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REVIEW ARTICLE

COMPARATIVE STUDY OF THREE METHODS FOR EXTRACTING TOTAL POLYPHENOLS FROM THE LEAVES OF *CYMBOPOGON CITRATUS (DC) STAPF*

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ABSTRACT

Extraction is a very important step in the isolation and identification of secondary metabolites. It is influenced by several factors, notably the method used and the presence of interfering substances. This work aims to compare three extraction methods by assessing the total polyphenol content and antioxidant activity of Cymbopogon citratus leaf extracts. Extraction rates were determined using three extraction methods: maceration, infusion and decoction. The total polyphenol content of the extracts obtained was determined using a spectrophotometric method, and antioxidant activity was quantified using the DPPH and ABTS methods. The best extraction yield was recorded by decoction, with an average of 8.9%. However, infusion and maceration remain good extraction methods. Decoction is the best method for extracting total polyphenols, with a level of 16.82±0.18 mg EAG/mL. Concerning antioxidant activity, the extract obtained by decoction showed the highest antioxidant activity whatever the analytical method used. This study shows that extraction by decoction is the best method for extracting total polyphenols and obtaining the highest antioxidant capacity for this plant.

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INTRODUCTION

Medicinal plants have always been an essential source of medicines. This source seems inexhaustible, with 400,000 known plant species, each containing several thousand different constituents (1). Indeed, the flora that has existed for millions of years has largely served as the basis for the discovery of many modern medicines. The use of medicinal plants in therapeutics throughout the world goes back a long way, and is currently enjoying a resurgence in popularity, despite advances in modern medicine. According to the annual report of the World Health Organization (WHO), some 80% of the world's population has turned at least once to traditional medicine to deal with health problems (2). This popularity is due to the fact that many illnesses can be treated satisfactorily and cost-effectively with plants. These plants owe their therapeutic power to the active substances they contain. To assess the biological activity of these plants, it is essential to use appropriate biological tests and chemical screening methods (3).

In most cases, the biological activity of secondary metabolites is recognized long before their chemical structures are determined (4). However, the active nature of these compounds can have both beneficial and harmful effects on living organisms. *Cymbopogon citratus (DC) Stapf is* a medicinal plant widely used by the Ivorian population, particularly in traditional medicine. The plant's therapeutic value is due to its secondary metabolites, notably essential oils and phenolic compounds. Phenolic compounds (mainly flavonoids, phenolic acids and tannins) are a rich resource widely exploited by the food, cosmetics and pharmaceutical industries (5). Extracting active ingredients from these metabolites is a very important step in their isolation, as well as in their identification (3). The food or therapeutic quality of a natural extract depends on the efficiency and selectivity of the extraction process used (3). The various processes used include aqueous maceration, infusion and decoction. It is within this framework that this work is situated. The aim is to compare three extraction methods for *Cymbopogon citratus* leaf extracts, to determine the best method for extracting total

polyphenols and obtaining the highest antioxidant capacity from the extracts studied.

MATERIALS AND METHODS

Plant material: The plant material consists of *Cymbopogon citratus* plant leaves. The leaves were harvested in March 2018 in Yamoussoukro (Côte d'Ivoire) and dried in the shade for ten (10) days. After drying, they were ground to powder using an electric grinder. Their botanical identification was carried out by Mr. N'GUESSAN Amani, taxonomist at the botany laboratory of the Agriculture and Animal Resources (ARA) department of the Institute National Polytechnique Félix Houphouët-Boigny de Yamoussoukro (INP-HB).

Extraction: The extracts were prepared according to the classic extract preparation processes used in village environments, i.e. infusion, decoction and maceration. This enabled us to obtain aqueous extracts. The powders obtained were used in the various extraction processes.

Decoction: A 10 g test sample of *Cymbopogon citratus* grind was introduced into a flask containing 100 mL distilled water. The mixture was boiled for 30 minutes on a soxhlet. After cooling and filtration, the filtrate was concentrated on an evaporative rotator under vacuum at 50°C. The extract was then oven dried at 46°C for 24 hours. The powders obtained were weighed and stored in a hermetically sealed sterile bottle untiluse.

Infusion: A 10 g test sample of *Cymbopogon citratus* grind was introduced into a flask containing 100 mL boiling distilled water. The mixture was kept for 30 minutes until it cooled. After filtration, the solvent was reduced under reduced pressure at 50°C. The extract was then oven dried at 46°C for 24 hours. The resulting powders were weighed and stored in a hermetically sealed sterile bottle until use.

Aqueous maceration: A 10 g test sample of *Cymbopogon citratus* grind was introduced into a flask containing 100 mL distilled water. The mixture was kept at room temperature under magnetic stirring for 24 hours. After filtration,the filtrate was concentrated on an evaporative rotator under vacuum at 50°C. The extract was then ovendried at 46°C for 24 hours. The powders obtained were weighed and then stored in a hermetically sealedsterile bottle until use.

Determining yield: The mass of the dry extract is determined by the difference between the mass of the full flask (after evaporation) and the weight of the empty flask (before transferring the filtrate to be evaporated) (2).

Yield (%) = ((Mass of dry extract) *100) / (Mass of plant matter)

Phytochemical screening: Phytochemical screening is carried out on the basis of characteristic staining tests to highlight the main chemical groups. Aqueous extracts of *Cymbopogon citratus* were used. The various chemical groups were characterized using the techniques described in the work of Békro *and al* (6). Alkaloids were identified by the Dragendorff reaction; flavonoids by the Wilstater reaction; tannins by the ferric chloride reaction; steroids and triterpenes by the Liebermann-Burchard reaction, and saponins by the foam index.

Spectrophotometric determination of total flavonoids: The method of Marinova *and al.* (7) was used for the determination of total flavonoids. In a 25 mL flask, 0,75 mL sodium nitrite (NaNO2) 5% (w/v) was added to 2,5 mL extract. The mixture was supplemented with 0.75 mL aluminum chloride (AlCl3) at 10% (w/v), then incubated for 6 minutes in the dark. After incubation, 5 mL sodium hydroxide (NaOH, 1N) was added and the volume made up to 25 mL. The mixture was shaken vigorously before being assayed on a Jasco V-530 spectrophotometer (JASCO, Japan). Readings were taken at 510 nm. Trials were carried out in triplicate. Flavonoid content was expressed in milligrams per liter of quercetin equivalent extract (mg EQ /mL).

Spectrophotometric determination of total polyphenols: The Wood *and al*, (8) was used for the determination of total polyphenols. A volume of 2,5 mL of diluted (1/10) Folin-Ciocalteu reagent was added to 30 µL of extract. The mixture was kept for 2 minutesin the dark at room temperature, then 2 mL of calcium carbonate solution (75 $g.L^{-1}$) was added. The mixture was then placed in a water bath at 50°C for 15 minutes, and rapidly cooled. Absorbance was measured at 760 nm, using distilled water as a blank. A calibration line was made with gallic acid at different concentrations. Analyses were performed in triplicate, and polyphenol concentration was expressed in grams per liter of gallic acid equivalent extract (mg. Eq GA/mL).

Assessment of antioxidant activity by DPPH: The anti-free radical activity of the extracts was measured using the 2, 2-diphenyl-1- picrylhydrazyl (DPPH) inhibition test according to the methods reported by Bekro et *al.* (9). and de Parejo et *al.* (10). This method assesses the extract's ability to bind free radicals by measuring the decrease in violet coloration due to the reduction of DPPH radicals. The dry aqueous extract of "Ahoutou" and ascorbic acid (reference antioxidant) dissolved in methanol at a concentration of 1 mg/mL were diluted to different increasing concentrations (0.025-0.05-0.1-0.2-0.3 mg/mL). DPPH was solubilized in absolute methanol to obtain a solution with a concentration of 6.34.10-5 M (0.0025 g DPPH in 100 mL methanol). The resulting solution was stored in a dark place. After 30 minutes incubation at room temperature, absorbance was read in a spectrophotometer at 517 nm against the blank sample. The free radical scavenging activity of the extracts tested, corresponding to the percentage inhibition (PI) of the DPPH radical, was calculated according to the following equation:

PI (%) = (A0 (Absorbance of blank) - A (Absorbance of extract) * 100) / (A0 (Absorbance of blank) Where A0 is the absorbance of the negative control, which contains DPPH and methanol without extract. The inhibition concentration IC50 (mg/mL), which corresponds to the concentration of the "Ahoutou" extract containing the reference antioxidant responsible for 50% inhibition of DPPH radicals, is used to assess the antioxidant power of the extract analyzed.

Assessment of antioxidant activity by ABTS: This TEAC (Trolox equivalent antioxidant capacity) method is based on the compounds' ability to reduce the ABTS radical$^{\circ+}$. The ABTS radical^{+}, in contact with an H- donor, leads to ABTS^{+}

and 734 nm decolorization of the solution (11). Antioxidant capacity in Trolox® equivalent (TEAC) corresponds to the concentration (mmol/L or mg/L) of Trolox® with the same activity as the same unit concentration of test substance. The test was performed according to the method described by Choong *and al* (12). The ABTS° radical-cation^{+} was produced by reaction of 8 mM ABTS (87.7 mg in 20 mL distilled water) and 3 mM potassium persulfate (0.0162 g in 20 mL distilled water) in a 1:1 (v/v) ratio. The mixture was then incubated in the dark at room temperature for 12-16 hours. This ABTS° solution^{+} was diluted with methanol to give a solution with an absorbance of 0.7±0.02 at 734 nm. A test portion of 3.9 mL of this diluted ABTS$^{\circ+}$ solution was added to 100 µL of the test compound. After stirring, the mixture was incubated for 6 minutes in the dark (T=30±2°C). The residual absorbance of the ABTS radical$^{+\circ}$ was then measured at 734 nm with a UV-visible spectrophotometer, and should be between 20%-80% of the absorbance of the blank. Trials were carried out in triplicate and results were expressed in µmol Trolox equivalent per liter of extract (µmol/L TE). Compound activity is expressed by the Trolox Equivalent Antioxidant Capacity (TEAC), which corresponds to the concentration of Trolox (a hydrophilic analog of vitamin E) giving the same antioxidant capacity as a 1 mM concentration of the compound tested. The higher the TEAC value, the more effective the antioxidant. The following concentrations of trolox: 3.75; 5; 6.25; 10; 11.25; 13.75 and 15.10^{-4} mM, were prepared and the inhibition rate (%I) of ABTS$^{\circ+}$ was expressed as follows:

(%I) = (A0 (Absorbance of blank) - A (Absorbance of extract) * 100) / (A0 (Absorbance of blank)

Where A0 is the absorbance of the control (diluted ABTS absorbance); A is the absorbance of the extract (diluted ABTS absorbance + sample)

Statistical analysis: Graph Pad Prism 7.0 and Microsoft Excel 2016 were used for statistical analysis. All experiments were performed in triplicates for total flavonoids assay and antioxidant activity. Results were represented by the mean with its standard deviation calculated on the average of three replicates for antioxidant activity and flavonoid assay. The difference between two values is considered significant when P<0.05. Statistical analysis of results was performed using analysis of variance (ANOVA). When a significant difference was observed, Tukey and Dunnetts multiple comparison tests were performed.

RESULTS AND DISCUSSION

Phytochemical screening: Qualitative phytochemical screening of *Cymbopogon citratus* plant extracts revealed the chemical family groups present in the plant extracts (Table 1).

Table 1. Phytochemical characterization of *Cymbopogon citratus* extracts

Secondary metabolites	*Cymbopogon citratus* (Leaves)		
	Maceration	Infusion	Decoction
Polyphenols	+	+	+
Flavonoids	+	+	+
Saponins	+	+	+
Tannins	+	+	+
Alkaloids	±	±	+
Terpenes and sterols	+	±	±

(-): Absent; (±): Low presence; (+): Presence

The results of phytochemical screening carried out on extracts (macerated, infused and decocted) of *Cymbopogon citratus* leaves show the presence of several families of chemical compounds. This analysis showed that flavonoids, polyphenols, tannins, saponins, terpenes and sterols, and alkaloids are present in the plant. This result is in line with that of Figueirinha *and al*, (13) in the *Cymbopogon citratus* plant. However, alkaloids are low in extracts obtained by maceration and infusion, while terpenes and sterols appear to be equally low in the extract obtained by decoction. The chemical constituents highlighted by phytochemical screening augur well for the interesting pharmacological activities of the plant studied. These include alkaloids, flavonoids, terpenes and polyphenols with anti-inflammatory (14) antibacterial (15), antidiabetic (16), antiplasmodial (17) and antioxidant (18) activities. The results of this phytochemical screening led us to carry out assays, in particular for total flavonoids and total polyphenols, in order to quantify the secondary metabolites of *Cymbopogon citratus* leaf extracts and assess their pharmacological activities, namely antioxidant activity. Comparative study of extraction techniques for polyphenols and total flavonoids. In this study, we used three extraction methods: aqueous maceration, infusion and decoction. A total of three dry extracts were obtained for the various tests. The comparative study of these three extraction methods focused on extraction yield, quantitative determination of polyphenols and total flavonoids, and antioxidant activity by DPPH and ABTS tests.

Plant extraction: Three extracts were obtained from the extraction methods studied. Table 2 shows the yields obtained for each extract.

Table 2. Dry extract yields for the three *Cymbopogon citratus* leaf extraction methods

Extracts	Extraction yield (%)		
	Decoction	Infusion	Maceration
Cymbopogon citratus	$8,9 \pm 0,26^{a}$	$8,40 \pm 0,20^{a}$	$7,8 \pm 0,26^{b}$

Mean values followed by their standard deviations (mean ± SD, n=3), assigned different letters on the same line, are statistically different (p<0,05). These results show that the best extraction yield of the three methods used is decoction with an average of $8,9 \pm 0,26^{a}$ followed by infusion and maceration with an average of $8,40 \pm 0,20^{a}$ and $7.8 \pm 0,26^{b}$ respectively. Statistically, there was no significant difference (P>0,05) between decoction and infusion. However, there was a significant difference between these two extraction methods and maceration (P<0,05). These results are in line with those obtained by (19) on *Psidium guajava* leaves. The result found by Roumeissa and *al*. (20) on the study of *Artemisia herba* differs from ours, instead finding that it is infusion that records the highest yield followed by decoction and maceration. The yield of extractions by decoction, maceration and infusion showed that the best yield was obtained with the decoction extract. Determining yields enables a quantitative assessment of the extracts that can be extracted from each species. These yields also make it possible to consider the quantity of organs to be harvested, should the need arise for a similar study, which would make the use of medicinal plants more rational (21). The choice of leaves is justified by the fact that they are the site par excellence of biosynthesis and even storage of the secondary metabolites responsible for the plant's biological properties (22). The results obtained in this study are better than those obtained by Tarkang and *al.* (23).

Indeed, these authors found a yield of 5,80 when extracting *Cymbopogon citratus* leaves by decoction. This difference could be due to the leaf harvesting periods and the origin of the leaves. Overall, these yields are quite high. This may be explained by the fact that water-soluble substances such as polyphenols and flavonoids are present in significant quantities.

Determination of total polyphenols: The total polyphenol content of extracts was obtained from a calibration curve (y = 1.129x + 0.0544; R^2 = 0.9935) established with quercetin concentrations (Figure 1). It is expressed in mg quercetin equivalent per mL extract. Acid results for total polyphenol content are given in the Table 3.

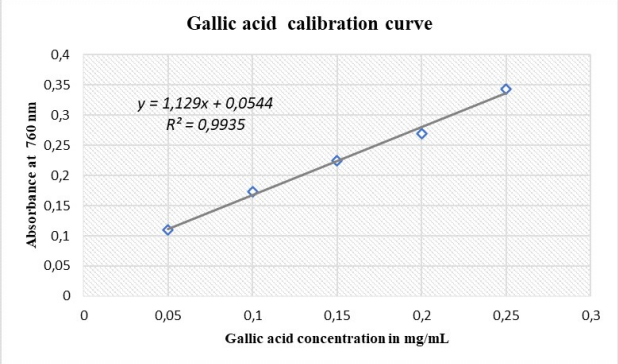

Figure 1. Calibration curve for polyphenols using gallic

Table 3: Total polyphenol content of *Cymbopogon* citratus leaf extracts

Plant (leaves)	Extraction mode	Polyphenol content (mg EAG / mL)
Cymbopogon citratus	Maceration	12,95± 0,07[b]
	Infusion	13,05± 0,02[b]
	Decoction	16,82 ± 0,10[a]

Mean values followed by their standard deviations (mean ± SD, n=3), assigned different letters in the same column per extract, are statistically different (p<0.05). The results show that decoction is preferable for extracting total polyphenols, with a value of 16.82±0.18 mg EAG/mL. However, the two extraction methods, infusion and maceration, did not show a significant difference (P>0.05). However, infusion appears to be the better method for extracting total polyphenols, with an average of 13.05±0.13 mg.EAG/mL, compared with an average of 12.95±0.05 mg.EAG/mL for maceration.\

Determination of total flavonoids: The flavonoid content of extracts was obtained from a calibration curve. (*y = 0.2716x + 0.0004*; R^2 = 0.9629) established with quercetin concentrations (Figure 2). It is expressed in mg quercetin equivalent per mL extract. Flavonoid content results are shown in the Table 4.

Table 4. Total flavonoid content of *Cymbopogon citratus* leaf extracts

Plant (leaves)	Extraction mode	Flavonoid content (mg. EQ / mL)
Cymbopogon citratus	Maceration	5,8± 0,12[b]
	Infusion	6,9 ± 0,12[a]
	Decoction	7,1± 0,38[a]

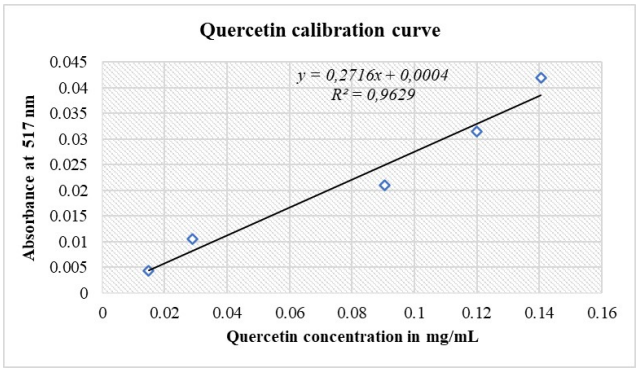

Figure 2. Flavonoid calibration curve using quercetin

Mean values followed by their standard deviations (mean ± SD, n=3), assigned different letters in the same column per extract, are statistically different (p<0.05). The results of the total flavonoid content of decoction, infusion and macerate obtained by the three extraction modes show that decoction and infusion are preferable for extracting flavonoids with an average of 7.1± 0.38 mg EQ/mL and 6.9 ± 0.12 mg EQ/mL respectively versus maceration with an average of 5.8± 0.12 mg EQ/mL.

Reduction of the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) by spectrophotometric assay: The antioxidant activity of *Cymbopogon citratus* extracts obtained by decoction, infusion and maceration was assessed using the DPPH free radical reduction method and the ABTS radical scavenging method. The results of the DPPH IC values50 of the ascorbic acid molecule (reference molecule) and the extracts studied, determined from Figure 3 and the ABTS test value, are shown in Table 5.

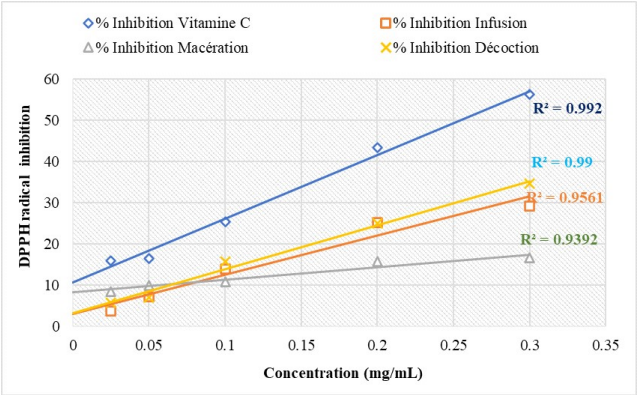

Figure 3. Percentage of DPPH radical inhibition as a function of ascorbic acid concentration andextracts studied

Table 5. CI values50 for DPPH test and ABTS of aqueous extracts

Plants (leaves)	Extraction mode	DPPH IC50 (mg/mL)	ABTS (µmol. L^{-1} TE)
Cymbopogon citratus	Maceration	1,36 ± 0,01[c]	45,6 ± 1,7[c]
	Infusion	0,49 ± 0,01[b]	55,6 ± 0,30[b]
	Decoction	0,44 ± 0,02[b]	102,6 ± 8,8[a]
Ascorbic acid	--	0,25 ± 0,00[a]	--

Results are expressed as mean ± SD, n=3 for each extract. Identical letters in the same column for each plant are not statistically significant (p>0.005).

The results of the DPPH test were expressed in terms of the IC parameter$_{50}$ and the extract with the lowest IC value$_{50}$ had the

highest free radical scavenging activity. Thus, according to the results, decoction has the highest antioxidant activity, followed by infusion and maceration. However, there was no statistical difference between infusion and decoction. Nevertheless, the results show that all extracts have antioxidant activity. Activities vary from one extract to another. The differences betweenthe extracts and the reference molecule (ascorbic acid) are statistically significant ($P < 0.05$). The reference molecule (Vitamin C) has a IC_{50} of 0,25 ±00 mg/mL. According to the results of the ABTS test, the decoction extract showed high antioxidant activity. Overall, the decoction extract showed the highest antioxidant activity in all DPPH and ABTS tests, which would justify the use of this extraction technique by traditional healers when using this plant totreat themselves, as the extracts obtained are sources of antioxidant activity

Variations in antioxidant activity and total flavonoid and polyphenol content with different extraction methods in *Cymbopogon citrates:* Variations in antioxidant activity by DPPH and ABTS tests, flavonoid and total polyphenol content in aqueous extracts of *Cymbopogon citratus* leaves are illustrated in Figures 4 and 5.

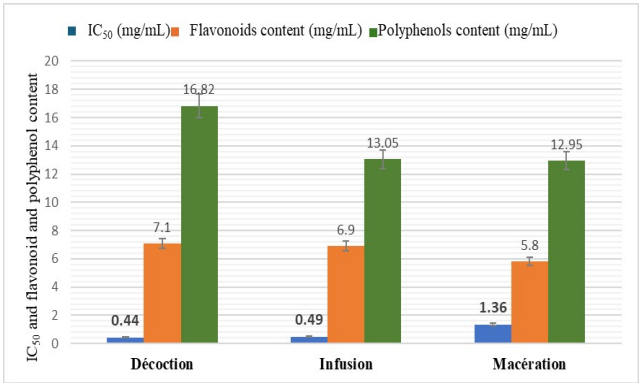

Figure 4. Histograms showing DPPH antioxidant activity and flavonoid and polyphenol contents in the aqueousextract of *Cymbopogon citratus* leaves using different extraction methods

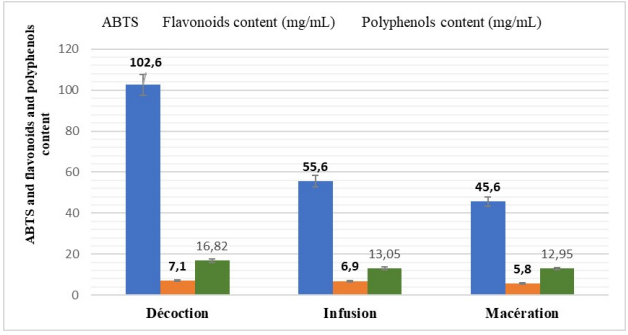

Figure 5. Histograms showing ABTS antioxidant activity and flavonoid and polyphenol content in the aqueousextract of *Cymbopogon citratus* leaves using different extraction methods

Our results show that antioxidant activity correlates with total polyphenol and total flavonoid content. This correlation explains why extracts with higher polyphenol and flavonoid contents give a lower50 CIand higher ABTS. This correlation between flavonoid content and free radical scavenging potential hasalso been demonstrated by *Yen and al.* (24).
This shows that extraction by decoction is the best method for extracting flavonoids and obtaining the highest antioxidant capacity from the extracts studied. Phenolic compounds and flavonoids quantified in extracts appear to be responsible for antioxidant activity. Phenolic acids and flavonoids are the polyphenolic compounds most frequently found in plant extracts (25). The antioxidant activity of phenols plays an important role in the absorption or neutralization of free radicals (26). Polyphenols also increase the level of the cellular antioxidant system and induce cytochrome P-450. This helps detoxify carcinogenic activity at the intracellular level (27). The abundance of polyphenolic compounds confirms the therapeutic properties attributed to them in ethnotherapy. Indeed, several studies have demonstrated that polyphenolic compounds confer several biological activities on the plant (28).

Correlation between flavonoid content and antioxidant activity of these plants: Figure 6 illustrates the correlation between total flavonoid content and antioxidant activity.

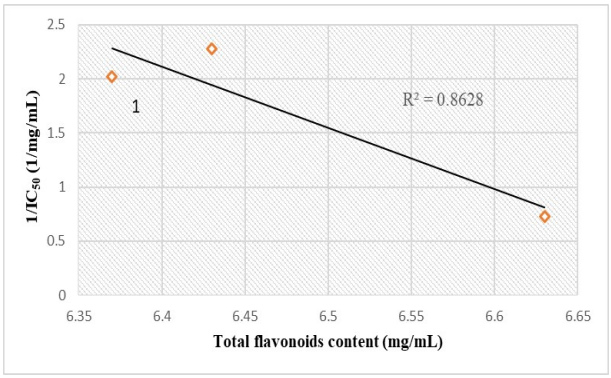

Figure 6. Correlation between antioxidant activity in *Cymbopogon citratus* and flavonoid content.

1: Maceration, 2: Infusion, 3: Decoction

Figure 6 shows that there is a good linear correlation (R^2=0.8628) between flavonoid content and antioxidant activity in extracts from the different extraction methods used for the plant studied. This correlation suggests that flavonoids are major contributors to antioxidant activity.

CONCLUSION

The present work involved a comparative study of three extraction techniques (decoction, infusion and maceration) for the polyphenols contained in *Cymbopogon citratus* leaves. The comparison focused on polyphenol extraction yield and antioxidant activity. Phytochemical screening showed that the extracts are rich in phenolic compounds and flavonoids. The results of the comparative study showed that the highest extraction yield of these compounds was obtained by decoction, followed by infusion and maceration. In terms of anti-free radical activity, the extract obtained by decoction was the most effective. This analysis therefore shows that the decoction extraction method is the best extraction method compared to the other two methods for extracting polyphenols and flavonoids and obtaining the highest antioxidant capacity from the aqueous extracts obtained.

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