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RESEARCH ARTICLE

IN VITRO ANTIOXIDANT ACTIVITY, PHYSICOCHEMICAL AND MICROGRAPHIC STUDIES OF THE LEAFY STEM OF IPOMOEA PES-CAPRAE (L) R. BR, A HALOPHYTE FROM IVORY COAST

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Abstract

Objective: To evaluate the *in vitro* antioxidant activity and determine the botanical and physicochemical characteristics of aqueous extracts of aerial parts of *Ipomoea pes-caprae*.

Methods: Aqueous extracts obtained by decoction of the leaves of *Ipomoea pes-caprae* were used to evaluate the antioxidant activity in vitro based on the capacity of the extracts to scavenge free radicals using the DPPH radical and the antioxidant reducing power test. ferric (FRAP). The botanical study of the plant was carried out following microscopic observation of the pulverized drug. As for the Physical studies, they focused on the residual humidity level after drying of the sample and plant at 103 ± 2°C, the water content after drying of the fresh drug for 5 weeks and the extraction yield. Phytochemical tests as well as the determination of polyphenols and total flavonoids were carried out on the leaves of *Ipomoea pes-caprae* according to standard methods.

Results: The extracts showed increasing antioxidant activity with concentration with an IC 50 = 46.23 ± 0.89 µg/ml and an oxidizing power estimated at 102.5 ± 12.3 µmol Eq Trolox/g EXS. Phytochemical screening revealed the presence of sterols, polyterpenes, polyphenols, alkaloids and saponosides. Polyphenols were estimated at 79.91±6.20 mg Eq AG/g dry extract and flavonoids were present at 249.2±17.25 mg Eq Q/g dry extract. The micrograph revealed the presence of elements entering into the anatomical structure of the plant studied.

Conclusion: This study showed that the aqueous extract of *Ipomoea pes-caprae* has dose-dependent antioxidant activity properties.

Introduction:-

Plants have always been used for their medicinal properties, even before the arrival of science. Even today, plants remain an alternative for the treatment of several conditions such as bacterial infections, malaria, and pain[1]. The WHO estimates that approximately 80% of the world's population uses herbal medicine to address several aspects of their primary health care[2]. However, only a fraction of the plant species presents on earth have ever been subjected to chemical and pharmacological studies with the aim of exploiting their medicinal properties.

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Ipomoea pes-caprae (IPC), is a plant belonging to the Convolvulaceae family and extremely widespread on all beaches and regions in tropical and subtropical areas. Tropical America is the most species-rich region, followed by tropical Africa, where around 150 species can be found. In Ivory Coast it grows along the coastline. The plant is used in various essences in popular and tribal medicines, for example in Ivory Coast for its anti-rheumatic and analgesic properties[3]. *Ipomoea pes-caprae*exhibits various biological activities such as anticancer, antioxidant, antidiabetic, antispasmodic, anti-inflammatory[4]. Pharmacological studies on antioxidant and phytochemical activity have already been carried out on this plant in certain countries[4], but none of them has been carried out in Ivory Coast. In the present work, the aim was to evaluate the antioxidant activity in vitro and to define some pharmacognostic and physicochemical parameters that could be used for quality control of *Ipomoea pes-caprae*.

Material and Methods:-

Plant material

The plant material consisted of leaves and stems of *I. pes-caprae*collected from the seaside of the town of Jacqueville, a town located 60 km from Abidjan (Ivory Coast). A specimen of the leafy stem was identified at the National Floristic Center (CNF) under the number UCJ004223.

Figure 1:- *Ipomoea pes-caprae*at the harvest site (Jacqueville, Ivory Coast) (Personal photo, TIA Eric, 2020).

Chemical and technical equipment

The chemical material consisted of, among others, potassium hydroxide, L-ascorbic acid, quercetin, gallic acid, DPPH, Folin-Ciocalteu reagent, Calcium Carbonate, methanol, FRAP reagent, Trolox, potassium hydroxide, Test tubes, micropipettes of 0-500 µL, 500-5000 µL, aUV-visible spectrophotometer (JENWAY 7315 spectrophotometer®), a water bath, an oven and a precision balance were used during this study.

Methodology:-

Preparation of the aqueous extract of *Ipomoea pes-caprae*

The freshly harvested plant was washed, dried at a temperature of 22°C for 5 weeks then roughly pulverized. A decoction was obtained from the powder of the dry plant material using distilled water as a solvent. After filtration, the decoction was put in an oven (45°C) for 48 hours. The dry extracts obtained were stored in the refrigerator at a temperature between 5°C.

Micrographic study

It consisted of observing the sprayed drug under a microscope. A small quantity of each powder is taken with a spatula and poured into a watch glass then triturated with a few drops of the reagent (KOH solution). A small quantity of the mixture is placed between slide and coverslip. The whole is observed under an optical microscope at magnifications 4, 10, 40. The different elements encountered were noted and photographed[5].

Physical parameters

Loss on drying (Residual humidity rate RHR)

The THR was calculated by the gravimetric method based on the drying of the sample in the oven at 103 ± 2°C. A test portion of 2g of powder is placed in a watch glass. The whole is placed under study at 100°C. After 24 hours the powder is cooled and weighed. The weight loss in the watch glass compared to the test portion gives its water content, expressed in%. Its estimation makes it possible to assess the state of conservation of the plant material. A content greater than 10% indicates that the sample cannot be stored without risk of deterioration because it promotes the oxidation of chemical compounds and the development of mold[6].

After drying, the water content (RHR) of the two plants was determined according to the following formula:

$$RHR = \frac{P_0 - P}{P}$$

P: weight of the vegetable powder after drying

P_0: initial weight of the test portion

Extraction yield

The extraction yield (R) was calculated with the following formula[7]:

$$R = \frac{\text{Mass of dry extract}}{\text{Mass of dry plant matter}}$$

Water content

This is the quantity of water evaporated from the plant after drying, it is determined according to the following formula[8]:

$$\text{Water content} = \frac{m_0 - m}{m}$$

m_0: initial weight of the fresh plant

m: weight of the plant after drying

Phytochemical screening

During this study, a phytochemical screening of the dry extracts from the decoction was carried out for the search for sterols, polyterpenes, polyphenols, flavonoids, tannins, alkaloids, quinines, saponosides. The various analyzes were carried out following the processes described in the literature [9]:

Sterols and polyterpenes

Sterols and polyterpenes were sought by the Liebermann reaction. 1 mL of the extract was evaporated to dryness. The residue is dissolved hot in 1 mL of acetic anhydride; 0.5 mL of concentrated sulfuric acid was added to the triturate. The appearance, at interphase, of a purple or purple ring, turning blue then green, indicates a positive reaction.

Polyphenols

The reaction with ferric chloride (FeCl3) made it possible to characterize the polyphenols. To 1 mL of the extract, a drop of 2% alcoholic ferric chloride solution was added. The appearance of a more or less dark blue-blackish or green color indicates the presence of polyphenols.

Flavonoids

Flavonoids were investigated by the cyanidin reaction. 1 mL of the extract was evaporated to dryness and the residue was taken up in 2.5 mL of hydrochloric alcohol diluted twice. By adding 2 to 3 magnesium shavings, there is

a release of heat then an orange-pink or purplish color. The addition of 3 drops of isoamyl alcohol intensified this coloring which confirms the presence of flavonoids.

Tannins

The search for catechic tannins was carried out using Stiasny's reagent. 1 mL of the extract was evaporated to dryness. After adding 3 mL of Stiasny's reagent to the residue, the mixture was maintained in a water bath at 80°C for 30 min. The observation of a precipitate in large flakes characterizes the presence of catechic tannins. For gallic tannins, we filtered the previous solution. The filtrate is collected and saturated with sodium acetate. The addition of 3 drops of FeCl3 causes the appearance of an intense blue-black color, a sign of the presence of gallic tannins.

Quinones

Quinones were investigated using Bornstraëgen's reagent. 1 mL of the extract was evaporated to dryness. The residue is taken up in 2.5 mL of 20% hydrochloric acid then brought to a water bath for 30 min.

After cooling, it is extracted with 10 mL of chloroform. Ammonia diluted 2 times (0.5 mL) was added to the chloroform solution. A red or purple color is a sign of the presence of quinones.

Alkaloids

The alkaloids were characterized using Dragendorff and Bouchardat reagents. Six (6) ml of the extract were evaporated to dryness. The residue is taken up in 6 mL of 60% alcohol. The addition of 2 drops of Dragendorff's reagent to the alcoholic solution causes a precipitate or an orange color.

Adding 2 drops of Bouchardat's reagent to the alcohol solution causes a reddish-brown precipitate and indicates a positive reaction.

Saponosides

10 mL of the extract is introduced into a test tube. After stirring for 15 seconds, the tube is allowed to settle. A persistent foam height greater than 1 cm indicates the presence of saponosides

Quantitative assays

Determination of total polyphenols

The method of Wood et al. was used for the determination of total polyphenols[10]. A volume of 2.5 ml of diluted Folin-Ciocalteu reagents (1/10) was added to 0.5 ml of extract. The mixture was kept for 2 minutes in the dark at room temperature, then 2 ml of calcium carbonate solution (75g/L) was added. Then, the mixture was placed for 15 minutes in a water bath at 50°C, then quickly. The absorbance was measured at 760nm with distilled water as a blank. A calibration line was produced with gallic acid at different concentrations. The analyzes were carried out in triplicate and the polyphenol concentration was expressed in milligram per gallic acid equivalent per gram of dry extract (mg Eq AG/g of dry extract).

Absorbance was measured to determine total polyphenol contents using the Equation:

$C = (C_1 \times V) / m$

C = Total polyphenol content expressed in mg gallic acid equivalent/g dry matter

C_1 = Concentration of gallic acid established from the calibration curve in mg/L

V = Volume of extract in L

m = Weight of the plant extract in g

Assay of total flavonoids

The method of Marinova et al. was used for the determination of total flavonoids[11]. In a 25 ml flask, 0.75 ml of 5% (m/v) sodium nitrite ($NaNO_2$) was added to 2.5 ml of extract. The mixture was added with 0.75 ml of aluminum chloride ($AlCl_3$) at 10% (m/v), then incubated for 6 minutes in the dark. After the incubation, 5 ml of sodium hydroxide (1N NaOH) were added and then the volume was made up to 25 ml. The mixture was shaken vigorously before being measured with a UV-visible spectrophotometer. The reading was taken at 510 nm. The tests were carried out in triplicate. The flavonoid content was expressed in milligram quercetin equivalent per gram of dry extract (mg Eq Q/g dry extract).

***In vitro* antioxidant assays**

Ability to scavenge free radicals using the DPPH radical. Antioxidant activity was determined using the 1,1-Diphenyl-2-picrylhydrazyl (DPPH) assay according to Brand Williams et al.[12]. The same proportions (1ml) of extract and DDPH reagent (100 µM in methanol) were mixed and kept in the dark for 30 min before reading the absorbance at 517 nm. Vitamin C was used as a reference. Radical scavenging activity was calculated as percent inhibition (I%) of the DPPH radical using the following equation:

I % = [(A0 – A1) /A1] × 100

I % = Percentage of inhibition in %

A0 = Absorbance of the sample,

A1 = Absorbance of the control witness.

Radical scavenging activity is presented as an IC_{50} value (defined as the concentration of the extract in µ/ml that causes 50% inhibition).

Ferric reducing antioxidant power test (FRAP):

The activity of ferric reducing antioxidant power was carried out using the modified method of Benzie & Strain[13]. This involves determining the ability of the extract to reduce iron (Fe3+ to Fe2+). In the presence of TPTZ, the Fe+2-TPTZ complex exhibits a blue color which can be measured. 3500 µl of the FRAP reagent was added to 140 µl of the test compounds dissolved in a methanolic solution. After incubation for 30 minutes at room temperature, absorbance was recorded at 593 nm relative to Trolox and values were expressed as micromol Trolox Equivalent per gram dry weight (µMol EqTrolox/g dry extract).

Physical parameters of *I. pes-caprae*

Table 1:- Physical parameters

Settings	Values
Residual humidity rate (RHR)	10.5%
Extraction yield (R)	7.48%
Water content (duration = 5 weeks)	13.18%

Phytochemical screening

Table 2:- Results of phytochemical screening of leafy stems of *Ipomoea pes-caprae*

Phytochemicals		*Ipomoea pes-caprea*
Sterols and Polyterpenes		+
Polyphenols		+
Flavonoids		+
Tannins	Catechical	+
	Gallic	-
Quinones		-
Alkaloids	Bourchardat	+
	Dragendorff	+
Saponosides		+

(-) : absence ; (+): presence; (++): scanty; (+++): very abundant.

Quantitative assays

Dosage of total polyphenols

The quantity of total polyphenols in the leafy stem of *Ipomoea pes-caprae*was evaluated at 79.91± 6.20 mg Eq AG/ g dry extract from the gallic acid calibration curve (Figure 3).

Results:

Micrographic study

a. Epidermis fragments with anisocytic stomata

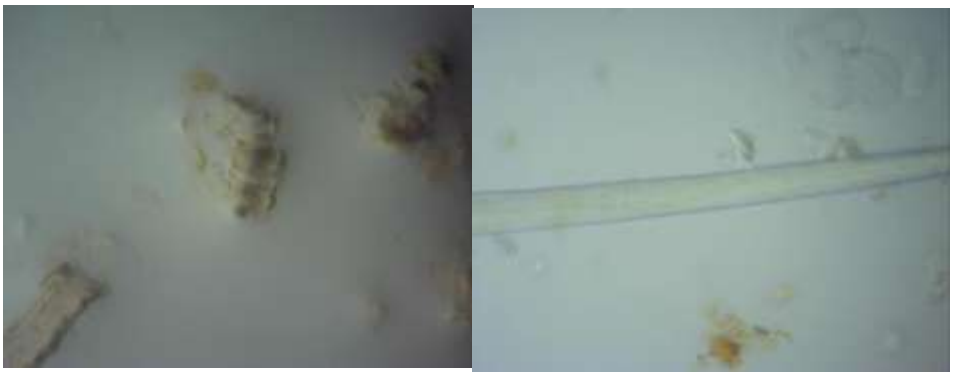
b.Epidermis of the upper surface of the leaf

c. Males

d. Lacunous parenchyma

e. Unicellular secretory hairs

f. Stoma



g. Palisade parenchyma

h. Unicellular protective hairs

Figure 2:- Micrographic elements observed (Personal photo, TIA Eric, 2020).

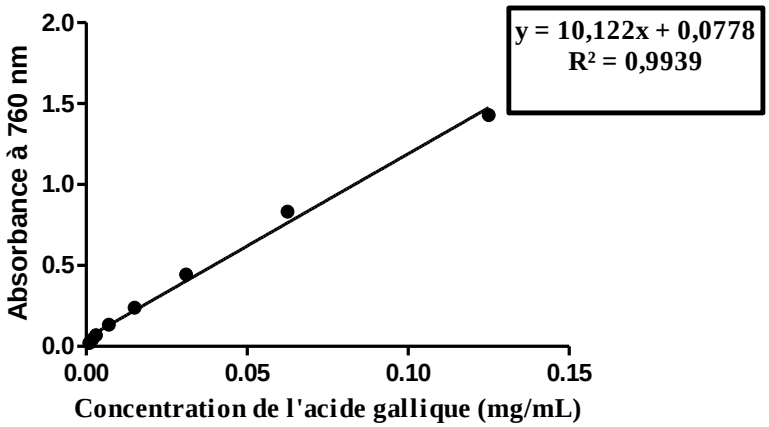


Figure 3:- Gallic acid calibration curve.

Dosage of total Flavonoids

The quantity of total Flavonoids from the leafy stem of *Ipomoea pes-caprae*was evaluated at 249.2 ±17.25 mg Eq Q/ g dry extract from the quercetin calibration curve (Figure 4).

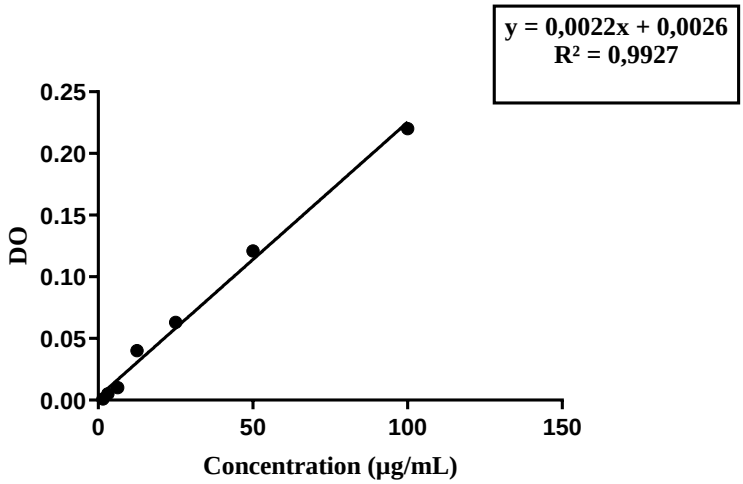


Figure 4:- Quercetin calibration curve

***In-vitro* antioxidant activity**

Ability to scavenge free radicals using the DPPH radical

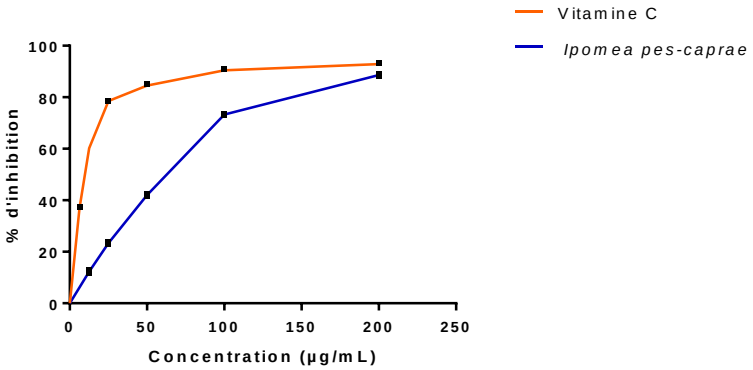


Figure 5:- Percentage of inhibition of the DDPH radical of aqueous extracts of leaves and stems of *Ipomoea pes-caprae* and vitamin C.

Ferric reducing antioxidant power test (FRAP):
The antioxidant power of the aqueous extracts of *Ipomoea pes-caprae* was evaluated using the FRAP test. The results show an antioxidant power estimated at 102.5 ± 12.3 µmol Eq Trolox/g EXS.

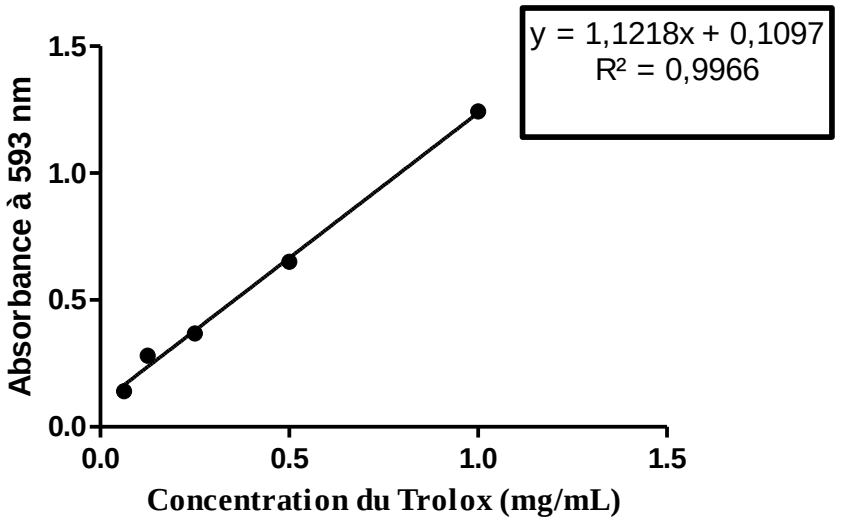


Figure 6:- Trolox calibration curve.

Discussion:-

Medicinal plants are an excellent source of natural antioxidants, and can act as potential medicines for several diseases such as cancer, autoimmune disorders, inflammation and diabetes. Antioxidants may also have a beneficial role in protecting oxidative stress, which is largely responsible for chronic and degenerative conditions[14, 15].Faced with the interest given to the discovery of new molecules from plants from African pharmacopoeias, this study consisted of evaluating the antioxidant activity and identifying the botanical, physical and chemical elements characteristic of *Ipomoea pes-caprae*leaves of Ivorian origin. Microscopic observation of the pulverized drug (aerial part) of *Ipomoea pes-caprae*noted the presence of several constituent elements, namely fragments of epidermis with anisocytic stomata, the epidermis of the upper face of the leaf, males, lacunous parenchyma, unicellular secretory hairs, stomata, palisade parenchyma, very long unicellular covering hairs. Compared to another pharmacognostic study conducted on the leaf and stem of *Ipomoea pes-caprae*in India, several other elements such as a spiral vessel, paracytic stomas, annular vessels, multicellular trichome, vascular bundles, sclerenchymatous tissue had been observed[8]. The micrographic elements found in our study could contribute to developing the monograph of this plant in Ivory Coast.

The qualitative phytochemical screening of the aerial parts of *Ipomoea pes-caprae*revealed the presence of sterols, polyterpenes, flavonoids, polyphenols, saponosides, alkaloids and catechic tannins with the exception of gallic tannins and quinones. In Brazil[16] and India[15], the aqueous extract of *Ipomoea pes-caprae*showed the presence of gallic tannins in the aerial part of *Ipomoea pes-caprae*. Another study carried out in India highlighted the presence of quinones in the leaf powder and stem of *Ipomoea pes-caprae*[17].Just as our study showed the presence of sterols, triterpenes, flavonoids, polyphenols and saponosides, the results of phytochemical sorting by Rokad et al.[8]in India had also shown that the dry powder of the leaf and stem of *Ipomoea pes-caprae* contained the same chemical compounds.

The quantitative dosage of the leafy stem of *Ipomoea pes-caprae*revealed the presence of total polyphenols (79.91± 6.20 mg Eq AG/ g dry extract) and total flavonoids (249.2 ±17.25 mg Eq Q/ g dry extract). Banerjee et al.[18] had shown that the aqueous extracts of aerial parts of *Ipomoea pes-caprae*collected in India contained a lower quantity of polyphenols (98 ± 0.58 µg Eq AG/mg of dry extract) than our study. The results obtained by Venkatesan et al.[15] in India in the aqueous extracts of aerial parts of *Ipomoea pes-caprae* had revealed the presence of a lower quantity of flavonoids (117.56 ± 0.60 µg Eq Q/mg of dry extract) compared to our study. In the same study, polyphenols (159.51 ± 0.86 µg Eq AG/mg of dry extract) were found to be in greater quantities than those obtained in our study. It is well known that the content and presence of biologically active constituents varies according to the stage of

development of the plant, according to the region, climate and even the type of soil[19]. These actors also have a significant influence on the physical qualities of medicinal plants.

The antioxidant activity was evaluated based on the ability of the extracts to reduce the concentration of DPPH by 50% and also to reduce ferric III to ferrous iron II. The inhibition of the DDPH radical was dose-dependent because it increased with the concentration of *Ipomoea pes-caprae* extracts. At 200µg/ml, the inhibition of the DDPH radical reached a value close to that of vitamin C with an IC_{50} of 46.23 ± 0.89 µg/ml. The antiradical effect of aqueous extracts of *Ipomoea pes-caprae*was identical to that of a study carried out in India on aqueous extract of leaves of *Ipomoea pes-caprae*[15] and superior to that of several studies carried out in other countries[18, 20]. The presence of phenols and flavonoids corroborate the antioxidant activity obtained with the aqueous extracts of *I. pes-caprae* leaves. Phenolic compounds (phenolic acids, flavonoids and proanthocyanidins) act in fact by free radical trapping mechanisms, with properties of hydrogen donors and hydrogen reducing agents as well as reducing agents 21, with abroad spectrum of biological activities and putative health effects[22]. Antioxidants are therefore of great interest in health. Indeed, several studies show that higher dietary intakes and circulating concentrations of antioxidants are associated with a lower risk of mortality from all causes[23].

Conclusion:-

The study showed that aqueous extracts of *Ipomoea pes-caprae* leaves have a favorable antioxidant effect in therapy. Also, the elements found in the micrograph and the physicochemical parameters could contribute to developing the monograph of this plant in Ivory Coast given that no study has been carried out in this context on *Ipomoea pes-caprae*.

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