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Phytochemical analysis and antioxidant activities of *Combretum molle* and *Pericopsis laxiflora*

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ABSTRACT

In Burkina Faso as in several African countries, diabetes is becoming a very serious disease and public concern. Patients use both drugs and plants for treatment. Thus, *Combretum molle* and *Pericopsis laxiflora* have been identified as plants used in Burkina Faso for the treatment of that disease. The aim of the study was to assess phytochemical components and antioxidant activities of those plants in order to highlight why diabetes patients refer to them. The phytochemical analysis of water, ash quantification and screening were done using the standard methods; the antioxidant activities of the ethanolic extract against 2, 2-diphenyl-1-picryl-hydrazyl (DPPH), nitric oxide (NO) and hydroxyl radical were performed using colorimetric or spectrophotometric methods. The Inhibitory Concentration (IC) was determined using GraphPad Prism version 5. The results showed 6.76±0.076% and 7.52±0.015% of water contents in *Combretum molle* and *Pericopsis laxiflora*, respectively. The ash content was 3.41±0.35% for *Combretum molle* and 3.87±0.15% for *Pericopsis laxiflora*. The screening revealed the presence of tannins, phenolic compounds, flavonoids and saponins in both plant materials. However, alkaloids were found in *Pericopsis laxiflora* and hydrolysables tannins and heterosides cardiotonics in *Combretum molle*. The IC_{50} for the extract plant of *Combretum molle* was 42 µg/mL and that of the *Pericopsis laxiflora* was 44.15 µg/mL for DPPH radical. The antioxidant effect of the extract plant of *Combretum molle* was 216 µg/mL and the extract plant of *Pericopsis laxiflora* was 225 µg/mL on the nitric oxide radical. It was concluded that the presence of tannins, flavonoids, phenolic compounds, saponin, alkaloids and cardiotonic heterosides, explains why traditional medicine uses both plants together to treat certain diseases. However, further investigations of the pharmacological potential for diabetes treatment are needed.

Keywords: Phytochemical, antioxidant activities, *Combretum molle*, *Pericopsis laxiflora*, Burkina Faso.

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INTRODUCTION

The use of plants for medicinal purpose is increasing in the developing world in spite of the available conventional drugs (Ouôba et al., 2006). Plants are used for several reasons and one of the strong reasons was found to be the cheapest price (Ouôba et al., 2006). In Africa, plants are affordable and many people believe also in the active principle by comparison with others. According to the World Health Organization (2002), nearly 80% of the populations depend on traditional medicine for health care.

In Burkina Faso, as in several African countries, diabetes is becoming a very serious disease and a public concern. Recently, it was found that many people were in the risk of diabetes when they reached age 40 and 66% of diabetes patients were women (Koevi et al., 2014). Other studies showed two types of treatments people refer to. (Unpublished survey data, 2013). The conventional treatment with the use of drugs monitored by a doctor and the traditional treatment with the use of plants without any helps of doctor.

Among the plants, *Combretum molle* and *Pericopsis laxiflora* were identified and regularly used by diabetes patients (Unpublished survey data, 2013). Previous research has shown that *Combretum molle* has positive effect on diabetes (Ojewole and Adewole, 2009). In Cameroon, Bum (2011) has recently shown that *Pericopsis laxiflora* had similar effect on diabetes when compared to *Combretum molle*. At the same time, it is known that the active principle of plants depends on area and agro-climatic conditions (Rajashekar et al., 2012). In Burkina Faso, the phytochemical and antioxidant activities of these plants have not yet been investigated. It is therefore important to look at that because of the increasing use by people and their belief.

The objective of the study was to determine phytochemical composition of those plants, their antioxidant activities and find why patients refer to them for diabetes treatment.

MATERIALS AND METHODS

Plant materials

Combretum molle (R. Br-ex G. Don) Engl & Diels (Combretaceae) and *Pericopsis laxiflora (Fabaceae)* (Benth. Ex Baker) Van Meeuwen, were selected on the basis of their use by diabetes patients in the city of Bobo-Dioulasso. The voucher specimen of each plant was identified as IFAN55790 for *Combretum molle* and IFAN02416 for *Pericopsis laxiflora*. The plants were collected in September 2014 in the peri-urban area of Bobo-Dioulasso. The leaves were air dried at room temperature, powdered and used for extraction.

Preparation of ethanolic extract

Fifty (50) grammes of each plant were put into a conical flask surmounted by a cooling device with backward flow, 500 mL ethanol 90 °C were added and heated to boil for 30 minutes. After cooling, the mixture was filtrated and the marc was discarded. The filtrate was subsequently evaporated and dried through rotative evaporator and stove. The extract was concentrated under reduced pressure at 50 °C, using a rotary evaporator. The semi solid extract obtained was dried in desiccators. The final weight obtained is P_{ex}'. The extractable one was calculated starting from the following equation.

Extraction = $P_{ex} – P_{ex}$' (Bassène, 2012).

P_{ex} = weight of the powder of dried plant

P_{ex}' = weight of the extractable product after desiccation

Ash quantification

Two (2) grammes of completely dried plant powders were incinerated at a temperature from +400 to +600 °C for one hour. Ashes representing the mineral

substances were weighed after cooling in desiccator. The weight is then brought back to 100 g of powder of dried plant. The yield of crude extract was calculated using the following equation (Bassène, 2012).

Yield = (P''/P) x 100

P = weight of the powder of dried plant

P'' = weight of the crude ashes

Phytochemical screening

The phytochemical investigation was carried out according to Bassène (2012).

Determination of phenolic and tannins compounds

One hundred (100) mg of dry ethanolic extract was boiled in 25 mL of distilled water and filtered on Whatman paper N°1. In five (5) mL of this filtered solution, few drops of phosphotungstic acid solution and few drops of 25% natrium carbonate were added. A blue color showed the presence of the polyphenol. For tannin, few drops of 2% ferric chloride were added to 5 mL of filtrate and brownish green or blue-black coloration was noted. Four (4) mL of STIASNY reagent were added to 8 mL of the ethanolic filtrated solution and boiled for 30 minutes. Then, 1 mL of HCl was added and the whole was boiled again. Red color was noted. The solution was filtered and then the filtrated solution was saturated by natrium acetate. After adding a few drops of 2% ferric chloride, a black color indicated the presence of hydrolysables tannins.

Determination of flavonoids

One hundred (100) mg of dry ethanolic extract was boiled in 25 mL of distilled water and filtered on Whatman paper N°1, the filtrate was used for the characterization. Two (2) mL of the filtrate was distributed in two test tubes. In the first tube, some drops of caustic soda (1/10 in water); in contact with the solution, a yellow color indicated the presence of flavonoid. In the second tube, two (2) mL of hydrochloric alcohol (alcohol 96% water/concentrated HCl 2/2/1) and magnesium pinches metal was added. A color change from pink to red was noted.

Determination of anthracene heterosides

Twenty (20) mL of distilled water were added to 100 mg of ethanolic extract and heated in a water bath for 30 minutes with constant agitation. And then, 1 mL of HCl was added to the solution and the mixture was heated in boiling water bath for 15 minutes. After filtration and cooling, the solution is thrown in a separator funnel with 10 mL of chloroform and wash for partition. The chloroformic phase was taken in a test tube and dried. Two (2) mL of 25% NH_4OH were then added and the tube was heated again for 5 minutes in the boiling water bath. The color changing from yellow to red indicated the presence of anthracenosides.

Determination of alkaloids

Five (5) mL of H_2SO_4 (1/10 in distilled water) were added to 500 mg of dry ethanolic extract. After 30 minutes, the solution was filtrated and the filtrate was poured into 2 tests tubes. The Dragendorff reagent was added in the first tube and the presence of black precipitates at the bottom of the test tube reveals a positive reaction. In the second tube, Valser Mayer reagent was added and the presence of precipitates shows the presence of alkaloids.

Determination of saponin

The process starts by adding 100 mL of distilled water to 1 g of powders of plant and the content was boiled in a conical flask of 500 mL under reflux system for 5 minutes. Then, the liquid was filtrated and completed to 100 mL with distilled water. This liquid was poured into 10 stoppered test tubes in successive portion of 1 mL, 2 mL, 3 mL, up to 10 mL and the volume of liquid of each test tube was adjusted to 10 mL with distilled water. Every test tube was vigorously shaken for about 15 seconds, and then allowed to stand in a vertical position. The appearance of foam with at least 1 cm in height persisting at

least for 15 minutes indicates the presence of saponosides. The foam index is the reverse of the concentration of the powder of plant being in the tube according to the formula.

$$\text{Foam Index} = \frac{1000}{X}$$

X is the volume in mL of the liquid used for preparing the dilution in the tube where foaming is observed.

Determination of cardiotonic heterosides

Five (5) mL of the chloroform/ethanol (4:1, v/v) solution were added to 100 mg of ethanolic extract. The solution was filtered and the filtrate was shared out in 3 test tubes. A few drops of the Baljet reagent were added in the first tube. A stable orange color was mentioned as a result. In the second tube, a few drops of the Kedde reagent were also added and a red crimson color was mentioned as a result. Finally, a few drops of Raymond-Marthoude reagent were added in the third tube and the results showed a fugacious violet.

Antioxidant activities

DPPH (2, 2-Diphenyl-1-picryl-hydrazyl) scavenging Assay

The protocol previously described by Bassène (2012) was used. Briefly, the ethanolic extract was dissolved in ethanol (1:1, w/v). From this solution, various dilutions were prepared with the ethanol. One hundred (100) µL of each dilution were distributed in test tubes. Nine hundred (900) µL of the solution of DPPH were added in each tube and incubated in the dark for 1 hour. The ascorbic acid, used as positive control, was carried out in the same condition. Optic Density (OD) was then read at 505 nm using the spectrophotometer. The percentage inhibition (%I) was calculated as follows:

$$(\%I) = \frac{(\text{OD control} - \text{OD sample}) \times 100}{\text{OD Control}}$$

%I is the percentage of inhibition of 2, 2-diphenyl-1-picryl-hydrazyl **(**DPPH**)**, OD control is Optic Density of control (DPPH in ethanol), OD sample: Optic Density of tested samples.

NO (nitric oxide) scavenging assay

For this test, the protocol used was previously described by Oyedemi et al. (2010) with a few modifications. In the tube containing 1 mL of nitroprussite and 250 µL of phosphate buffer, 250 µL of each concentration (1 to 0.0039 mg/mL) of extract was added and the mixture was incubated for 150 minutes. In the negative control, the extract was replaced by ethanol while in blank Nitroprussite and extract was replaced by water and ethanol respectively. After incubation, 500 µL of each tube was collected and mixed with 1 mL of sulfanilic acid following by 1 mL of N-(1-Naphthyl)-ethylenediamine (NED). The mixture was incubated for additional 30 minutes and the pink color was read at 540 nm. The percentage of inhibition was given through the following equation:

$$\%I_{NO} = \frac{(OD_t - (OD_{Ex} - OD_{Ec})) \times 100}{OD_t}$$

$\%I_{NO}$ is the inhibition percentage of the radical nitro-oxide, OD_t is the Optic density of the negative control, OD_{Ex} is the Optic density of the test in the presence of the extract, OD_{Ec} is the optical density of control extract without hydrogen peroxide.

Activity on the radical hydroperoxide (H_2O_2)

The method used was described by Kalyani and Anuradha (2013). On 50 µL of the hydrogen peroxide solution, 50 µL of each ethanolic extract were added and then incubated for 30 minutes. Then 900 µL of the ferrous oxidation in xylenol (FOX) reagent solution was introduced into each and incubated for 30 minutes in dark. Briefly, the FOX orange reagent was prepared by mixing 88 mg of butylated hydroxytoluene, 7.6 mg of xylenol orange, 9.8 mg of ammonium

sulphate, 90 mL of methanol and 10 mL of sulphuric acid, 250 mM. The negative control was prepared in the same conditions but ethanolic extracts is replaced by 50 µL methanol. The blank is prepared by replacing the ethanolic extracts and hydrogen peroxide by 10 µL of methanol. The extracts controls were prepared under the same conditions as the tests with the difference that the hydrogen peroxide is replaced by 50 µL of distilled deaerated water. All of the tubes were read at 560 nm on a spectrophotometer. The percentages of inhibitions were calculated using the following equation:

$$\%I_{H2O2} = \frac{(OD_t - (OD_{Ex} - OD_{Ec}))}{OD_t} \times 100$$

%I is the percentage of inhibition of hydrogen peroxide, OD_t is the Optic density of the negative control, OD_{Ex} is the Optic density of the test in the presence of ethanolic extract, OD_{Ec} is the optical density of extract ethanolic control without hydrogen peroxide.

Statistical analysis

The results of the test were expressed as Means ± Standard Deviation (SD) of triplicate determination. The IC was obtained using GraphPad Prism version 5. The data were subjected to analysis of variance (ANOVA) and the means were compared using F-test and the difference was considered to be significant at the level of $p < 0.05$.

RESULTS

Phytochemical screening of ethanolic extract

The results showed 6.76 ± 0.076% of water in *Combretum molle* and 7.52 ± 0.015% in *Pericopsis laxiflora* (Table 1). The ash contents were 3.41 ± 0.35% for *Combretum molle* and 3.87 ± 0.15% for *Pericopsis laxiflora* (Table 1). Tannins, phenolic compounds, flavonoids and saponins were found in both plants. Alkaloids have been found only in *Pericopsis laxiflora* but not in *Combretum molle* leaves. Hydrolysable taninins and heterosides cardiotonics were found only in *Combretum molle*. Anthracene heterosides were not found in either plant. The foam index was 200 and 100 for *Combretum molle and Pericopsis laxiflora*, respectively.

Antioxidant activities

DPPH (2,2-diphenyl-1-picryl-hydrazyl) antioxidant assay

The IC_{50} was 42 ± 0.07 µg/mL for *Combretum molle* and 44.15 ± 1.10 µg/mL for *Pericopsis laxiflora*, 2.785 ± 0.8 µg/mL for ascorbic acid and 1.207 ± 0.6 µg/mL for gallic acid. The results showed that both plants seemed to have the same IC_{50} and this was lower than that of control material (ascorbic acid and gallic acid). It should be noted that antioxidant activity of gallic acid is better compared to that of ascorbic acid (Table 2).

Nitric oxide (NO) antioxidant assay

The IC_{50} was 216 ± 2.7 µg/mL for *Combretum molle* and 225 ± 2.96 µg/mL for *Pericopsis laxiflora*. The two plants showed a low antioxidant activity in comparison to the reference of ascorbic acid. The IC_{50} of gallic acid was 100 ± 0.08 µg/mL (Table 2).

H_2O_2 antioxidant assay

At 0.5 mg/ml of the extract, the Optical densities (OD) for *Combretum molle* activity were 1.458 ± 0.00 and were 1.457 ± 0.002 for *Pericopsis laxiflora* activity on the hydroxyl radical scavenging; at the same time, the value of the Optical density of the negative control ($OD_{t)}$ was 1.459 ± 0.02; the OD of the reference compound of gallic acid was 1.413 ± 0.000. Then the percentage inhibition obtained from OD would be very low also and the IC_{50} was not calculated. This means inhibition was not obtained for H_2O_2 using fox reagent with both plants and for the ascorbic acid (Figure 1).

Table 1: Chemical components of plant materials.

Names	***Combretum molle***	***Pericopsis laxiflora***
Water	6.76 ± 0.076	7.52 ± 0.015
Ash	3.41 ± 0.35	3.87 ± 0.15
Tannins	++	++
Hydrolysables tannins	++	ND
Phenolic compounds	++	++
Flavonoid	++	++
Saponin	++	+
Alkaloids	ND	++
Heterosides cardiotonics	++	ND
Anthracene heterosides	ND	ND
Foam index	200	< 100

++: medium amount; +: small amount or present and ND: not detected

Table 2: IC_{50} in µg/mL of *Combretum molle*, *Pericopsis laxiflora*, gallic acid and ascorbic acid on DPPH and NO tests.

Radical scavenging assay	***Combretum molle***	***Pericopsis laxiflora***	**gallic acid**	**ascorbic Acid**
DPPH	42 ± 0.07	44.15 ± 1.10	1.207 ± 0.6	2.75 ± 0.8
NO	216 ± 2.7	225 ± 2.96	100 ± 0.08	NC

NC: not carried out. Values are mean ± Standard deviation of 3 replicates. The both extracts and the reference control was significantly different with p = 0.000

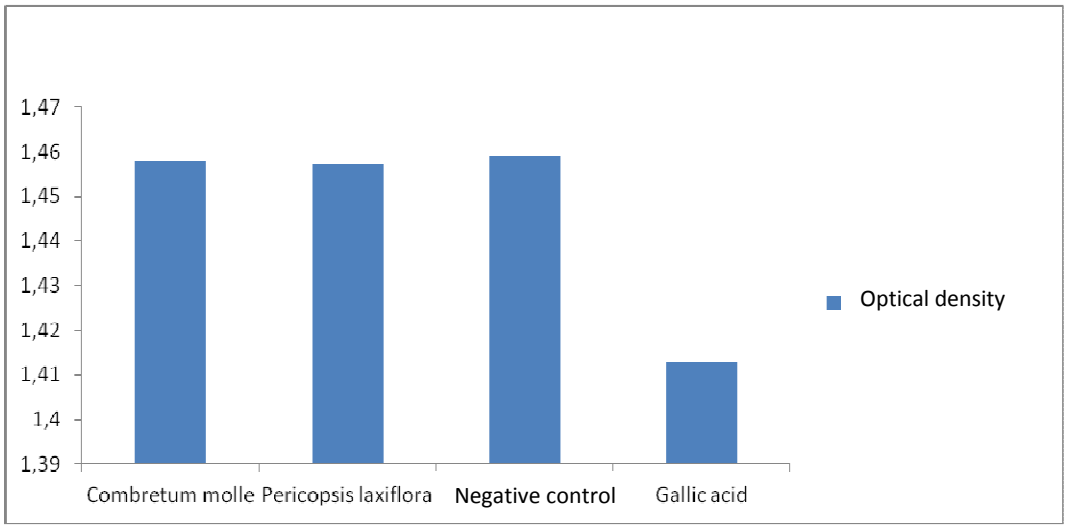

Figure 1 : Optical density of *Combretum molle*, *Pericopsis laxiflora*, Negative control and gallic acid on H_2O_2 test. Each value represents means ± Standard deviation (n = 3).

DISCUSSION

The current findings revealed phytochemical compound of tannins, phenolic compounds, flavonoids and saponins. Similar components were found by Zintchem et al. (2013); Saidu and Abdullahi (2011) who concluded that their major biological effect on human health was against chronic diseases. It was also recorded that tannins, flavonoids and alkaloids have hypoglycemic activities which may explain why patients in Burkina Faso refer to *Combretum molle* and *Pericopsis laxiflora*. Furthermore, Saleem and Basha (2010) reported that flavonoids could inhibit the low-density lipoprotein (LDL) cholesterol oxidation and prevent atherosclerosis and decrease the cardiovascular disease risk. Since there is positive correlation between diabetes and cardiovascular disease (Koevi et al., 2014), this probably explains why the diabetes patients refer to *Combretum molle* and *Pericopsis laxiflora*. According to Ajibesin (2011), several reasons may explain the utilization of the plant materials used in the treatment of diseases. Tannins are useful to prevent cancer and treat inflamed or ulcerated tissues. Alkaloids have analgesic, antimalarial, antiseptic and bactericidal activities which could be used to treat other chronic diseases. Saponins are responsible for most biological effects on cell growth and division in human body and have an inhibitory effect on inflammation. Now, we could understand why most patients refer to the current plants in this study. However, the toxicity of those plants is not yet well highlighted. It could be concluded that, regarding the biological effect of each phytochemical compound, both plants could be used to treat several diseases. The library search did not show a result on the phytochemical study of the plant extracts of *Pericopsis laxiflora*. On the other hand, according to the study of Saidu and Abdullahithe (2011), the leaf extract plant collected from Nigeria indicated the presence of alkaloids while with Burkina Faso sample leaf extract collected does not contain any. In addition, cardiotonic heterosides were found in the leaf plants from Burkina Faso but not in those from Nigeria.

Additional information is needed on those plants which could effectively treat diseases without strong antioxidants. This means that, when the patient uses it, the effect on diseases depends on the amount of anti-oxidants in the plant material as reported by Adedapo et al. (2008). The current study was not able to show which one is strongly needed if we have to recommend the plant to patient. The ability of a substance to act as an antioxidant depends on its capacity to reduce RONS (reactive oxygen and nitrogen species) (Adedapo et al., 2008) by donating hydrogen atom for instance. Both plant materials used in the current study has very low activity on the DPPH antioxidant. Then, this study showed that the DPPH antioxidant activity of *Combretum molle* is higher rating than that of *Pericopsis laxiflora*. Both plants had shown not much antioxidant activity regarding the result of the reference compounds of ascorbic acid and gallic acid, the difference was significant ($p = 0.000$). A study carried out by Ozgen et al. (2010) showed that phenolic compounds are known to exhibit strong antioxidant activity. This means that the quantity of the phenolic compounds is also low in the plant extract which we have used in this study. 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 (Zintchem et al., 2013). Further study of the phenolic compounds and of the flavonoids quantification could be done to obtain more information about that. 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 (Parul et al., 2012). In the present study, both extracts showed a low

inhibitory effect. The phytochemical constituent of the plant extract can be responsible of the observed effect. In fact, Ozen et al. (2010), working on *Sambucus canadensis* L containing the same components, have obtained a good inhibition on these radicals but not with the same quantities of phytochemical compounds because for the same author, fruit provide more of phenolic compounds than leaf. The variability of the phytochemical compound of each plant may explain why traditional medicine uses the combination of both plants to increase the antioxidant activities. The inhibition absence of both plants on the hydroxyl radical scanvenging comes from the very low antioxidant activity of the both plants and according to the method which have been used in this study. It will be noted that DPPH radical scavenging is much higher than Nitric oxide which is higher than hydroxyl radical. Regarding the different results obtained from the antioxidant, we know that the antioxidants are able to neutralize the radical growth during the chronic diseases. The low antioxidant activity and the variability of the phytochemical compound of each plant could explain why traditional medicine uses the combination of both plants to increase the antioxidant activities. The quantities of phytochemical of different constituents have not yet been assessed.

Conclusion

Several extractible matters were found in *Combretum molle and Pericopsis laxiflora*. They were mainly tannins, phenolic compounds, flavonoids and saponins, alkaloids hydrolysable tannins, heterosides cardio-tonics and anthracene heterosides. These components could be responsible for the biological effects abstained from both plants. The extract from this plants also reveal antiradical activity against DDPH and Nitric oxide **(**NO) but no inhibition was obtained on Hydro-peroxide. Further study of both plants could be done to know if the quantity of the different phytochemical is still low or not and to explain why the antioxidant activity was not strong.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHOR'S CONTRIBUTIONS

Individual contribution of each author has been noted during the study. KKKA prepared the extract, carried out the assays, conducted the literature search, participated in data collection and analysis and prepared the first draft of the manuscript. VM assisted with data collection, analysis and interpretation, and assisted with the drafting of the manuscript. AS Carried out the assays. JBHF generated the concept, gave reagents, carried out the assays and assisted with the drafting of the manuscript. GAO coordinated the study. EB conceived the study, designed the research protocol and coordinated the study. All authors read and approved the final manuscript.

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