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Phytochemical Screening and Antioxidant Activity of "KROATISANE", a Traditional Recipe Used in the Management of COVID-19 Symptoms

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Abstract: In Côte d'Ivoire, more than 340,000 infected people and more than 15,000 deaths have been recorded during the COVID-19 pandemic. In order to make a significant contribution to this problem, practitioners of traditional medicine (PMT) have used in Côte d'Ivoire a medicinal formulation composed of leaves of *Alchornea cordifolia* (euphorbiaceae), the bark of *Alstonia boonei* (apocynaceae), leafy stems of *Ocimum gratissimum* (lamiaceae), and rhizomes of *Zingiber officinale* (zingiberaceae) named "Kroatisane" used for the management of symptoms of COVID-19. The present study aims to determine the phytochemical composition and evaluate the antioxidant activity of the aqueous extract of "Kroatisane". To do this, an aqueous extract of the four medicinal plants was prepared. Classes of secondary metabolites were identified according to the methods described by Evans (2009) and several other authors. Antioxidant activity was determined by that of free radical scavenging using DPPH. The aqueous extract of "Kroatisane" (KTI) showed DPPH scavenging activity dependent on its effective concentration (EC50) of 100.3 µg/mL. This antioxidant activity has an inhibitory concentration (IC50) of 100.3µg/mL compared to that of ascorbic acid of 3.025 µg/mL and the IC50 of gallic acid of 0.8968 µg/mL. The activity of the aqueous extract of "Kroatisane" is 33 and 104 times less powerful than those of ascorbic acid and gallic acid respectively. Furthermore, the phytochemical screening of "Kroatisane" revealed the presence of several secondary metabolites in particular, alkaloids, tannins, flavonoids, terpenoids, saponosides and reducing compounds. These results could partly justify the traditional use of this recipe in traditional medicine for the management of symptoms of COVID-19.

Keywords: Kroatisane, Traditional Recipe, Phytochemical Screening, Antioxidant Activity, Phytocompound

Introduction

Although conventional medicine has made significant advances in recent decades, 80 to 90% of the world's population still uses traditional medicine today (Jiofack *et al.,* 2010). Indeed, according to the World Health Organization (WHO), approximately 65 to 80% of the population of developing countries depends on plants for their primary health care due to poverty and inaccessibility to modern medicine. (Awoyemi *et al.,* 2012). Traditional medicine is now experiencing spectacular development in most countries around the world (Kroa *et al.,* 2014). In rural areas, it constitutes the most preferred means of solving health problems (WHO, 2002; WHO, 2003).

Considering the place of traditional medicine in human health and to provide concrete answers based on scientific data, medicinal plants have been the subject of several research projects in recent years with a view to determining their therapeutic effectiveness. on so-called "emerging and new" diseases (Abdollahi *et al.,* 2003). These diseases include COVID-19 (Raoult *et al.,* 2020). It is a SARS-CoV infection from the coronavirus family that causes mild or severe respiratory tract infections in humans (Lone and Ahmad, 2020). This virus is very contagious and spreads quickly from person to person, but mainly through physical contact (Mbunge, 2020).

The coronavirus pandemic with its corollary of high morbidity and mortality among immunocompromised individuals has posed great challenges to the health systems of countries around the world. Thus, country governments have implemented a wide range of prevention and containment measures to break chains of transmission with a view to containing the spread of the disease and managing cases of infections (WHO, 2020). These measures range from social distancing, isolation and quarantine of infected people, closing borders and restricting international travel (WHO, 2020). With this in mind, the Ivorian government has implemented several prevention, screening and patient care actions based on WHO recommendations (Communiqué of the Council of Ministers of the Ivorian government of 23-12- 2020). In addition to disease prevention and treatment actions, vaccines have been developed to slow the spread of disease. However, these vaccines have raised questions among most populations in Africa in general and in Ivory Coast in particular. Faced with this situation and in view of the increasing number of cases of contamination and deaths around the world (WHO, 2021), medicinal plants and medicinal plant-based medicines whose safety has already been demonstrated have been used to treat symptoms of COVID-19 (Tachema *et al.,* 2020).

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This is how, in Ivory Coast, practitioners of traditional medicine, through their ancestral knowledge, offered the community an opportunity to create a recipe composed of a mixture of medicinal plants for the management of symptoms of the disease.

However, the use of medicinal plants still raises many questions about the availability, safety, effectiveness and quality of these so-called traditional recipes. It is with regard to all these questions that this study was undertaken, the general objective of which was to contribute to ensuring and guaranteeing the quality of this recipe based on medicinal plants called "Kroatisane" by the determination of the phytochemical composition and its antioxidant power for its use in the management of symptoms of COVID-19.

Materials And Methods

Framework and duration of the study

This study was carried out over a period of 7 months, from March 2022 to October 2022, at the Traditional Medicine Research Institute in Ghana, Department of Science and Technology, Kwame Nkrumah University, Kumasi with the collaboration of the Program National Promotion of Traditional Medicine (PNPMT) of Côte d'Ivoire and practitioners of traditional medicine.

Plant material

The plant material, a phytomedicine called "Kroatisane" was developed based on four medicinal plants. The plants and parts of plants used are composed of:
-*A. cordifolia* leaves (euphorbiaceae);
-the bark of *A. boonei* (apocynaceae);
-leafy stems of *O. gratissimum* (lamiaceae);
-rhizomes of *Z. officinale* (zingiberaceae).
These plants were collected at Takra Adiekro in the Botro department, 381 km from Abidjan. Samples of each plant were sent to the National Floristic Center (CNF) of Félix Houphouët-Boigny University to be identified with the CNF specimen: *A. cordifolia* UCJ 018635, *A. boonei* UCJ 001553, *O. gratissimum* UCJ008882, *Z. officinale* UCJ 019001.

Methodology

Methods for preparing TISANE

Each plant material (harvested part of the plant) was washed 3 times with clean water. It is then dried in the open air at room temperature in a dry place protected from direct sunlight for 10 days. The dry plant material obtained was crushed separately using an electric grinder until a fine powder was obtained and sifted using a 0.2 mm mesh sieve. The powder obtained from the four plants was mixed homogeneously according to the proportions presented in Table 1 and stored at room temperature in glass bottles protected from light.

Table 1: Formulation of "Kroatisane"

Plants used	Quantity per 1000g of powder	Dry matter rate (%)
A. cordifolia	300	30
A. boonei	250	25
O. gratissimum	250	25
Z. officinale	200	20

Phytochemical analysis

Extraction by decoction

The extraction was carried out according to the method described by Dellaoui (2021). One hundred (100) grams of "Kroatisane" powder were introduced into a glass flask containing two liters of distilled water heated to 100°C. The mixture was then cooled for four hours to 32 C°. The mixture was immediately filtered three times successively using Wattman No. 3 paper. The filtrate obtained was dried in a Rotavapor then freeze-dried to obtain the aqueous extract.

The yield (Yield) of the extract was determined according to the method described by (Bssaibis *et al.* (2009).

$$\text{Yield (\%)} = \frac{\text{final mass}}{\text{initial mass}} \text{X } 100$$

Yield: extract yield
final m: quantity of the final extract in grams (g).
initial m : quantity of dry matter used in grams (g).

Phytochemical screening of "KROATISANE"

Classes of secondary metabolites were identified according to the methods described by several authors. Alkaloids and tannins were studied according to the method of Dohou *et al.* (2003), flavonoids according to Bruneton, (1993), saponins according to Yves et al. (2007), terpenoids according to Koffi *et al.*(2009) and reducing sugars according to Amonkan *et al.* (2010).

Test for alkaloids

An amount of 0.5 g of powdered materials was extracted with ammoniacal alcohol, filtered and evaporated to dryness. The residue was extracted with 1% sulfuric acid (H2SO4), filtered and the filtrate was made typically alkaline with dilute ammonia (NH3) solution. It was then shaken with chloroform and the chloroform layer evaporated. After that, the residue was dissolved in 1% sulfuric acid (H2SO4) and used for other tests including that of Dragendroff. The color of the precipitate generated after adding a drop of Dragendroff's reagent to the 1% sulfuric acid (H_2SO_4) extract was observed. An orange precipitate after 15 minutes indicates a positive reaction including the presence of alkaloids.

Test for saponins

50 mg of powder was added to a tube containing 25 mL of distilled water. The resulting solution was shaken vigorously and left to stand for more than 5 minutes (persistent foam indicates positive results). The moss index was determined on the filtrate of the 1% decoction of plant organ powder. Dilutions of the filtrate were made in tubes which were then shaken 30 times for 15 seconds. After 15 minutes, the height of the foam formed was measured, the value of which was used to calculate the foam index.The formula for calculating the foam index I is as follows: I= h x 5/t x10-2, h being the height of the foam (in cm) in the tube t. The presence of saponins is confirmed by a foam index greater than 100

Test for tannins

A. The color of the precipitate generated is obtained after adding ten (10) drops of a 1% lead acetate solution to one (01) mL of the aqueous extract of “Kroatisane”.

B. To one (01) mL of the aqueous extract of “Kroatisane”, ten (10) mL of distilled water, 2 to 10 drops of a 1% ferric chloride solution were added. The color of the precipitate formed was noted. The blue-black precipitate was observed in drops for hydrolyzable tannins and the dark green precipitate suggested the existence of condensed tannins.

Test for flavonoids

The aqueous extracts of “Kroatisane” were applied to strips of Whatman No. 3 filter paper and allowed to air dry at room temperature. The paper strips were subjected to strong ammonia (NH_3) vapors in the fume hood. An intense yellow color was noted. The yellow filter paper strips were exposed to intense hydrochloric acid (HCl) vapors as a confirmatory test. The disappearance of the intense color validates the presence of flavonoids in the sample.

Test for triterpenoids

A quantity of 5 g of “Kroatisane” powder was weighed and extracted with chloroform. Concentrated sulfuric acid was added to 5 mL of the chloroform extract in the test tube to form a layer. The reddish brown or cherry red color at the interface indicates the presence of a triterpenoid core.

Test for reducing sugars (general glycoside test)

A sample of 0.2 g of “Kroatisane” powder is heated for 2 minutes in a water bath in 5 mL of diluted sulfuric acid (H_2SO_4), then filtered. After adding four drops of 20% NaOH solution, the filtrate became noticeably alkaline (confirmed with litmus paper). The filtrate was mixed with 1 mL of Fehling's solutions A and B and warmed in a water bath for approximately 2 min, and the color of the precipitate was observed (brick red precipitate indicates positive results).

Evaluation of the antioxidant activity of kroatisane

The aqueous extract of ‘Kroatisane’ was analyzed for its ability to scavenge free radicals using DPPH radical scavenging and total antioxidant capacity tests.

The DPPH radical scavenging activity of Kroatisane aqueous extract (KTI) and the reference antioxidant compounds ascorbic acid and gallic acid were evaluated using the standard protocol described by Govindappa *et al.* (2011).

DPPH in methanol was used as a negative control. The percentage of free radical scavenging activity was calculated according to the following equation:

$$\%\,DPPH\ inhibition = \left\| 1 - \left[\frac{A_{agent}}{A_{control}}\right] x\ 100 \right\|$$

A_{agent} indicates the absorbance of the extract,
$A_{control}$ indicates the absorbance of the control.

Assay of the total antioxidant capacity (TAC) of phosphomolybdenum

The total antioxidant capacity of the aqueous extract of “Kroatisane” (KTI) was determined according to the methods described by Prieto *et al.* (1999). Thus, 1 mL of aqueous extract of "Kroatisane" at 125 µg/mL was added to 3 mL of a reagent of ammonium molybdate (4 mM), di sodium hydrogen phosphate (28 mM) and 6 mL of sulfuric acid (mM) and incubated at 95°C for 90 minutes. 200 µL of the mixture cooled to 32 C° was transferred to a microtiter plate and the absorbance was measured at 695 nm. A standard calibration curve was generated from reference compounds such as ascorbic acid (100 – 0.78 µg/mL) then with gallic acid (25 – 0.78 µg/mL) (figure 2). The experiments were repeated 3 times and expressed as ascorbic/gallic acid equivalent (AAE and GAE respectively) in mg per g and the aqueous extract of Kroatisane.

Statistical analysis

The statistical analysis was carried out using XLSTATPRO 7.1 software. Results were analyzed using Dunnett's post hoc tests combined with one-way ANOVA. The different statistical tests are considered significant when $p < 0.05$ and very significant when $p < 0.01$.

Results

Phytochemical screening

The results of characterization of the chemical groups present in the aqueous extract of Kroatisane highlighted the presence of alkaloids, tannins, flavonoids, terpenoids, reducing compounds and saponosides. The identification of the different classes of essential bioactive compounds of this Kroatisane extract showed more presence of bioactive compounds (+) (Table II).

Table II: Phytochemical compounds in Kroatisane aqueous extract

Phytocompounds	Characterization reaction	Inference
Alkaloids	Potassium iodomercurate, Dragendorff (potassium iodobismuthate)	+
Tannins	Ferric chloride	+
Flavonoids	Cyanidine, Shinoda	+
Terpenoids	Libermann-Burchard Kedde specific to cardenolides	+
Saponosides	Moss height	+
Reducing compounds	Fehling liqueur	+

+: presence

Antioxidant activity of the aqueous extract of “Kroatisane”

At different concentrations of the antioxidant tested (ascorbic acid, gallic acid, aqueous extract of "Kroatisane"), the kinetics of reduction of DPPH° is followed over time until an equilibrium is obtained translated by the presence of a bearing. The results are shown in Figure 1.

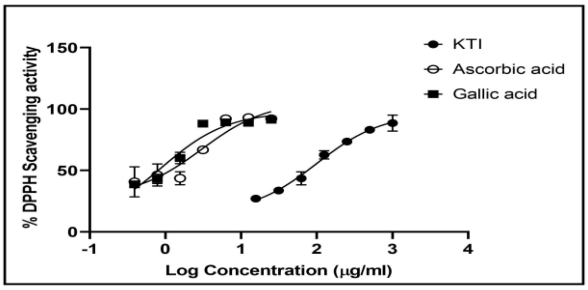

Figure 1: DPPH scavenging effect of the aqueous extract of “Kroatisane” compared to ascorbic and gallic acid as reference antioxidant compounds.

Indeed, the antioxidant activity of “Kroatisane” showed an effective concentration (EC 50) of 100.3 µg/mL. The kinetics of DPPH reduction for the two compounds tested including ascorbic and gallic acid showed that gallic acid was the most active compound with an EC50 = 0.8968 µg/mL. The EC50 of ascorbic acid was EC50 = 3.025 µg/mL). In short, the activity of the “Kroatisane” extract was 33 and 104 times less powerful than ascorbic acid and gallic acid respectively. The total antioxidant capacity of the extract was 65,765±5,757 mg/g, compared to that of ascorbic acid 54,472±6,522 mg/g, and gallic acid 54,472±6,522 mg/g, obtained under the same conditions.

The total antioxidant capacity of ascorbic and gallic acid is presented in Figure 2.

The trapping of the free radical DPPH was accompanied by the transition from violet color to yellow color. The aqueous extract of "Kroatisane" (1000 µL each) at a concentration range of 15.625 to 1000 µg/mL, ascorbic acid and gallic acid (0.3906 to 25 µg/mL), prepared by double dilution), were mixed with 3000 µL of DPPH (0.004% DPPH in methanol).

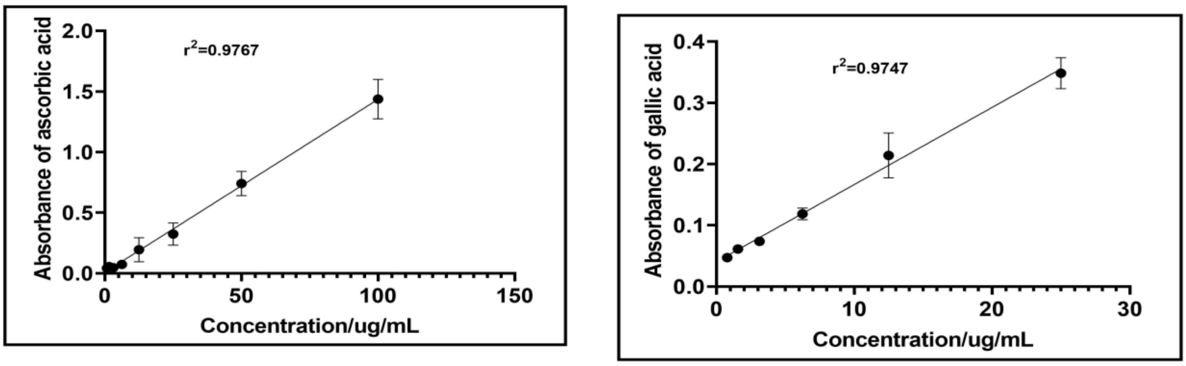

Figure 2: Calibration curve for ascorbic acid and gallic acid

Discussion

This study revealed the presence of several bioactive compounds including alkaloids, tannins, flavonoids, terpenoids, saponosides and reducing compounds in the aqueous extract of "Kroatisane". These results are particularly considerable due to the physiological effects of these compounds in pharmacology.

According to Bruneton, 1999, alkaloids like cycloserine, mytomycin have antibacterial properties. These different properties give alkaloidal extracts a place of choice in both traditional and modern pharmacopoeia. In addition, alkaloids are involved in important biological activities, notably antiproliferative and analgesic (Aimé, 2010).

As for flavonoids, they have antioxidant and anti-inflammatory activities and play a positive role in the treatment of cardiovascular and neurodegenerative diseases (Wang and Mazza, 2006). In some cases, they are known for their antiviral, antimicrobial and antitumor activity (Narayana *et al.,* 2001; Seyoum *et al.,* 2006). Furthermore, the best described property of flavonoids is their antioxidant power and their ability to trap free radicals. Several studies have shown the role of flavonoids in the deactivation of free radicals. Flavonoids are also known for their antiviral activity, mainly against the HIV retrovirus, the Influenza virus, the Herpes virus, the Adenovirus and the influenza A virus (Spedding *et al.,* 1989, Choi *et al.,* 2009). Also antibacterial and antiviral properties of flavonoids on different bacterial strains have also been demonstrated by Ghedira, (2005).

The tannins present in the aqueous extract of "Kroatisane" are considered good remedies in the treatment of respiratory diseases and coughs (Bouchet *et al.,* 2000). Internally, tannins exert a certain anti-diarrheal activity. Its antiseptic, antibacterial and antifungal properties are clearly demonstrated in the treatment of infectious diarrhea and dermatitis (Aron *et al.,* 2008). Tannins therefore have strong antioxidant activity. They are very good scavengers of free radicals and inhibit the formation of superoxide radicals.

The presence of terpenoid derivatives in the aqueous extract of "Kroatisane" could explain the beneficial effects of these terpene extracts against the resistant form of malaria (Benoit, 1996).

The saponosides present in the extract of "Kroatisane" constitute a large group of glycosides very common in plants. They are characterized by their surfactant properties. They dissolve in water forming foaming solutions. These surfactant properties are very marked in "Kroatisane" extracts. The foaming character is very important in the aqueous extract with the foam index which reflects the presence of saponosides more visible. Often devoid of antibacterial activities, saponosides are sometimes inactive in vitro on viruses (for example: glycyrrhizin) (Bruneton, 2009).

The presence of flavonoids in the aqueous extract of "Kroatisane" demonstrates the strong antioxidant power of this extract. Flavonoids are known as potentially antioxidant substances (Guignard, 1994).

According to Turkmen *et al.* (2005), the antioxidant activity of an extract could be linked to its richness in flavonoids which seem to be effective hydrogen donors for the DPPH radical, due to their ideal chemical structure. Additionally, these studies reported a positive correlation between antioxidant compounds and antioxidant activities in plant parts (flowers, fruits, leaves, seeds).

The study of the variation of the antiradical activity as a function of the concentration of the extract from the linear regression equation of graph makes it possible to determine the concentration which corresponds to 50% of the inhibition concentration (CI_{50}). It is a parameter used to estimate antioxidant activity. The smaller the value of the inhibition concentration, the greater the antioxidant activity of a compound (Khoudali *et al.,* 2014). The aqueous extract of "Kroatisane" (KTI) has an inhibitory concentration (CI_{50}) of 100.3 µg/mL, a value much higher than that of ascorbic acid of 3.025 µg/mL and gallic acid (CI_{50}) = 0.8968 µg/mL.

Conclusion

Screening of the aqueous extract of "Kroatisane" indicated the presence of alkaloids, tannins, flavonoids, terpenoids, reducing compounds and saponosides. However, the level of total polyphenols has not been determined. Also, the presence of bioactive phytocompounds demonstrate that the aqueous extract of "Kroatisane" has considerable pharmacological properties, which justifies its use in traditional medicine.

The analysis of the phytochemical compounds of this "Kroatisane" extract showed that it has a low antioxidant power compared to ascorbic acid and gallic acid.

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