agoè market), Vo City (Vogan and Akoumapé), Bas-mono City (Kpetsou), Lacs City (Aného and Anfoin) and Notsè in Haho City (Figure 2)

Survey methodology: To cover the five prefectures, the survey was conducted in 9 randomly selected locations (Ardilly, 2006). The randomly selected respondents are mostly adults (Assogbadjo *et al.*, 2008). Nevertheless some people from the classes of young and old are also taken into account. The survey was done thanks to a sheet developed after an exploratory survey. The sections of the survey concern in particular the medicinal uses of *Cleome viscosa* and the used parts of the plant. To facilitate the interview with the people surveyed, the survey was conducted in the local language (Ewe) of Togo.

Sample and preparation of the extracts: *Cleome viscosa* was collected in march 2016 from kpetsou near Afagnan in Togo. The aerial portions were identified and authenticated by the Herbarium of Lome University. The plant samples were left at room temperature for two weeks. The different parts of the plant were then separated. Each part was pulverized using an electric grinder.

The powder of the leaves, fruits and the whole stem and root are stored. After 10 g of the powder of leaves, 10 g of the powder of fruits and 10 g of the powder of a mixture of stem and root were macerated with 100 ml of of differents kinds of solvents as dichloromethane, ethanol and the water-ethanol mixture in order to increase their polarities. The mixtures obtained are incubated for 3 days. All the mixtures are then

filtered on the Whatman filter paper. Rotavapor is used to evaporate our mixtures. We obtained the dry extracts. The dry extracts were stored in refrigerator at 4 ° C till further analysis.

Phytochemical tests: Phytochemical screening refers to all methods and techniques for the detection of active chemical groups in the plant. The whole phytochemical screening was performed according to standard methods described by (Badiaga *et al.* 2008).

Alkaloids detection: Alkaloids were checked using BOUCHARDAT reagent. Two drops of Bouchardat reagent were addea to 6 mL of the extract. The appearance of a brown-black, brown-earth or yellow-brown precipitate indicates a positive reaction of presence of alkaloids. The presence of brown-black, brown-earth or yellow-brown colour precipitate means a positive reaction.

Flavonoids detection: To isolate flavonoids, a few drops of the 10% soda are added to 6 mL of the extract. The presence of a yellow-orange colour indicates a positive reaction.

Saponins detection: To indentified saponins 10 mL of the extract is made in the tube. The foam persistence obtained after a few minutes shows the presence of the saponins.

Tannins detection: To detecte the amount of tannins, 1 mL of the 10% lead acetate is added to 3 mL of the extract.

The presence of blue, blue-black, whitish or brownish colour precipitate indicates a positive reaction.

Anti-radical tests

Diphenyl-2-Pycril Hydrazil(DPPH) Assay

Kinetic study: For the kinetic study of the reaction of the DPPH• with the sample, a mixture was made in a tube containing 100 µl of the extract at 100 g/L and 2 ml of the solution of the DPPH• at 100 µmol/L. This preparation was homogenised with the vortex. We also examined the regular time interval which is 40 minutes. The optical density is then recordedat 517 nm with the spectrophotometer UV-VIS 5100B SPECTROPHOTOMETER. Ethanol is used as control and is recordedunder the same conditions.

Antiradical power evaluation with DPPH

A study was performed to evaluate the antiradical power. The method, inspired by the works of Molyneux (Molyneux, 2004) and Constantin *et al* (Dabire *et al.*, 2011), is based on the reduction of the DPPH radical by a molecule (AH) leading to the formation of the 2,2-diphenyl-1-picrylhydrazine (DPPH-H) and the radical (A•) according to the following equation :



The reagent is prepared daily with an optical density which must be between 0.85 and 1. We also prepared a quercetin solution at a range of concentrations between 5-30 mg/L. After, 2 mL of the DPPH • 10⁻⁴ mol.L⁻¹ solution is added to 100 µL of the extract. The mixture is homogenised with the vortex and is incubated at room temperature for 40 minutes. The UV- VIS Spectrophotometer is used to recorder the optical density at 517 nm. Ethanol is also used as control and is recorded under the same conditions. The analysis is repeted trice in a very short time. The absorbance mesures help us to established the

"anti-radical potential". Based on the calibration regression equation established, the equivalent of quercetin per gram of dry extract mg EQ/g plant material) was determined.

Ferric Reduction Antioxidant Power (FRAP) assay: The reduction capacity of *Cleome viscosa* extract was evaluated by FRAP (Ferric Reduction Antioxidant Power) assay that analyzes the blue-colored Fe^{2+} -TPTZ formed by the reduction of Fe^{3+} -TPTZ. The test was carried out according to the method described by Nair *et al* (23). We prepared 25 μ L of sample which is incubated 5 minutes in the darkness with 175 μ L FRAP reagent (300 mM acetate buffer, pH 3.6: 10 mM TPTZ in 40 mM HCl: 20 mM $FeCl_3 \cdot 6H_2O$ in 40 mM HCl, 10:1:1, v/v/v). Quercetin was used as an external standard (calibration curve obtained for 200–2000 μ mol/L). The absorbance was measured at 593 nm with UV-VIS 5100B SPECTROPHOTOMETER. The test was performed in triplicate from which the standard deviations were calculated. The result is expressed as μ mol/L equivalents of Fe^{2+} .

Statistical analysis: Results were analyzed statistically using the OriginPro 9.0 software. Data were expressed as means \pm standard deviation (SD) of experiments performed in triplicate. One-way analysis of variance (ANOVA), principal component analysis (PCA) and Pearson correlation coefficient (ρ) were used to evaluate and correlate the results with each other. The statistical significance between results for different samples and between results for samples and controls were set at $P < 0.05$.

RESULTS AND DISCUSSION

RESULTS

Ethnobotanical data: In our study, 53% of the subject were women and 47% were men. Our data indicated that 88% of the subject were adult aged between 30 and 60 years old (figure 3). In 82% of the cases, *Cleome viscosa* is used to fight diseases. *Cleome viscosa* could also be used combined with other plants such as *Hyptis suaveolens* and *Azadirachta indica* to fight different kinds of diseases (Figure 4). All the subject said that *Cleome viscosa* treatment is taken by inhalation or by decoction using the whole plant or the leaves. To evaluate the effect of *Cleome viscosa* on different kinds of diseases, an investigation was conducted. We note that *Cleome viscosa* is used to treat malaria in 83% of cases. In 71% cases, *Cleome viscosa* treated headache. The plant treated hemorrhoids, wounds and abscesses in 59% of cases. Our survey indicated that *Cleome viscosa* treated vomiting in 30%. Our study showed that sinusitis and toothaches are treated by the plant in 6% of cases (figure5).

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Phytochemical studies of *Cleome viscosa*: A study was carried out to evaluate the phytochemical compound of *Cleome*

viscosa. Our result shows that in the dichloromethane, alkaloids and tannins were present in the fruits, leaves, stems and roots. Flavonoid and saponins were present in the leaves stems and roots but were absent in fruits. The ethanolic extract revealed that the flavonoids and tannins were present in all the three extracts (fruits, leaves and stems-roots). Alkaloids and saponins were present in the leaves but were absent in the fruits, stems and roots. The mixture water-ethanol indicated that flavonoids and tannins were present in all the three extracts (fruits, leaves and stems-roots). We note that alkaloids were present in the leaves but were absent in fruits and stems-roots. Saponins are present in leaves and stems-roots but are absent in fruits. The majority of the compounds (alkaloids, flavonoids, saponins and tannins) is found in the leaves extracts (table 1).

Kinetics of the reaction between *Cleome viscosa* L. and DPPH•

The kinetic study of the reaction between *Cleome viscosa* L. and DPPH• show a rapid reduction between 0 and 20 minutes. We note that it becomes slow between 20 and 40 minutes. Our observation shows that the extract reacted with the DPPH free radical reaching a steady state in about 40 minutes (Fig 6).

Determination of the quercetin equivalent with DPPH•

Using our measurements, we draw the curve optical density as a function of quercetin concentration (Figure 17). We note that the correlation coefficient is $R^2 = 0.9957$. The equation obtained is $Y = -0.00733 X + 0.94859$. We finally determined the anti-radical power which is 16.18 mg of quercetin per gram of plant material (Figure7).

FRAP (Ferric-Reducing Antioxidant Power) assay: As determined by the FRAP assay we draw the curve optical density as a function of Fe^{2+} concentration (Figure 17). We note that the correlation coefficient is $R^2 = 0.9984$. The equation obtained is $Y = 0.0008 X + 0.0605$. We finally determined the anti-radical power which is $986.88 \pm 0.012 \mu$ mol / L of Fe^{2+} per gram of plant material (Fig 8).

DISCUSSION

The present study was aimed to investigate the phytochemical screening and anti-radical activity of *Cleome viscosa*. We note that, 53% of the subject were women and 47% were men. This result means that women use *Cleome viscosa* than men to fight diseases. All the subject said that *Cleome viscosa* treatment is taken by inhalation or by decoction using the whole plant or the leaves. This result confirms those resulting from the work of Yedomonhan *et al* who shows that *Cleome viscosa* treatment is taken by inhalation or by decoction (Adomou, 2012). Additionally our investigation shows that *Cleome viscosa* is used to fight malaria and headache. Our result is in agreement with that reported by Yedomonhan *et al* which indicated that *Cleome viscosa* is used to treat malaria and wounds (Adomou, 2012). The phytochemical screening is used to identify some bioactive substances that can be derived from plants. The phytochemical compounds identified in *Cleome viscosa* are alkaloids, flavonoids, saponins and tannins. The leaves extracts accumulate all the compounds identified (alkaloids, flavonoids, saponins and tannins). Our study confirms that Ghosh's *et al* who shows that *Cleome viscosa* L. contains flavonoids, tannins, saponins and alkaloids (Packialakshmi, 2014; Sivaraman, 2014; Bose *et al.*, 2011).

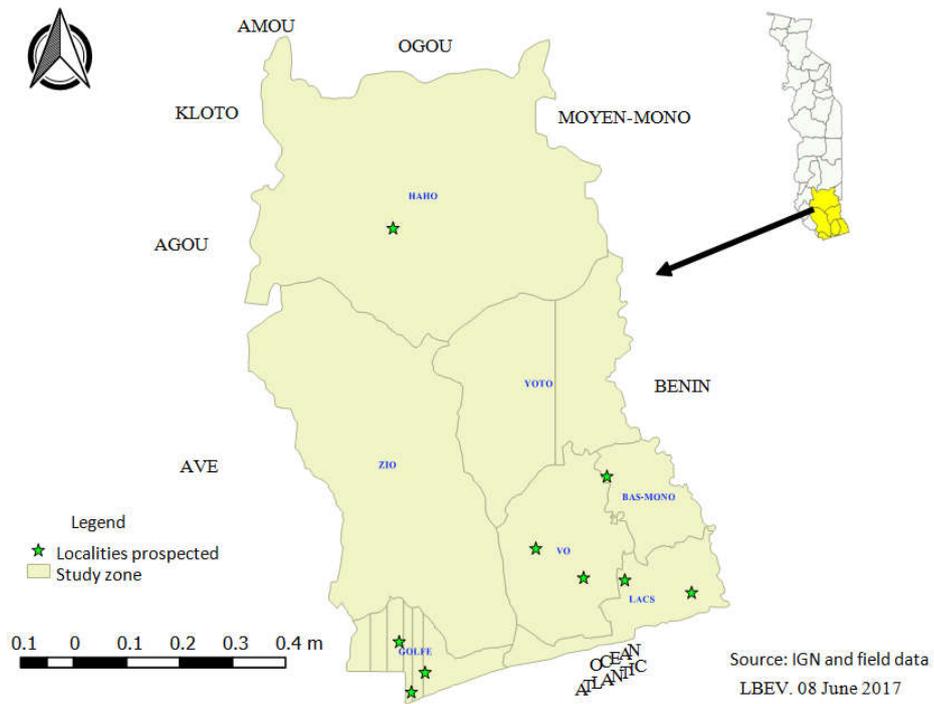


Figure 2. Map of the ethnobotanical study area

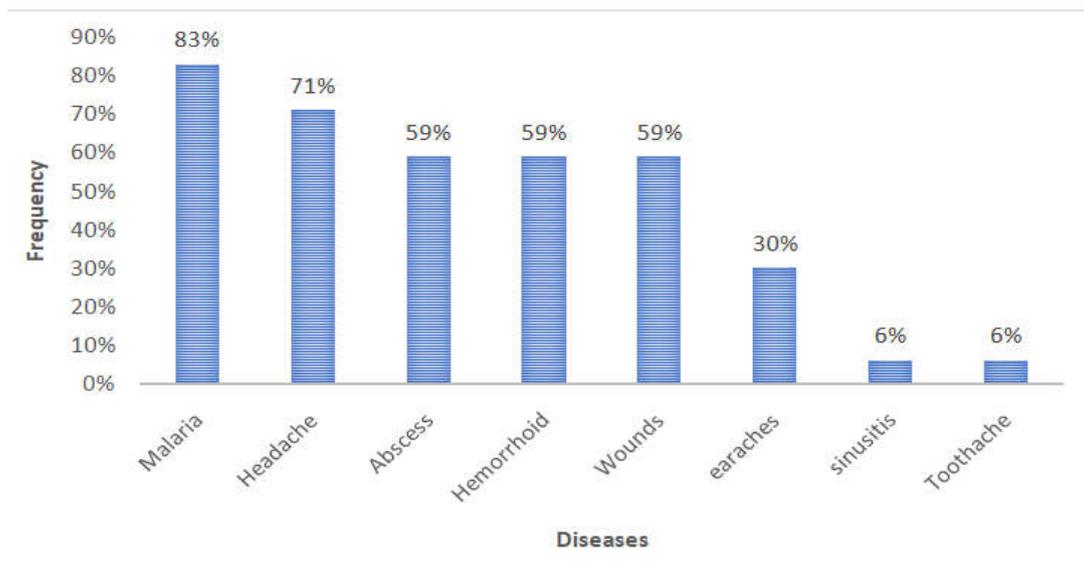
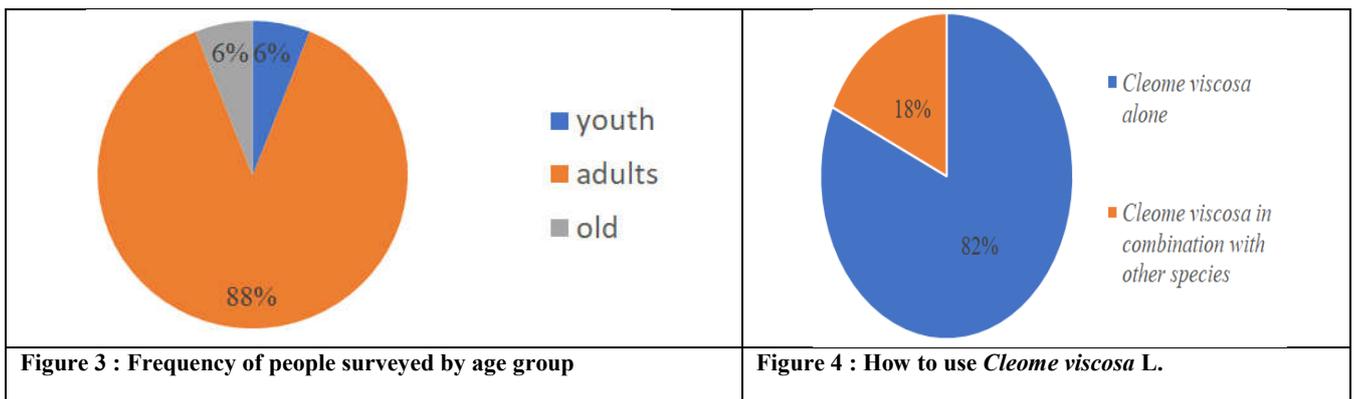


Figure 5. Diseases treated with *Cleome viscosa* L.

Table 1 : Results of the phytochemical study of *Cleome viscosa*

Family of compounds	Dichloromethanolic extracts			Ethanolic extracts			Hydroethanolic extracts		
	Fruits	Leaves	Stem - Roots	Fruits	Leaves	Stem - Roots	Fruits	Leaves	Stem - Roots
Alkaloids	+	+	+	-	+	-	-	+	-
Flavonoids	-	+	+	+	+	+	+	+	+
Saponins	-	+	+	-	+	-	-	+	+
Tannins	+	+	+	+	+	+	+	+	+

+ : Detected; - : Not detected.

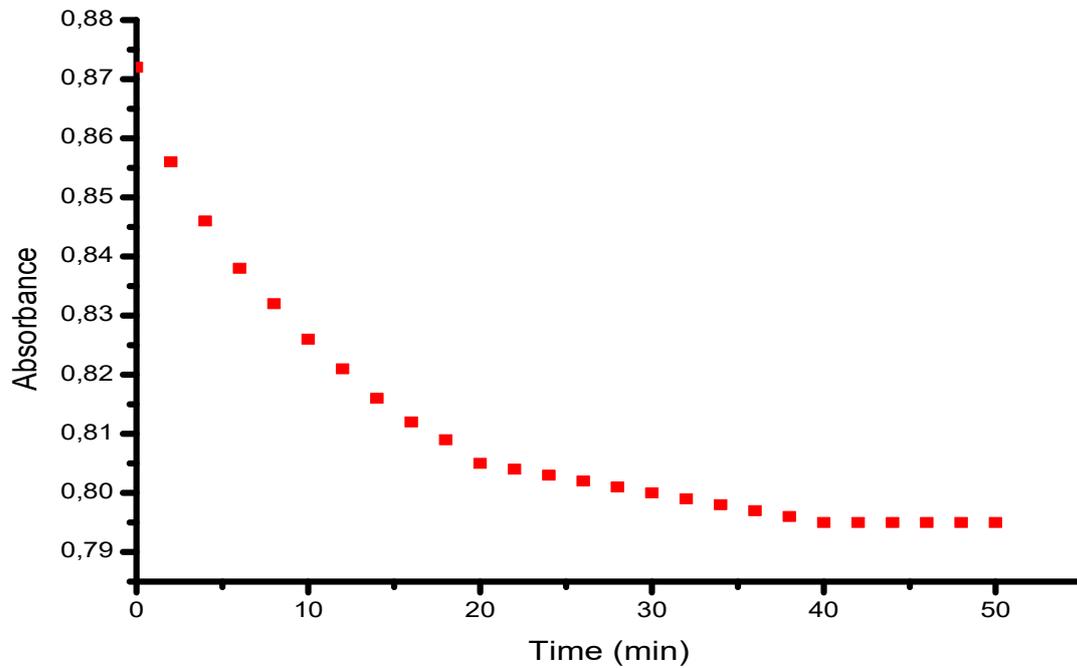


Figure 6. Curve of the kinetics of the reaction with the radical DPPH

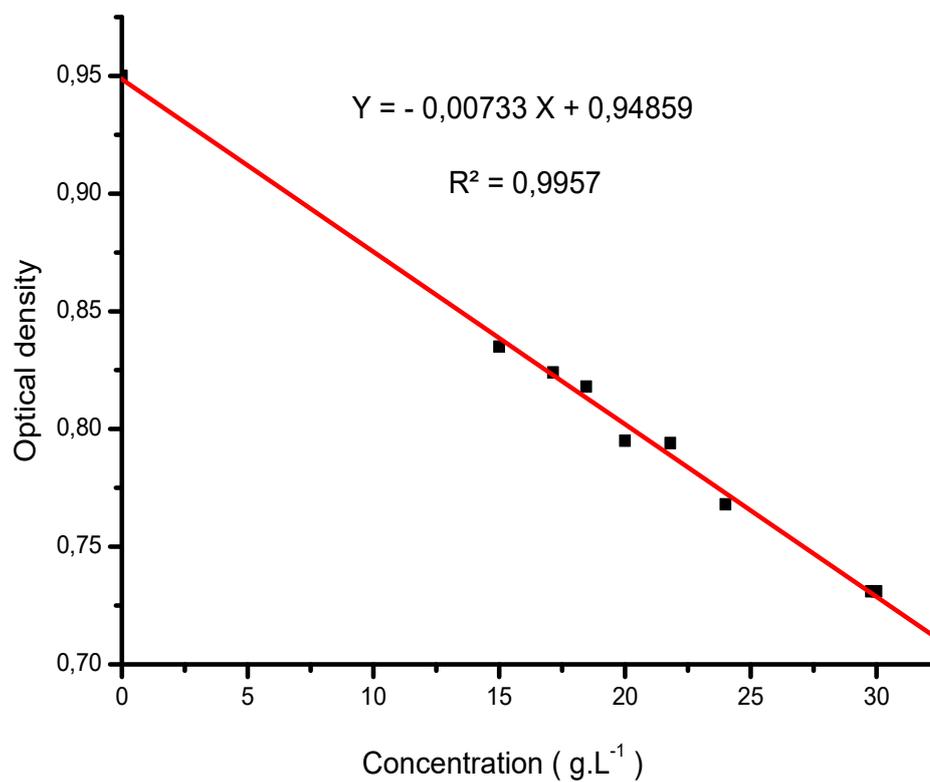


Figure 7. Calibration curve for quercetin

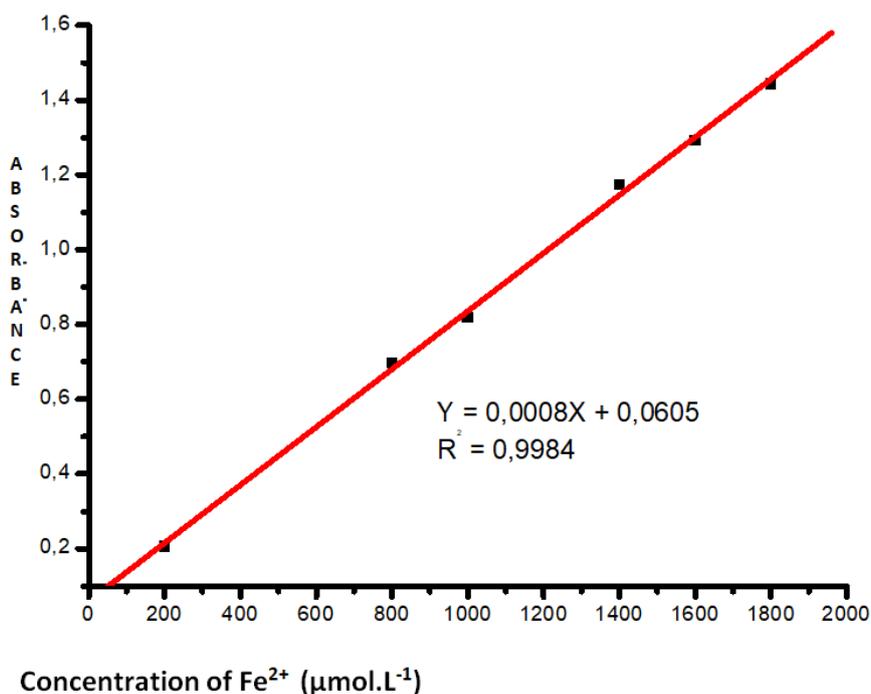


Figure 8. Calibration curve of Fe²⁺

Table 2. Results of the anti-radical tests of *Cleome viscosa*

Reaction time (min)	Equivalent quercetin (mg/g)	Equivalent to Fe ²⁺ (µmol/L/g)
40	16,18 ± 0.024	986,88 ± 0.012

The reason why *Cleome viscosa* is used in traditional medicine is that, plant contains alkaloids, flavonoids, saponins and tannins, which works as potent antioxidants, antiradicals, diuretics, purgatives, disinfectants, analgesics, anti-inflammatory, antipyretics and antispasmodics. Similar development has been obtained by Ngene, *et al.* (Ngene, *et al.*, 2015 ; Maestri, 2006). The antioxidant activity of *Cleome viscosa* was evaluated by the free radical DPPH and the FRAP methods. The DPPH free radical method offers an approach evaluating an antioxidant potential of a compound, an extract, or biological sources. In our study based on the measurement of the scavenging capacity of *Cleome viscosa* the value obtained is 16.18 ± 0.024 mg of quercetin per gram of plant material indicating the antioxidant property of *Cleome viscosa* (fig.6). Additionally the FRAP assay uses antioxidants as reductants in a redox-linked colorimetric method employing an easily reduced oxidant as Fe(III). Also the FRAP assay can rank the reducing power and the antioxidant potential of a wide range of test compounds. By the FRAP test the result is $986.88 \mu\text{mol} / \text{L}$ of Fe²⁺ per gram of plant material indicating that *Cleome viscosa* has an antioxidant potential (fig 7). In our knowledge this is the first study reporting the anti-radical activity of *Cleome viscosa*.

The presence of flavonoids and tannins revealed by phytochemical tests may explain the anti-radical potency of *Cleome viscosa* L.

Conclusion

The present study was aimed to investigate the phytochemical screening and anti-radical activity of *Cleome viscosa*.

The ethnobotanical investigations conducted shows that women use more *Cleome viscosa* than men to fight diseases such as malaria, fever, abscess, hemorrhoids, sinusitis and wounds. The findings of the study revealed that *Cleome viscosa* possesses alkaloids, flavonoids, saponins and tannins which confer to the plant its anti-radical activity. The majority of the compounds (alkaloids, flavonoids, saponins and tannins) is located in the leaves extracts. This could provide a strong argument in favor of the use of *Cleome viscosa* to fight malaria, fever, abscess, hemorrhoids, sinusitis and wounds. Further studies are needed to quantify some chemical groups and isolate the active principles responsible for the treatment.

Conflict of interests: Authors declare no conflict of interests. We declare that the work described in the manuscript is new and has not been published previously.

Ethical considerations: Ethical consideration has been completely observed by the authors.

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Author Contributions

Authors' contributions: Komi Michael Fulbert ADANLEMEGBE conducted the full work. Kafui KPEGBA, Kokou Agbékonyi AGBODAN, Pakoupati BOYODE and Oudjaniyobi SIMALOU supervised the experiments; Kossi Issa SALOUFOU, participated in the writing process. Essowè Badenèzi POTCHO and Kodjo Selom EVENAMEDE gave

technical support via the using of apparatus. all authors contributed to the writing of the manuscript and confirmed publication of the final version.

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