EXCELLENT PUBLISHERS

International Journal of Current Research and Academic Review

ISSN: 2347-3215 Volume 2 Number 6 (June-2014) pp. 67-73

www.ijcrar.com

Determination of trace elements and antioxidant compounds contained in the aqueous extract of leaves of *Centella asiatica* (Apiacee)

Lohoues EEC[1], Trébissou JND[2]*, Adjé F[3], Konan AML[1], Yapo AF[2], Yapi HF[2], Djaman AJ[2]

[1]Biochemical Laboratory, UFR medical Sciences, Felix Houphouet Boigny University, POBox, 582, Abidjan 22- Côte d'Ivoire
[2]Pharmacodynamics Biochemical Laboratory, UFR Biosciences, Felix Houphouet Boigny University. PO Box, 582, Abidjan 22- Côte d'Ivoire
[3]Research department of water and natural sciences, INP-HB, PO Box, 1093, Yamoussoukro, Côte d'Ivoire
***Corresponding author**

KEYWORDS

Centella asiatica (Apiacee), Trace Elements, Antioxidants, Free Radicals

A B S T R A C T

To determine the content of trace elements and antioxidants contained in the aqueous extract of leaves of Centella asiatica (Apiacee). This plant is traditionally used to treat diseases such as yellow fever, dysentery, diarrhea and mental illness. For the determination of trace elements, the proposed method of International Institute of Tropical Agriculture (IITA) was used. For flavonoids and phenolic compounds content, the colorimetric and Folin-Ciocalteu method were used respectively. The aqueous extract of leaves of Centella asiatica contains minerals such as magnesium (11,048 mg/g), Sodium (6,823 mg /g) , zinc (3,251 mg /g) and iron (1,348 mg /g). This plant extract is rich in phenolic compounds (130 µg Eq AG / L) and flavonoids (90 µg Eq Isorham/L) in concentrations water /citric acid of 2N and 0.01 N respectively. The aqueous extract of leaves of Centella asiatica (Apiacee) contains phenolic and flavonoid compounds as well as trace elements, which are involved in the mechanisms established by the body to get rid of free radicals. This plant can be used to find a cure against oxidative stress which is responsible for the presence of free radicals in the human body.

Introduction

Oxygen is recognized as one of the origins of the deleterious effects caused by Oxygen Free Radicals (OFR) and other activated oxygen species (ROS) in the body (1). Formed during the biochemical processes of the body, these reactive oxygen species are beneficial as well as toxic (2).

The excess free radicals in the body attack biological molecules such as (lipids, proteins, nucleic acids) that are important to tissues cells (3). Oxidative stress is a consequence of the presence of free radicals, the cause of several diseases such as cardiovascular disease, diabetes, cancer

(4). Yearly there is a number of increasingly growing cases of patients suffering due to oxidative stress in Côte d'Ivoire. Period of healing usually very long and expensive therefore remains inaccessible to most of the patients. This worrying situation reflects a real and serious public health problem in Côte d'Ivoire (5).

The antioxidants in the body fight against damages caused by free radicals and ROS (6, 7). Although living organisms produce antioxidants and systems to fight against free radicals, antioxidants from food and medicinal plants are supplementary antioxidants, they are very effective and promoters of the body's defense against the ROS (4, 8).

Today, all over the world and particularly in Africa, medicinal plants play an important role in therapy both in scientific circles and in rural and urban areas. (9, 10). African Floristic heritage is very rich in medicinal plants whose effectiveness is well proven. Indeed, it has been documented in the Africa continent more than 5000 medicinal plant species (11). In other for more efficient exploitation of this heritage, research works has been undertaken to provide scientific basis of actions of these plants (12, 9).

Extracts of *Centella asiatica* (*Apiacee*) are used in traditional medicine to treat diseases such as yellow fever, dysentery, diarrhea and mental illness (4). In order to contribute to the search for remedies against free radicals, we will extract the active ingredients contained in the aqueous extract of *Centella asiatica (Apiacee*) by maceration method to determine its' trace elements and antioxidants content.

Materials and Methods

Plant Material

The plant material used is some powder obtained from the aerial parts (leaves) of *Centella asiatica (Apiacee).* This plant has been identified by an expert from the National Floristic Center Côte d'Ivoire, where samples have been kept.

Maceration Method of *Centella asiatica (Apiacee*) leaves

The leaves of *C. asiatica* were harvested in Dabou (southern Côte d'Ivoire). The fresh leaves were dried in the laboratory at 18°C for one week and ground by a mechanical grinder. The dry powder (leaves) of *Centella asiatica* was extracted with water (100g /L) using a magnetic stirrer (IKAMAG RCT) for 24 hours, the extract obtained was filtered on a Buchner funnel with Wattman 3 mm paper and evaporated at reduced pressure at 30°C using a rotary evaporator.

Acidification Method of the aqueous extract of leaves of *Centella asiatica (Apiacee*)

Powder obtained from leaves of *Centella asiatica* was dissolved for 2 hours into a water / citric acid solution at a ratio of 1/100 at different concentrations of acid (0.01 N , 0.05 N , 0.08 N , 1N , 2N) . Acidification of the extract solution with citric acid, we try to imitate the traditional practice of adding lemon juice to the extract solution.

Determination of Trace Elements

The analysis of soil and plant samples method described by International Institute of Tropical Agriculture (13) was used to determine the content of trace elements contained in the aqueous extract. 0,3 g of dry sample was burnt at 600°C for 5 hours until white ashes. After cooling, 5 mL of nitric acid (1N) was added and evaporated to dryness. To the residue was added 5 mL of hydrochloric acid 1N and the mixture was put into the oven again for 30 minutes. The residue was recovered in 10 mL of hydrochloric acid and the solution was recovered in a 50 mL volumetric flask. The operation was repeated three times and the flask was completed to the mark. The elements contained in the solution are then determined by flame atomic absorption (AAS) spectrometry.

Determination of total phenols by Folin-Ciocalteu method

A 30 µL sample aliquot was added to 2,5 mL of Folin- Ciocalteu 1/10 according to the method of Singleton (14**).** The mixture was then incubated for 2 minutes in the dark. To the solution was added 2 mL of Na_2CO_3 (75 g / L) and the mixture was heated to 50 ° C for 15 minutes. After cooling in an ice-water bath, the absorbance is determined at 760 nm in a spectrophotometer. Gallic acid was used as standard.

Determination of total flavonoids in a colorimetric method at 510 nm

A solution was prepared from 0,3 mL of sodium nitrite ($NaNO_2$) of 5 % (m /v) and 0,3 mL of aluminum trichloride ($AlCl_3$) of 10% (m/v) and 1 mL aliquot of extract. After 5 minutes of incubation, the mixture was brought into contact with 2 mL of normal sodium hydroxide (NaOH). The volume was adjusted to 10 mL. The absorbance was measured at 510 nm, after vigorous stirring. The total flavonoids content was determined by isorhamnetin equivalent mass of dry weight (µg /L Isorham eq).

Statistical Analysis

Results are expressed as mean ± SEM (Standard Error of Mean). Statistical programs used were Stat View ®4.01 (Mind Vision Software Concepts, Inc., Berkeley , CA, USA) and Graph Pad Prism ® version 4.00 (GraphPad Software Inc., San Diego , CA , USA).

Result and Discussion

Determination of trace elements contained in the aqueous extract of *C. asiatica*

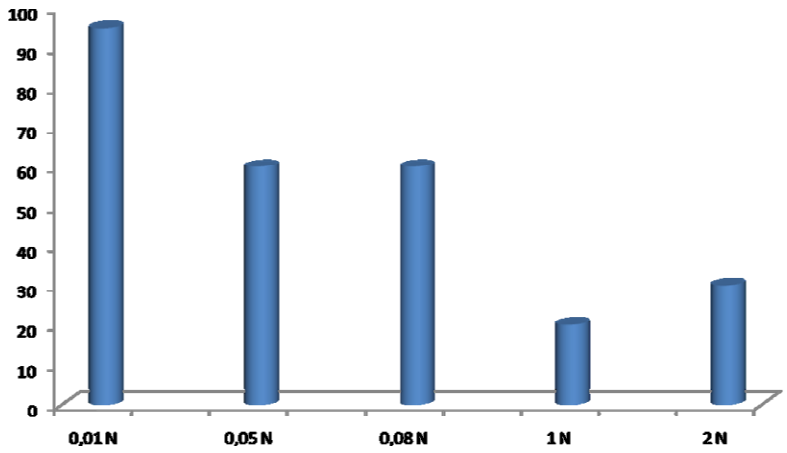
Table I shows the content of trace elements contained in the aqueous extract of *C. asiatica*. Magnesium, Sodium , Zinc and iron are present in high concentration which are respectively 11,048 ; 6,823 ; 3,251 and 1,348 mg/g. Against the trace elements such as copper, nickel and lead present in very low concentration 0,059 ; 0,026 and 0,011 mg/g respectively.

Determination of total flavonoids in the aqueous extract of *C. asiatica*

Whatever the concentration of water / citric acid, the measured value of flavonoids was high.

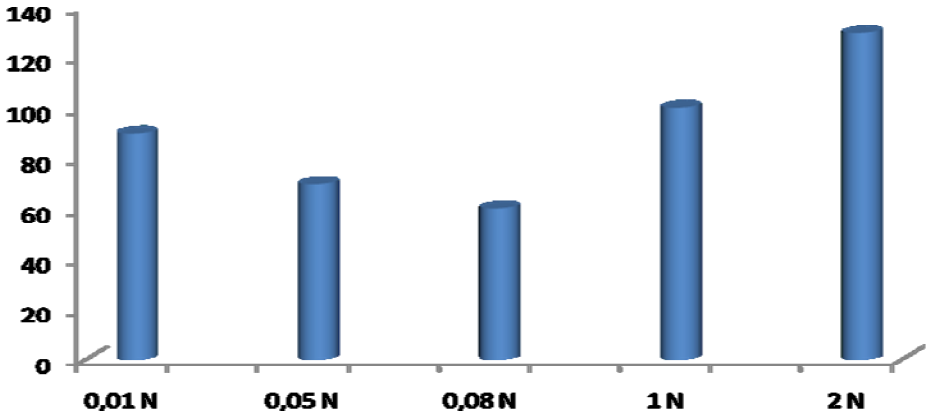
Table.I Determination of the Trace elements content

Trace elements (mg/g)	Aqueous extract of *C. asiatica*
Sodium	6,823
Ion	1,348
Copper	0,059
Magnesium	11,048
Zinc	3,251
Nickel	0,026
Cadmium	0,005
Lead	0,011



Solution water/ citric acid (N)

Fig.1 Concentration of total flavonoids in leaves of *C. asiatica* based on the concentration of citric acid solution



Solution water/ citric acid (N)

Fig.2 Concentration of total phenol in leaves of *C. asiatica* based on the concentration of Citric solution

This measured value was between 20 and 90 µg Eq Isorham / L. The highest value was measured in the water / citric acid solution of 0,001 N which gave 90 µg Eq Isorham / L. As against the concentration of citric 1N which was 20 µg Eq Isorham / L (Figure 1).

Determination of the phenolic compounds contained in the aqueous extract of *C. Asiatica*

Figure 2 shows the content of phenolic compounds in different concentrations of water /citric acid. The highest value was 130 µg Eq AG / L obtained in a concentration of 2 N. The lowest concentration of phenolic compounds was 60 µg Eq AG / L, it was obtained in the water / citric acid concentration of 0,08 N.

The aim of our study is to determine the trace elements content and antioxidants compounds in the aqueous extract of leaves of *C. asiatica (Apiacee*). This plant is used in the traditional medicine to treat diseases such as yellow fever, dysentery, diarrhea and mental illness (4). We have determined the trace elements content, minerals such as Magnesium, Sodium, Zinc and iron are the most important with values of 11,048 ; 6,823 ; 3,251 and 1,34 mg/g respectively. This study revealed the presence of antioxidant compounds which are phenolic compounds and flavonoids whose highest values obtained are 130µg Eq AG / L and 90 µg Eq Isorham / L respectively. Recently, many research teams around the world have conducted studies on the determination of antioxidant compounds and trace elements contained in plant extracts. This is motivated by the devastating effects of free radicals contained in our body. Despite the production of antioxidants in our body organisms, those of plant origin are supplements that help our bodies fight against diseases caused by oxidative stress (3). A study was conducted in 2011 on the determination of trace elements contained in six different extracts of *C. asiatica (Apiacee*). The results showed presence of trace elements. They have shown that trace elements such as Iron and Calcium are the majority with Iron content of 74 mg/100 g of dry weight. It was also determined in these extracts Potassium content of 6,165 mg/100 g of dry weight. This study showed that trace elements such as Iron, Calcium and Potassium combined with antioxidant compounds to scavenge free radicals who are responsible for several pathologies in our body organism (15). A team of researchers led by Frederico et al in 2009, conducted a study on the determination of phenolic compounds and flavonoids in the extract of *C. asiatica* and these compounds were evaluated on human cancer serum (MDA-MB 231). Results showed that this plant is rich in phenolic compounds (2.86 g/100g) and flavonoids (0,36 µg/100g). These two compounds are powerful antioxidants they scavenge free radicals contained in our body organism (16). The *in vitro* evaluation of these compounds showed the inhibitory action on human cancer serum with an IC_{50} equal to 648 ng / ml . This study revealed a positive correlation between the antioxidant compounds content and antitumor activities (16, 3). The human body produces antioxidants to fight against free radicals. The plant antioxidants are additional source to help the body fight ROS which represent a real public health problem in Côte d'Ivoire. Consumption of fruits and vegetables helps prevent diseases caused by oxidative stress (17). These fruits and vegetables contain phenolic compounds that scavenge free radicals (17, 18, 19). These compounds are powerful antioxidants (6, 17). It has been proved that

the flavonoids are also powerful antioxidants found in the plant extracts. Free radicals damage the various metabolic pathways of lipids, proteins and nucleic acids. Flavonoids activate key enzymes of mitochondrial respiration and prevent brain aging (20).

Conclusion

This study has allowed us to determine the trace elements content and antioxidant compounds in the aqueous extract of *C.asiatica*. This study showed that the antioxidant compounds of plant origin are supplementary source of antioxidants being used by the body organism to scavenge free radicals that are responsible for many diseases in our body. The aqueous extract of *C. asiatica* can be used to fight against the effects of oxidative stress.

References

1) Yan MH, Wang X, Zhu X, (2013). Mitochondrial defects and oxidative stress in Alzheimer disease and Parkinson disease. Free Radical Biology and Medicine. 62, 90–101.
2) Panieri E, Gogvadze V, Norberg E, Venkatesh R, Orrenius S, Zhivotovsky B, (2013). Reactive oxygen species generated in different compartments induce cell death, survival, or senescence. Free Radical Biology and Medicine. 57, 176–187.
3) Madamanchi NR, Runge MS, (2013). Redox signaling in cardiovascular health and disease. Free Radic Biol Med. 61C, 473-501.
4) Mijanur R, Shahdat H, Asiqur R, Nusrat F, Taslima N, Borhan U and Mafroz AB, (2013). Antioxidant Activity of *Centella asiatica* (Linn.) Urban: Impact of Extraction Solvent Polarity. Journal of Pharmacognosy and Phytochemistry. 1(6): 27-32.
5) AB Effi, KJ N'Dah, AA N'Guiessan, B Doukouré, M Kouyaté, AD Abouna, BS Koui, KE Koffi, E Troh, AB D'Horpock, MIJM Diomandé, M Hondé, (2012). Épidémiologie et histopathologie des cancers en Côte-d'Ivoire. Journal Africain du Cancer. 4 (1): 41-47.
6) Jin D and Mumper RJ, (2010). Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. Molecules. 15,7313-7352.
7) Feng-Lin S, Ren-You O, Yuan Z, Qin X, Lei K and Hua-Bin L, (2010). Total phenolic contents and antioxidant capacities of selected Chinese medicinal plants. Internatonal Journal of Molecular Sciences. 11, 2362 – 2372.
8) Olivier R, (2011). Traitement antioxydant associé à la trithérapie = Antioxidative treatment associated with tritherapy. La phytothérapie européenne. 61, 23-26.
9) Trébissou JND, Bla KB, Yapo AF, Yapi HF and Djaman AJ, (2014). Therapeutic survey on traditional treatment of Buruli ulcer in Côte d'Ivoire. J. Microbiol. Biotech. Res.4(2): 52-56.
10) Yapi GY, KRA AKM, Ackah JAAB, et Djaman AJ, (2011). Evaluation de l'activité antifongique et essai de purification des principes actifs des extraits de *terminalia mantaly* (h.perrier), une combretacée, sur la croissance *In vitro* de *Candida albicans*. Bulletin de la Société Royale des Sciences de Liège. 80, 953 – 964.
11) Adjanohou, JE, (1979). Contribution au recensement des plantes médicinales de Côte-d'Ivoire. CRES, Université CI, Centre national de floristique. 358 p.

12) Trebissou JND and Djaman AJ, (2013). Epidemiological study on the mode of transmission of Buruli ulcer in Côte d'Ivoire. World Journal of Pharmaceutcal Research (WJPR). 3(2): 1579-1584.
13) International Institute of tropical Agriculture (IITA), (2014). Manuel N°1, PMB5320. Ibadan. Nigeria. 3p.
14) Singleton VL and Rossi J, (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enology and Viticulture.16, 144 - 158.
15) Chandrika UG, Salim N, Wijepala GDDJ, Perera KSU, Goonetilleke AKE, (2011). Carotenoid and mineral content of different morphotypes of *Centella asiatica* L. (Gotukola).International Journal of Food Sciences and Nutrition. 62(5): 552-557.
16) F Pittella, RC Dutra, DD Junior, MTP Lopes and NR Barbosa, (2009). Antioxidant and Cytotoxic Activities of *Centella asiatica* (L) Urb. Int J Mol Sci. 10(9): 3713-3721.
17) MA Mohd Shukri, C Alan and AR Site Noorzuraini, (2011). Polyphenols and antioxidant activities of selected traditional vegetables. Journal of Tropical Agriculture and Food Science. 39, 69-83.
18) Song FL, Gan RY, Zhang Y, Xiao Q, Kuang L, Li HB, (2010). Total phenolic contents and antioxidant capacities of selected chinese medicinal plants. Int J Mol Sci. 11(6): 2362-72.
19) Reihani SFS and Azhar ME, (2012). Antioxidant activity and total phenolic content in aqueous extracts of selected traditional Malay salads (Ulam). International Food Research Journal. 19(4): 1439-1444.
20) Milan SS, Neda N, Vladimir M, Marina T, Slavica S, (2011). Antioxidant activity, total phenolic content and flavonoid concentrations of different plant parts of *Teucrium polium* L. subsp. Polium. Acta Soc Bot Pol. 81(2):117-122.