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Research Article

Antioxidant and Anti-Inflammatory Activities of *Caralluma Acutangula* (Decne.) N.E.Br Extracts, a Medicinal Plant from Burkina Faso

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ABSTRACT

Plants have always played an important role in health care in Africa. The stress, a situation of imbalance between oxidizing and antioxidant systems in favor of the prooxidants is responsible for the installation of several pathologies such as cancers, cardiovascular diseases, diabetes ... The objective of this study was to highlight the presence Secondary metabolites in *C. acutangula* extract and determine its antioxidant and anti-inflammatory potential. For the determination of the acute toxicity of the extract, a dose of 2000 mg / kg body weight was administered to the NMRI Mouse. The methods of screening were used to detect secondary metabolites like tannins, steroids and terpen, flavonoids, coumarins. The antioxidant capacity was evaluated in vitro by determining the ability of the extract to inhibit lipid peroxidation, hydrogen peroxide, degradation of deoxyribose. The anti-inflammatory potential was evaluated on lipoxygenase and xanthine oxidase. Acute toxicity evaluated in NMRI mice showed that the ethanolic extract of *C. acutangula* show no toxicity. Tannins, steroids and terpen, flavonoids, coumarins have been detected in the extracts. *C. acutangula* showed good activity with an inhibition of $50.71 \pm 2.51\%$ at $100 \mu\text{g} / \text{ml}$ on lipid peroxidation, of $66.105 \pm 1.26\%$ on deoxyribose degradation and $8.625 \pm 1.09\%$ on hydrogen peroxide. It showed good activity on xanthine oxidase with an $81.5 \pm 5.5\%$ inhibition. For the effect on lipoxygenase it gave an inhibition of the enzyme at $43.11 \pm 3.4\%$. This potential could be used in the fight against inflammatory diseases and that due to oxidative stress.

Keywords: antioxidant, anti-inflammatory, oxidative stress, lipid peroxidation

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INTRODUCTION

Oxygen, a vital molecule for life, is likely to cause damaging effects in the body via the formation of reactive oxygen species (ROS). In biological systems, an imbalance between oxidizing and antioxidant systems in favor of prooxidants can occur. This imbalance is favored by the effect of certain endogenous pathological stimuli (hyper-LDLemia, hypertension, diabetes, obesity ...) or exogenous (environmental pollutants, smoking ...) leading to what is commonly called oxidative stress. It results from oxidation-reduction processes involving oxygen and leads to the excessive production of reaction intermediates called reactive oxygen species: superoxide ion ($\text{O}_2 \bullet^-$), hydroxyl ($\text{OH}\cdot$), perhydroxyl ($\text{HO}_2 \bullet$) and nitric oxide ($\text{NO}\cdot$) (Figure 1) ¹. Among the ROS, some species called free electron radicals or "free radicals" are characterized by one or more unpaired electrons on their outer layer, which gives them some instability. Indeed, these free radicals seek to match their

single electron by reacting with many types of molecules in their environment, causing damage to lipid (lipid peroxidation) proteins, DNA; compromising their functionality.

The oxygenated and nitrogenous reactive species can be of exogenous source or endogenous source. Exogenous sources are mainly of physical and chemical origin (for example X or gamma radiation, UV radiation [315-400 nm], radiolysis of water, photochemical reactions, etc.) ². In the body, there are many sources of reactive oxygen species that are enzymatic or non-enzymatic. The auto-oxidation of certain molecules such as dopamine, adrenaline, flavines and hydroquinones most often produces the superoxide anion which plays an important role in the formation of reactive oxygen species such as peroxide. hydrogen (H_2O_2), hydroxyl radical ($\text{OH}\cdot$), or singlet oxygen ($\text{O}_2 \bullet$). The superoxide anion can be formed from the enzymes found in the vascular wall such as NADPH oxidases that involve NADH or NADPH. The oxidation of

arachidonic acid during its metabolism by lipoxygenases or cyclo-oxygenases allows the formation of hydro-peroxydides essential for the formation of leukotrienes. Oxygen-reactive species are produced during the leukotriene biosynthetic steps. Lipoxygenase also contributes to the development of cardiovascular diseases as well as other sources of ERO production.³ Xanthine oxidase plays a very important role in the production of oxygenated reactive species such as superoxide anion and hydrogen peroxide during infusion or ischemia and thus contrasts with increased oxidative stress in the mitochondria the reduction of oxygen by the enzymatic pathways allows the formation of H₂O₂ which undergoes a mono-electronic reduction to give the superoxide anion, the latter intervenes in other reactions by producing the hydroxyl radical⁴. Lipid peroxidation is a chain reaction which is an example of very dangerous oxidation for cells, caused by oxygen derivatives, in particular hydroxyl radicals (OH•) or peroxy radicals (ROO•). In vivo, lipid peroxidation is also a very important phenomenon. Cell membranes are particularly rich in polyunsaturated fatty acids (30-50%) present in phospholipids, sphingolipids, cardiolipins. The lipoperoxidation of the membranes alters their functionality (modification of their permeability, their fluidity, loss of activity of enzymes, receptors...). Many other pathologies are associated with lipid peroxidation. This is the case of neurodegenerative diseases (Alzheimer's, Parkinson's), diabetes, cancers, inflammatory diseases, aging⁵. To combat the generation of ERO, living organisms have antioxidant systems. Antioxidants can be enzymes or simple molecules. Some are produced by the body, they are endogenous antioxidants and others come from the diet or medication and are therefore called exogenous. Endogenous antioxidants include:

- Enzymatic antioxidant systems: superoxide dismutase (SOD), catalase, glutathione peroxidase (GPX), heme oxygenase.
- Endogenous non-enzymatic antioxidant systems: glutathione, uric acid.
- Vitamin E (α-tocopherol), vitamin C (ascorbic acid), polyphenols are non-enzymatic antioxidants and food-derived (exogenous).

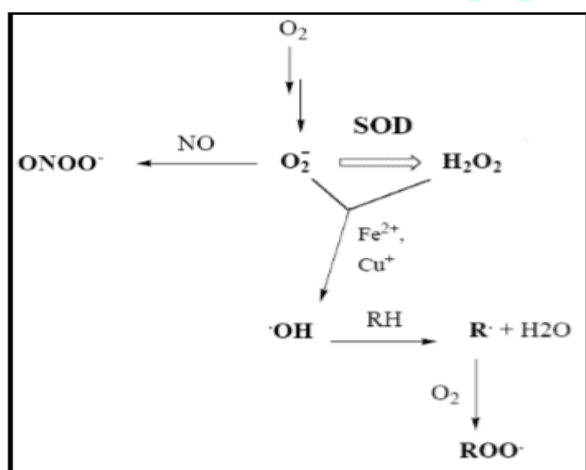


Figure 1 : Formation of reactive oxygen species (ROS).

Medicinal plants remain the main resort for a large majority of people to solve their health problems. Many studies have shown that plants have antioxidant properties due to the presence of secondary metabolites. they can prevent oxidative modification by neutralization of free radicals,

oxygen scavenging or peroxide decomposition through their antioxidant activities⁶.

C. acutangula is a medicinal plant used traditionally in Burkina Faso against weight gain, it is also used against boils to treat wounds and is considered a magical plant to protect livestock⁷ chest pain⁸.

Very few studies have been done on the ability of *C. acutangula* extracts to reduce oxidative stress, hence the purpose of this study is to determine the antioxidant and anti-inflammatory potential of *C. acutangula* extracts.

MATERIAL AND METHODS

Plant material and extraction:

The whole plants of *C. acutangula* were harvested at Goromgorom (a locality located in northern Burkina Faso at 400 Km on the road Ouagadougou (12 ° 29'42.7 N, 1 ° 24'1.2 W) during the period of March to April 2017. The specie was authenticated and a herbarium was deposited in the UFR / SVT under identification codes ID 17049. Samples were dried under laboratory conditions away from the sun and then pulverised and stored in freezer bags for extractions. The powder (50 g) of plant material *Caralluma acutangula* was placed in bottles containing 500 ml of a ethanol (100%). The bottles were subjected to mechanical stirring for 24 hours at room temperature. The macerated were filtered and then concentrated in an evaporator equipped with a vacuum pump and then evaporated to dryness. These extracts preserved and used for different tests

Experimental Animals :

MNRI mice aged 5 to 6 weeks were obtained from the Pet Shop of Department of Animal Physiology at University Ouaga I Professor Joseph Ki Zerbo -Burkina Faso. Before the experiment, the animals were acclimatised to laboratory conditions for one week at the Animal Transit Room of Research Institute for Health Sciences (IRSS) Burkina Faso. The animals are placed 12 hours in the light ; 12 hours in the dark and they had free access to food and water. All experimental animal protocols had complied with the instructions of the Institutional Animal Ethics Committee (directive 2010/63/EU on the protection of animals used for scientific purposes). Ethical approval code : 2010/63/EU, Date of approval: 20 October 2010. The institutional animal ethical guidelines were strictly observed. All authors hereby declare that "Principles of laboratory animal care were followed, as well as specific national laws where applicable.

Oral Acute Toxicity Study :

Acute toxicity was determined according to the method described by the OECD⁹. This is a sequential method. The mice will be randomized into batches of 6 animals (females). Each animal is identified by a different brand. The animals are fasted for 12 hours, then the weight of each mouse is taken, and they receive a single dose of extract (2000 mg / kg of body weight). The extracts were administered orally by gavage to the different test batch against a control batch which received only the water. The signs of toxicity (writhing, panic, moribund state, death ...) were noted by lot after 2h, 24h, 48h, 72h and the animals are kept under observation for two weeks.

Phytochemical study of the Extract

Screening test for secondary metabolites :

The purpose of the tests is to detect the main phytochemicals present in plant extracts. These tests were performed on the extracts of the plant studied. The procedures described by

Ciulei ¹⁰ have been used for the demonstration of the different chemical groups. So :

The reaction with iron trichloride (FeCl₃) is used for the detection of tannins and polyphenols,

The Shibata test for flavonoids,

The Feiggl-Frehden test for coumarins

The Liebermann / Buchard test for triterpenes / steroids,

The foam test for saponosides.

Determination of triterpenic compounds :

The total triterpene content was evaluated according to the method described by Chang et al. ¹¹. A volume of 300 µl of vanillin-glacial acetic acid (5%) is mixed with 200 µl of the extracts dissolved in 100% methanol (final concentration 1 mg / ml) ; 1 ml of perchloric acid is added and all incubated at 60 degrees for 45 minutes. 4.5 ml of glacial acetic acid are then added and the reading is made at 548 nm with the spectrophotometer. In total, three (3) analyzes are performed for each extract and the result given is an average of the three readings. The results are expressed in equivalent milligram of ursolic acid per milligram of dry extract (mg E.A.U./mg)

Antioxidant Potential

Inhibition of deoxyribose degradation :

The ability of the plant extract to trap the hydroxyl radical was evaluated using the deoxyribose degradation scavenging assay as described by Perjési et al. ¹². The reaction mixture was constituted by 100 µl of extract (1 mg / ml in 50 mM phosphate buffer, pH 7.4), 100 µl of EDTA (1.04 mM aqueous), 100 µl of iron sulfate (100 µl aqueous mM), 100 µl of deoxyribose (60 mM aqueous) and 100 µl of hydrogen peroxide (10 mM). The volume was made up to 1 mL with phosphate buffer, and the mixture was incubated (37 ° C for 1 hour). Trichloroacetic acid (1 mL, 15% aqueous) and thiobarbituric acid (1 mL, 0.675% in 25 mM aqueous NaOH) were added, and the whole was then incubated (100 ° C. for 15 minutes). After cooling in an ice bath (5 minutes), the tubes were centrifuged (3000 rpm for 10 minutes) and then 200 µL of the supernatant were transferred to 96-well microplates. Trapping of deoxyribose degradation was measured spectrophotometrically at 532 nm against a blank. Quercetin has been used as a reference substance. The activity of the extract to trap deoxyribose degradation was expressed as a percentage of deoxyribose degradation trapping compared to control without extract.

Lipid peroxidation :

Inhibitory activity of lipid peroxidation (LPO) extract or fractions were determined by the method of 2-thiobarbituric acid ¹³. Iron dichloride (FeCl₂) with hydrogen peroxide H₂O₂ was used to induce peroxidation of rat liver homogenate. In this method 0.2 mL of the extracts (1.5 mg / mL) was mixed with 1.0 mL liver homogenate in 1% Tris-HCl buffer (50Mm, pH 7.40), 50 µL FeCl₂ (0.5 mM) and 50 µL of H₂O₂ (0.5 mM) were added. The mixture was incubated at 37° C for 60 minutes, then 1 mL of trichloroacetic acid (15%) and 1 mL of 2-thiobarbituric acid (0.67%) were added and the mixture is heated in boiling water for 15 minutes. Absorbances were read at 532 nm using the spectrophotometer. The percentage inhibition of lipid peroxidation is calculated according to the formula:

% Inhibition = ((A₀-A₁) / A₀) × 100 With A₀ the percentage of inhibition of the negative control, A₁ the absorbance of the sample. Quercetin is used as a positive control.

Inhibition of hydrogen peroxide :

Hydrogen peroxide has a relatively long life and can cause damage far from its place of production. It diffuses easily through the cell membrane. The ability of the plant extract to trap hydrogen peroxide was evaluated according to the method described by Mohan et al. ¹⁴. The reaction mixture consisting of 100 µl of extract (200 µg / ml in 10 mM phosphate buffer, pH 7.4) and 100 µl of hydrogen peroxide (100 mM) was incubated for 10 minutes at room temperature. Residual hydrogen peroxide was measured at 230 nm against a blank containing only the phosphate buffer. The activity of the hydrogen peroxide trapping extract was expressed as a percentage of trapping hydrogen peroxide relative to the control without extract. Gallic acid was used as a reference substance

Anti-inflammatory Potential

Inhibition test of lipoxygenase :

The inhibitory activity of extracts and fractions on lipoxygenase was determined by the spectrophotometric method developed by Malterud et al. ¹⁵. Briefly, the reaction medium consisted of a mixture of 100 µL of extract or fraction prepared in borate-methanol buffer (1%) and 400 µL of LOX (167 U mL⁻¹). The mixture was incubated at room temperature for 2 minutes and the reaction is initiated by adding 500 µl of the substrate solution (linoleic acid, 250 µM in borate buffer). The kinetics of the reaction are monitored at 234 nm for 2 min. The inhibitory activity, expressed as percentage inhibition of lipoxygenase is calculated as follows: Percentage of inhibition (%) = (1 - /) 100

A = activity of the enzyme without inhibitor (Δabs with enzyme-Δabs without enzyme) B = activity of the enzyme with inhibitor (Δabs with enzyme-Δabs without enzyme)

Inhibition of xanthine oxidase :

The inhibitory activity of extracts on XO (EC.1.1.3.22) was evaluated according to the method described by Filha et al. ¹⁶. The reaction mixture consists of 50 µL of extract or fraction at the final concentration of 100 µg / mL, 150 µL of phosphate buffer (pH 7.5, 1/15 M) and 50 µL of enzyme solution (0.28 U / mL prepared in buffer). After preincubation of the mixture at 25 ° C for 1 min, the reaction is initiated by adding 250 µl of a substrate solution (0.6 mM) and the absorbance is measured for three minutes. A blank is prepared without extract. Quercetin and gallic acid are used as positive controls. The inhibitory activity of xanthine oxidase, expressed as percent inhibition, is calculated using the formula below:

Percentage of inhibition (%) = (1 - /0) 100

V₀: variation of the absorbance per minute of the test without the extract; V: variation of the absorbance per minute of the test with the extract.

RESULT AND DISCUSSION

RESULT

Toxicity of extract

Oral gavage was the method of administering the extracts of the plants used. On batches of six (06) mouse we did not observe any mortality or sign of intoxication after seventy-two hours (72 H) of observation following the administration of extracts (Table 1).

Table 1 : Extract toxicity

Plant specie	Dose	Number of Mice used	Number of deaths after				% of death after 72h	Signs of toxicity
			2h	24h	48h	72h		
<i>C. acutangula</i>	2000mg/kg.bw	06	00	00	00	00	00	No sign of toxicity

The extracts thus seem very low in toxicity (LD50 greater than 2000 mg / kg for *C. acutangula*, this allows us to have a margin of safety in the use of the extracts for the biological activity.

Phytochemistry

Qualitative phytochemical tests have demonstrated the presence of large group of secondary metabolites in plant extracts. Flavonoids, steroids, saponosides, alkaloids, polyphenols and tannins have been demonstrated in *C. acutangula* extracts (Table 2). The extract also exhibited a triterpene compound content of 3.57 ± 0.08 mg E.A.U./mg.

Table 2 : Result of phytochemical study

Test	Saponosids	Tannin and polyphénols	Flavonoïds	Steroids and triterpènes	Coumarins	Triterpenic compounds (mg E.A.U./mg)
Specie						
<i>C. acutangula</i>	+	+	+	+	+	3,57 ±0,08

+ = Presence - = Absence

Mg E.A.U./mg= equivalent milligram of ursolic acid per milligram of dry extract

Antioxydant activity

The antioxidant capacity of extracts of both plants and their fractions were evaluated in vitro by the use of different tests of antioxidant activities involving different mechanisms of action.

Figure 2 shows the ability of the extract to inhibit lipid peroxidation. *C. acutangula* showed good activity with an inhibition of $50.71 \pm 2.51\%$ at $100 \mu\text{g} / \text{ml}$, but it is lower than that of gallic acid ($90.67 \pm 0.98\%$) and quercetin quercétine ($91,78 \pm 1.52\%$) used as a reference.

For degradation of the deoxyribose, the ethanolic extract of *C. acutangula* gave an inhibitory capacity of $66.105 \pm 1.26\%$ deoxyribose degradation. Quercetin and gallic acid, two reference molecules, showed a higher inhibitory capacity (Figure 3).

Figure 4 shows the inhibitory potential of the extract on hydrogen peroxide. *C. acutangula* extract gave good activity with $8.625 \pm 1.09\%$ inhibition and equal to that of quercetin as the reference molecule.

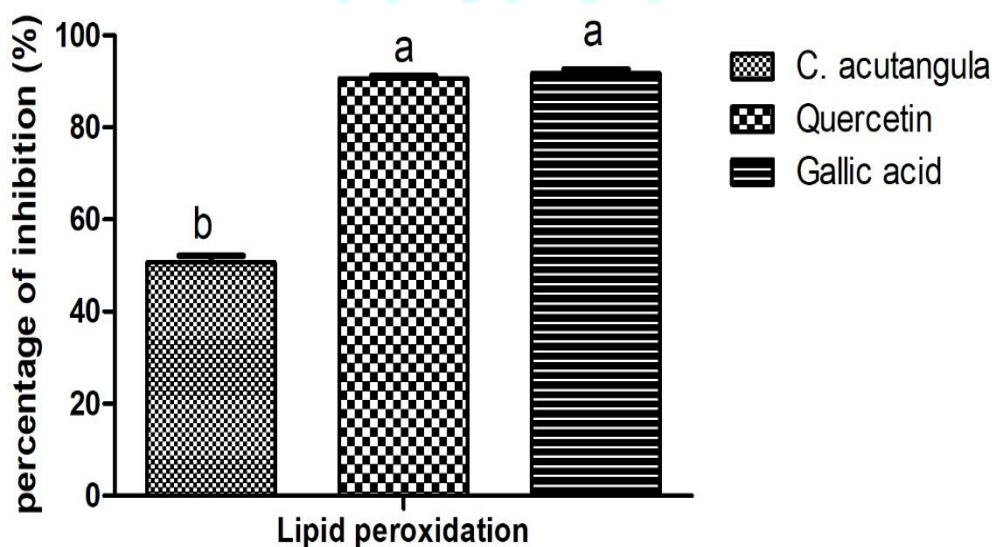


Figure 2 : Inhibition of lipid peroxydation

Mean \pm S.E.M = Mean values \pm Standard error of means of three experiments. Values with different superscript letters (a, b) differ significantly ($P < 0.05$) for each measured parameter

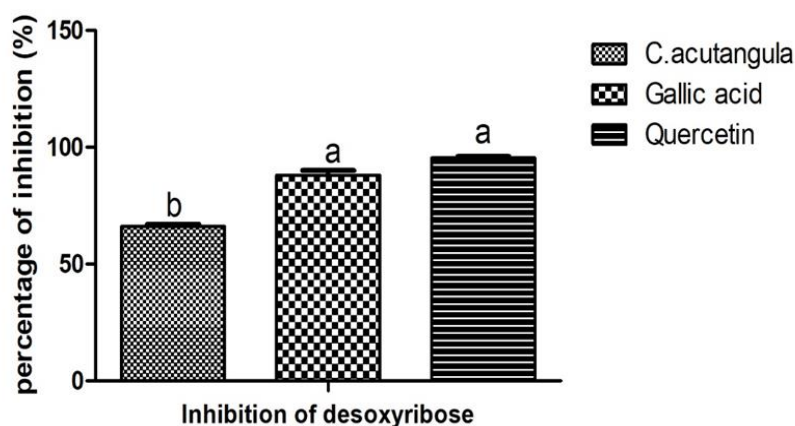


Figure 3 : inhibitory potential of Desoxyribose degradation.

Mean ± S.E.M = Mean values ± Standard error of means of three experiments. Values with different superscript letters (a, b) differ significantly (P < 0.05) for each measured parameter

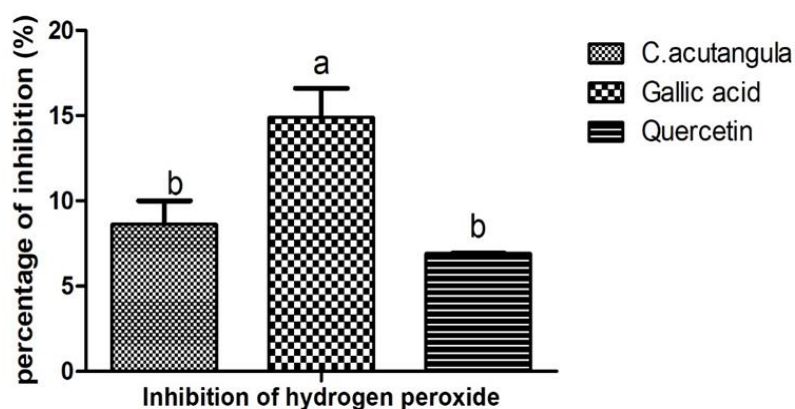


Figure 4 : Inhibitory activity of hydrogen peroxide

Mean ± S.E.M = Mean values ± Standard error of means of three experiments. Values with different superscript letters (a, b) differ significantly (P < 0.05) for each measured parameter

Anti inflammatory activity

The study consisted of an evaluation of the inhibitory power of extracts on xanthine oxidase and lipoxygenase in vitro. Both of these enzymes are involved in inflammatory processes.

C. acutangula showed good activity on xanthine oxidase with an 81.5 ± 5.5% inhibition not statistically different with the inhibition of quercetin as a reference substance (Figure 5).

For the effect of the extract on lipoxygenase at the concentration of 1 mg / ml, it gave an inhibition of the enzyme at 43.11 ± 3.4%, but this activity is lower than that of quercetin and gallic acid (Figure 6).

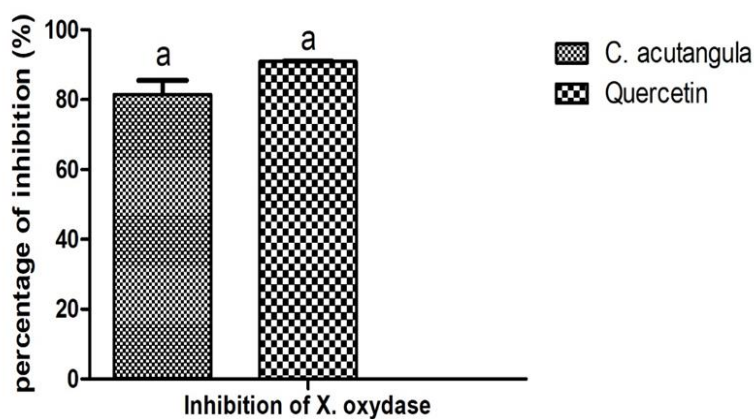


Figure 5 : Inhibition of Xanthine oxidase

Mean ± S.E.M = Mean values ± Standard error of means of three experiments. Values with different superscript letters (a, b) differ significantly (P < 0.05) for each measured parameter

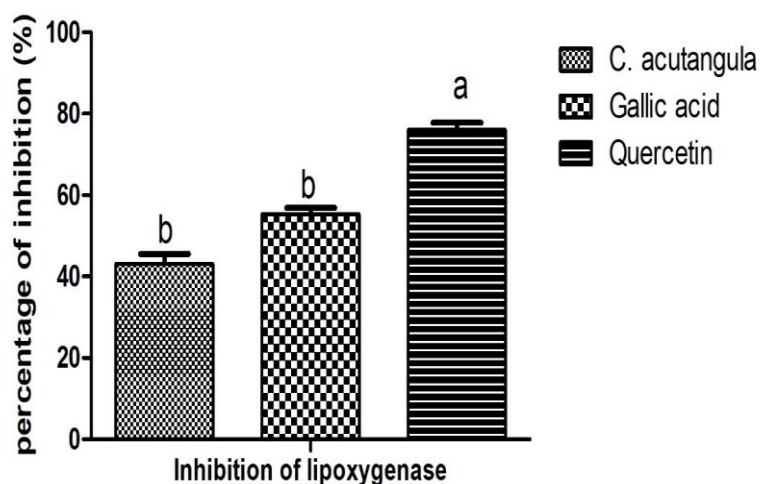


Figure 6 : Inhibitory activity of lipoxygenase

Mean \pm S.E.M = Mean values \pm Standard error of means of three experiments. Values with different superscript letters (a, b) differ significantly ($P < 0.05$) for each measured parameter

DISCUSSION

The ethanolic extract of *C. acutangula* did not show any mortality at a dose of 2000 mg / kg body weight, so *C. acutangula* has an LD₅₀ above 2000 mg / kg body weight. According to the WHO classification, extracts with an LD₅₀ greater than 2000 mg / kg body weight are very low in toxicity. Hence, the extract of *C. acutangula* has a very slight acute toxicity.

Phytochemical screening results showed that the extract contained polyphenols, flavonoids, triterpenes, steroids, saponosides.

These different secondary metabolites have already shown in several studies good therapeutic activities. Some flavonoids exhibit antiobesity activities, indeed flavonoids such as rutin, quercetin, naregenine inhibit the activity of glycerol-3-phosphate dehydrogenase (GPDH) and the expression of genes PPAR γ , C / EBPR involved in differentiation adipocytes¹⁷. Which contributes to the decrease of the body weight. Coumarins are lipolytic on adipocytes, anti-cancer, anti-inflammatory, antioxidant, anti-diabetic, antidepressant¹⁸. There is a relationship between wound healing, anti-inflammatory activity and tannins¹⁹. According to Jassim and Naji²⁰, calanolide A and Inophyllum are coumarins belonging to a new subclass of non-nucleoside compounds with a strong reverse anti-transcriptase and therefore endowed with antiviral activity. The presence of these metabolites in the species *Caralluma acutangula*, could explain the traditional use of the species against different ailments.

C. acutangula extract exhibited good antioxidant activity by inhibiting lipid peroxidation, deoxyribose degradation and hydrogen peroxide.

Lipid peroxidation induces a chain mechanism of degradation of fatty acids within the membrane, leading to the formation of hydroperoxides (ROOH) which are themselves unstable and reactive. Other products are formed during this lipid oxidation process: isoprostane, malondialdehyde (MDA) and 4-hydroxynonenal (4 HNE)²¹. The main pathologies associated with lipid peroxidation are cancers, cardiovascular diseases and Alzheimer. Polyphenols most often react with peroxy (ROO) or alkoxy (RO) radicals, thus interrupting the spread of lipid peroxidation²². The polyphenols found in the extract could justify the

inhibitory activity of lipid peroxidation observed. The extract would also contain substances that protect biological molecules including DNA, RNA. Indeed, cancer initiation and progression have been associated with oxidative stress by increasing DNA mutations or inducing DNA damage, genome instability, and cell proliferation²³; so *C. acutangula* extract could therefore be used for the protection and preservation of genetic material.

Inflammation is a set of defense reaction mechanisms by which the body recognizes, destroys and eliminates all foreign substances. But this inflammatory reaction sometimes exceeds its objectives and leads to the appearance of various disorders and inflammatory diseases. Several enzymes such as xanthine oxidase and lipoxygenase are involved in the process of inflammation. *C. acutangula* showed good anti-inflammatory activity by inhibiting xanthine oxidase and lipoxygenase. Oxidative stress and inflammation are key factors in the pathogenicity of obesity-related diseases, such as type 2 diabetes, cardiovascular diseases and cancers²⁴. The anti-inflammatory actions of flavonoids involve the inhibition of the synthesis and activities of various proinflammatory mediators such as eicosanoids, cytokines, adhesion molecules and C-reactive protein²⁵.

Lupeol, a triterpene is recognized primarily for its anti-inflammatory properties²⁶. Tannins have antioxidant, anti-inflammatory, anti-thrombotic and antibacterial properties²⁷. The presence of tannins and flavonoids, terpene could explain the anti-inflammatory activity obtained and this species of plant could therefore be used to reduce the pathogenicity of diseases such as obesity, cancer, cardiovascular diseases.

CONCLUSION

In conclusion, the ethanolic extract of *C. acutangula* showed a presence of polyphenol, tannins, flavonoids and triterpenes. This species has shown good anti-oxidant and anti-inflammatory activity. This potential could be used in the fight against inflammatory diseases and that due to oxidative stress. This study confirms the traditional use of *C. acutangula* and thus allows a valuation of traditional medicine

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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