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In vitro comparative screening of anti-inflammatory effect of crude extracts from *Cassia sieberiana* DC. (Ceasalpiniaceae) and Khaya *grandifoliola* C. DC. (Meliaceae)

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ABSTRACT

The inflammatory process is triggered for tissue repair following an attack of pathogens, chemicals or cell damages. Although beneficial, chronic inflammation may be the cause of certain diseases. Non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids have many side effects. Plants with anti-inflammatory properties may lead to the discovery of new anti-inflammatory agents with fewer side effects and cost. In this study, in vitro anti-inflammatory effect of glycosidic and aglyconic crude extracts of Cassia sieberiana and Khaya grandifoliola was evaluated using membrane stabilization of erythrocytes and inhibition of proteins's denaturation tests. For this purpose, Acetylsalicylic acid has been used as a reference like NSAID. The results obtained showed an anti-inflammatory effect more pronounced of aglycones that respective glycosides. This suggests that the deglycosilation can enhance the anti-inflammatory activity.

Keywords: screening, *in vitro*, anti-inflammatory effect, crude extracts, *Cassia sieberiana*, *Khaya grandifoliola*

INTRODUCTION

Inflammation is an immune reaction phenomenon implemented by the organism whenever the integrity of its morphological and biological constants is threatened. The occurrence of inflammation has many causes which include infections, burns, toxic products, allergy or other toxins. Its symptoms are pain, swelling, redness, fever, sensation of heat [1]. Although a defense mechanism, inflammation produces a variety of disorder that is characterized by vascular permeability, denaturation of proteins and alteration of cellular membranes [2]. Thus, uncontrolled and persistent inflammation may be an etiologic factor of various diseases chronic [3] as vasomotor rhinitis, rheumatoid arthritis, atherosclerosis [4], cardiovascular diseases and cancer [5].

The current treatments of inflammation are essentially The NSAIDs and The steroids. Although their effectiveness is established in some cases, they are not suitable for all cases of inflammation especially as their use cannot be extended because of their many side effects and their toxicity [6]; hence the necessity to search for new anti-inflammatory drugs presenting less inconveniences.

Medicinal plants are an important source of bioactive phytocompounds. The study of those used to treat inflammation, seems like a logical strategy that may lead to the discovery of new anti-inflammatory therapeutics [7].

Cassia sieberiana DC. (Caesalpiniaceae) is tree of savanna and dry zones of forest. It is found in multiple parts of West Africa (from Senegal to Nigeria) and East Africa. In traditional therapy, its extracts are used to treat fever, malaria, diarrhea, leprosy, schistosomiasis, stomach pains, inflammation, fatigue, jaundice, articular pains, and also used as diuretics and wormers [8, 9].

Khaya grandifoliola DCD. (Meliaceae) is a plant species distributed in West Africa from Guinea to Cameroon and also in East Africa. It is found in Benin, the Democratic Republic of Congo, Côte d'Ivoire, Ghana, Nigeria, Sudan, Togo, and Uganda. It is used in combination with other herbs to treat convulsions, cough, stomach ache, fever, rheumatism, ringworm and malaria [10, 11]. Previous works have demonstrated *in vivo* anti-inflammatory activity of aqueous, methanol and ethyl acetate extracts of these two plants[12-14]. The aim of our study was to compare the anti-inflammatory effect *in vitro* of glycosic and aglyconic crude extracts from drugs of *Cassia sieberiana* and *Khaya grandifoliola*.

MATERIALS AND METHODS

All chemicals used were of analytical grade.

PLANT MATERIAL

The plant material consists of root bark of *Cassia sieberiana* and Stem of the trunk *of Khaya grandifoliola*. Harvest took place in Vavoua, a town in west-central of Côte d'Ivoire. The samples of plant material were identified at the National Floristic Center (CNF) of Côte d'Ivoire by the emeritus botanist Professor Laurent-AKE ASSI and samples have been deposited at our laboratory for future reference. Plant samples were dried in the open air, away from the sun for 30 days. They were cleaned, cut, crushed in a mortar and sieved. The obtained powders were then sampled and then subjected to extractions with water and an organic solvent to give crude extracts to be analyzed.

Preparation of crude extracts of glycosides

A decoction of 250 g plant powder is performed in distilled water (500 ml) for 30 min. After filtration, the decoction was concentrated with a rotary evaporator under reduced pressure. The concentrate is then dried for 72 hours in a steam room at 50° C to provide the crude extracts of glycosides (CS1) for *C. sieberiana* and (KG1) for *K. grandifoliola*.

Preparation of crude extracts of aglycones

A mixture (150 g of plant powder in 500 ml of HCl, 2N) was heated at reflux for 40 min. After cooling and filtration, the aglycones were extracted by ethyl acetate. The organic phase was dried over anhydrous MgSO4, then concentrated with a rotary evaporator to give the dried crude extracts of aglycones (CS2) for *C. sieberiana* and (KG2) for *K. grandifoliola.*

STUDY OF THE ANTI-INFLAMMATORY EFFECT

Reagents

Aspirin (anti-inflammatory drug reference) available in pharmacies under trade name "Ciphaspire", EDTA (Ethylene diamine tetra acetic acid, Sigma-Aldrich), Tris Buffer (MP Biomedicals), egg albumin (Prolabo) and NaCl (Park) have been used. The negative control is a white without plant excerpt.

Preparation of the suspension of erythrocytes

Freshly collected sheep blood is added to a solution of EDTA (anticoagulant), to equal volume. The mixture is centrifuged at 3000 rpm for 10 min. The erythrocyte pellet was washed three times with iso saline solution (0.85% NaCl; pH = 7.2). A suspension of erythrocytes (10%, v/v) was constituted with an iso saline solution.

Hemolysis activity induced by hypotonic solution or membrane stabilization

This test was performed according to the method of Shinde et al., [15] with some modifications. Various concentrations of plant extracts (125-2000 micrograms / ml) were prepared with the iso saline solution. A mixture of 0.5 ml of plant extract, 1ml of Tris buffer (pH 7.4; 0.15 M), 2 ml of hyposaline NaCl (0.36%) and 0.3 ml of the suspension of erythrocytes (10%, v/v) was incubated at 37° C for 30 min before being centrifuged at 3000 rpm for 10 min. The absorbance of the supernatant is read with UV-visible spectrophotometer (BioMerieux) at 560 nm. The percent inhibition of hemolysis was calculated with formula:

$$\% \text{ hemolysis inhibition} = [(A1-A2) / A1] \times 100$$

A1: absorbance of control
A2: Absorbance of the extract or of the reference

Inhibition of albumin denaturation

Proteins denaturation was performed according to the method of Elias et al., [16] with some modifications. 1ml of each plant extract at different concentrations (125-2000 µl/ml) and 1 ml of egg albumin solution are added and the whole is incubated at 27° C for 15 min. Protein degradation is induced by maintaining the mixture in a water bath at 37° C for 10 min. After cooling, turbidity was measured using UV-visible spectrophotometer (BioMerieux) at 660 nm. Inhibition of protein denaturation caused by heat was calculated using the formula:

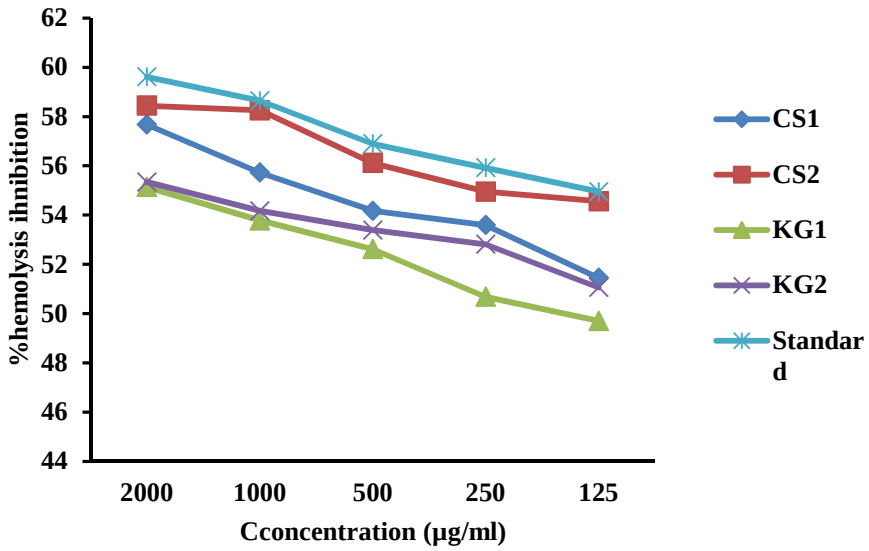
% Protection Protein = [(A1-A2) / A1] × 100

A1: absorbance of control
A2: Absorbance of the extract or of the reference

RESULTS AND DISCUSSION

Inhibition of lysis of erythrocyte membranes induced by hypotonic medium that means stabilization of erythrocyte membranes was used to test for our study. In fact, the erythrocyte membranes resemble lysosomal membranes and stabilization of erythrocyte membranes can be extrapolated to the lysosomal membranes [17]. The vitality of the cells depends on the integrity of the membrane, and when the erythrocytes are exposed to aggressive substances such as isotonic medium (0.36% NaCl) for example, it results in a lysis of the membrane, followed by the hemolysis and the oxidation of hemoglobin [18, 19]. Plant extracts like acetylsalicylic acid are expected to preserve the integrity of erythrocyte membranes and therefore of the cell membranes against these aggressive substances [20, 21]. Several studies showed further that there is a correlation between the potential of stabilization of cell membranes of a substance and its anti-inflammatory activity [22, 23]. The stabilization of lysosomal membranes is important in limiting inflammation in that it prevents the release of lysosomal constituents activated of neutrophils such as bactericidal enzymes and proteases that cause inflammation of other tissues and affect extra cellular activity [23]. *In vitro* study of the anti-inflammatory effect of different crude extracts of glycosides (CS1, KG1) and aglycones (CS2, KG2) from *C. sieberiana* and *K. grandifoliola*, was performed. The results are shown as curves (Figure 1) highlighting the percentage of hemolysis inhibition according to varied concentrations of CS1, CS2, KG1, KG2 and that, compared to acetylsalicylic acid, a NSAID taken as standard. The Figure 1 clearly shows that KG1, KG2, CS1 and CS2 are effective in the inhibition of the hemolysis at different concentrations. Maximum stabilization (58.44%) is obtained at 2000 µg/ml with CS2. Also, let's note ourselves that when the concentration increases, the hemolysis rate decreases and stabilization/protection of membranes increases, suggesting that the membrane stabilization follows a dose-response relationship. Concerning *C. sieberiana* and *K. grandifoliola*, their crude extracts of aglycones have anti-hemolytic activity more pronounced than that of their crude extracts of glycosides. This finding suggests that the sugar fragment of the glycosides inhibits anti-haemolytic activity.

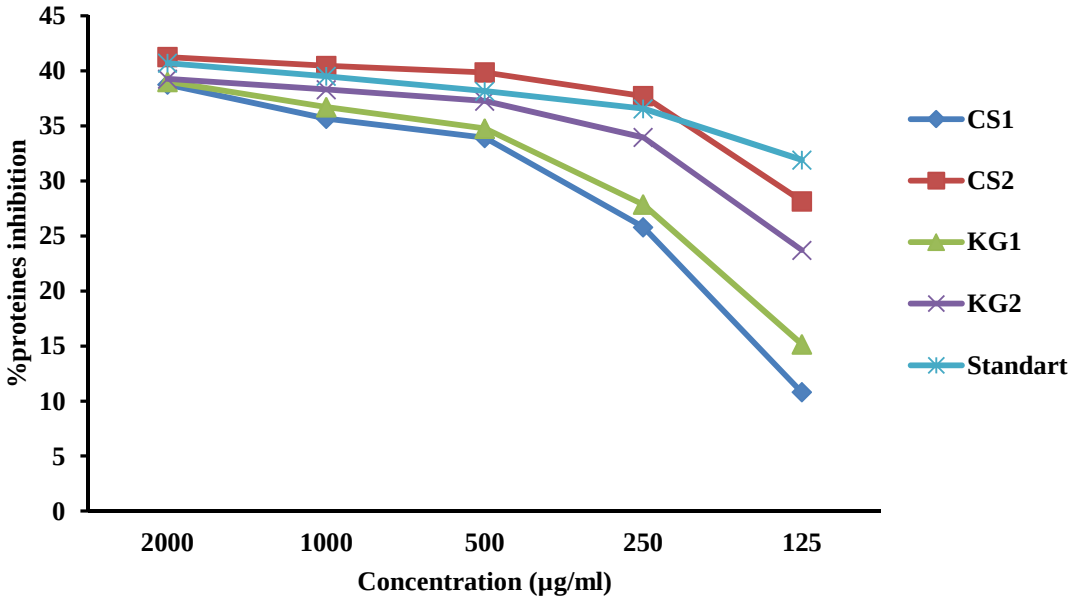
Figure 1 : Percentage of hemolysis inhibition according to the concentrations of CS1, CS2, KG1, KG2 and the standard



The test of proteins's protection is a suitable method for assessing the anti-inflammatory activity. Indeed, it was verified that the denaturation of proteins causes inflammation and chronic diseases such as rheumatoid arthritis [24]. Moreover, the protection of proteins denaturation is the main mechanism of action of classic NSAIDs [25]. The

excerpts tested CS1, CS2, KG1 and KG2 showed an efficiency to inhibit the denaturation of proteins led by the heat (Figure 2). The maximum percentage inhibition (41.25%) is observed with CS2. Thus, the total crude extracts of aglycones obtained from our study plants, exhibit a protective effect of the proteins clearly perceptible that the one of aglycones obtained from the aforesaid plants. Consequently, we believe that the glycone of glycosides would be responsible for the inhibition of the protective effect of proteins.

Figure 2 : Percentage of inhibition of proteins denaturation according to the concentrations of CS1, CS2, KG1, KG2 and the standard



Several studies have demonstrated the anti-inflammatory activity of plant extracts by membrane stabilization and proteins protection [2, 22, 26]. The crude extracts of *C. sieberiana* and *K. grandifoliola* are undoubtedly gifted anti-inflammatory power. Our expected results are consistent with those obtained in earlier work *in vivo* [12-14]. The anti-inflammatory effect of these two plants could be explained by their wealth in secondary metabolites [14, 27] among which some are proving to be endowed with anti-inflammatory activity with different mechanisms action.

CONCLUSION

Our survey carried on the screening of the *in vitro* anti-inflammatory effect of crude extracts of glycosides and aglycones obtained from drugs of *Cassia sieberiana* and *Khaya grandifoliola* and that by membrane stabilization and proteins protection tests. It not only permitted to show but also to confirm that these two plant species possess a considerable anti-inflammatory effect. Furthermore, our expected results suggest firstly, a better pharmacological efficacy of aglycones compared to glycosides and secondly, highlight the scientific basis for the ethnopharmacological use of root bark of *C. sieberiana* and those of the trunk of *K. grandifoliola* in traditional medicinal practice in Côte d'Ivoire.

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REFERENCES

[1] Joachim DG, Judith FA, Pierre L, Wilfrid KA, Richard T, Pierre D, Mansourou M., *International Journal of Biological and Chemical Sciences.* **2011**, 5 (2), 634-641.
[2] Umapathy E, Ndebia EJ, Meeme A, Adam B, Menziura P, Nkeh-Chungag BN, Iputo JE., *Journal of Medicinal Plant Research*. **2010**, 4 (5), 789-795.
[3] Kumar V, Abbas AK, Fausto N, *Pennsylvania 7th edition*, **2004**, pp 47-86.
[4] Henson PM and Murphy RC, *Amsterdam Elsevier*, **1989**, pp 404.
[5] Macarthur M, Hold GL, El-Omar EM., *American Journal of Physiology-Gastrointestinal and Liver Physiology*. **2004**, 286, 515-520.
[6] Ahmadiani A, Fereidoni M, Semnanian S, Kamalinejad M, Saremi S. *Journal of Ethnopharmacology*. **1998**, 61 (2), 229-232.
[7] Robbins, Cotron, Vinay K, Abdul KA, Nelson F., *7th edition*, **2008**, pp 47-53.

[8] Odugbemi TO, Odunayo R, Akinsulire EA, Peter OF., *African Journal of Traditional Complementary and Alternative Medicines*. **2007**, 4 (2), 191-198.
[9] Tamboura HH, Bayala B, Lompo M, Guissou IP, Sawadogo L., *African Journal of Traditional, Complementary and Alternative Medicines*. **2005**, 2, 13-24.
[10] Olowokudejo JD, Kadiri AB, Travih VA., *Ethnobotanical Leaflets*. **2008**, 12, 851-65.
[11] Madusolomuo MA, Nadro MS, Wurocheke AU., *Nigerian Journal of Biochemistry and Molecular Biology*. **1999**, 14, 21-25.
[12] Donkor K, Okine LNK, Abotsi WKM, Woode E, *Pharmacologia b*. **2013**, 4 (4), 301-310.
[13] Sy GY, Fall AD, Diatta W, Gueye M, Badji K, Bassène E, Faye B, *African Journal of Pharmacy and Pharmacology*. **2009**, 3 (12), 651-653.
[14] Abiodun F, Ching FP, Sunday AAO, *International Journal of Pharmacy and Technology*. **2009**, 1 (4), 1061-1064.
[15] Shinde UA, Phadke AS, Nair AM, Mugantiwar AA, Dikshit VJ, Saraf VO., *Fitoterapia*. **1999**, 70 (3), 251-257.
[16] Elias G and Rao MN, *Indian Journal of Experimental Biology*. **1988**, 26 (10), 540-542.
[17] Omale J and Okafor PN., *African Journal of Biotechnology*. **2008**, 7 (17), 3129-3133.
[18] Augusto O, Kunze KL, Montellano PR., *Journal of Biological Chemistry*. **1982**, 257 (11), 6231-6241.
[19] Ferrali M, Signorni C, Ciccoli L, Comporti M., *Biochemical Journal*. **1992**, 285 (1), 295-301.
[20] Maxwell SRJ., *Drugs*. **1995**, 49 (3), 345-361.
[21] Liu GT, Zhang TM, Wang BE, Wang YH., *Biochemical Pharmacology*. **1992**, 43 (2) : 147-152.
[22] Chopade AR, Sontakke PM, Sayyad FJ., *Journal of Karad Institute of Medical Sciences University*. **2012**, 1 (1), 67-72.
[23] Kumar V, Bhat ZA, Kumar D, Bohra P, Sheela S., *International Journal of Drug Development and Research*. 2011, 3 (3), 176-179.
[24] Mizushima Y., *Lancet*. **1966**, 2, 443.
[25] Grant NH, Alburn HE, Kryzanauskas C., *Biochemical Pharmacology*. **1970**, 19: 715-722.
[26] Gambhire M, Juvekar A, Wankhede S., *The Internet Journal of Pharmacology*. **2009**, 7 (1), 1-4.
[27] Kpegba K, Agbonon A, Petrovic AG, Amouzou E, Gbeassor M, Proni G, Nesna N., *Journal Natural Products*. **2011**, 74 (3), 455-459.