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Original Research Article

Phytochemical, Antioxidant and pharmacological study of *Heterotis Rotundifolia* (SM.) JACQ.- FEL. (Melastomataceae)

Sylvestre Dossou Etekpo[1], **Kohue Christelle Chantal N'Gaman-Kouassi**[1,*], **Treya Rodrigue Yao**[2], **Janat Akhanovna Mamyrbekova-Bekro**[1], **Yves-Alain Bekro**[1]

[1]*Dept. of Basic and Applied Sciences Training and Research, Nangui Abrogoua University, Abidjan, Côte d'Ivoire*
[2]*Dept. of Animal Physiology, Félix HOuphouet-Boigny University, Côte d'Ivoire*

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ABSTRACT

In developing countries, particularly those located south of the Sahara, research in both phytochemistry and phytopharmacology is a means of valorization endogenous medicinal plants into accessible phytomedicines for the populations. Our study focused on the species Heterotis rotundifolia, a Melastomataceae growing in Côte d\'Ivoire, and used in traditional medical care for various diseases. Qualitative (color reaction and TLC identification tests) and quantitative (elemental analysis) phytochemical tests as well as antioxidant and pharmacological evaluations on the species were carried out. All the results obtained showed that the study plant contains, on the one hand, mineral elements and secondary metabolites that condition its antioxidant, analgesic, and anti-inflammatory properties.

1. Introduction

The Ivorian flora is a reservoir of plants of pharmacological interest. From this point of view, it represents a natural pharmacy from which new molecules for therapeutic purposes can be discovered in the expectation of obtaining drugs with reduced side effects.[1] In recent years, research on plants of therapeutic interest has experienced a meteoric rise in view of the many scientific data collected, mentioning their all-round benefits.[2] Many authors[2,3] believed that the therapeutic effect of these plants for the treatment of various diseases is governed by their phytochemical compositions. The present study was carried out on *Heterotis rotundifolia* (Sm.) Jacq. -Fél. (Melastomataceae) used in traditional Ivorian medicine to treat measles and gestational diabetes. Apart from Etekpo et al. work[4], there is no scientific data on the phytochemical composition of the Ivorian species. We recall, however, that works on the leaves of the plant have been published. Indeed, Agwu et al.[5] showed that *H. rotundifolia leaves* reduce the osmotic fragility of human hemoglobin S (HbS) erythrocytes. Ogunka-Nnoka et al.[6] demonstrated by GC-MS analysis of the fat extracted from the same organs of this plant, that it contains 83.79% saturated fatty acid and 9.72% unsaturated fatty acid. The overall aim of this study was to highlight the phytochemical composition, the antioxidant, peripheral analgesic and anti-inflammatory activities of *H. rotundifolia* growing in Côte d'Ivoire.

2. Materials and Methods

2.1. Plant material

The whole plant of *H. rotundifolia* was the matrix of the study carried out. It was harvested in May 2018 on

* *Corresponding author*.
E-mail address: kohuechristelle@yahoo.fr
(Kohue Christelle Chantal N'Gaman-Kouassi).

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Table 1: Developers used for CCM

Selective fraction	Developer
Hexane	Hexane / AcOEt (5: 1.5 (v/v))
$CHCl_3$	Hexane / AcOEt / $CHCl_3$ / AcOH (3.5 / 3 / 2.5 / 0.5 (v/ v/ v/ v))
AcOEt	Hexane / AcOEt / $CHCl_3$ / AcOH (2 / 1.5 / 4 / 0.5 (v/v/v/v)) + 5 drops of NH_4OH
n-BuOH	AcOEt / $CHCl_3$ / AcOH / EtOH (2 / 4.5 / 0.6 / 0.5 (v/v/v/v))
H_2O	AcOEt / $CHCl_3$ / H_2O / MeOH (2 / 4.5 / 0.6 / 0.5 (v/v/v/v))

AcOH: acetic acid, MeOH: methanol, EtOH: ethanol

Table 2: Phytochemical families present in *H. rotundifolia*

Phytochemical family	Type of Test	Results
Polyphenols	$FeCl_3$ (2%)	Positive
Flavonoids	Shinoda test	Positive
	Basic lead acetate	Positive
Coumarins	KOH (2%)	Positive
Tannins	Stiasny	Positive
Alkaloids	Dragendorff	Negative
Sterols, Polyterpenes	Acetic anhydride + H_2SO_4	Negative
Reducing compounds	Fehling liquor	Positive
Saponins	Foam Index	130

Positive: Presence Negative: Absence

Table 3: Phytocompounds of *H. rotundifolia* identified by TLC

Selective fraction	Identified phytocompound [Rf] color	
Hexane	Lupane triterpenes^{c,d}: [0.05] Y^{c2}-B^{c1} ; [0.22 et 0.47] O^{d2}	Oleanane and ursanne triterpene^{d}: [0.27] R^{d2}. [0.4] P^{d1}-R^{d2}. [0.57] B^{d1}-R^{d2}
	Sterols^{d}: [0.05] Y^{d2}, [0.18] G^{d1} ; [0.5] Y^{d1}- G^{d2}	Terpene^{c} : [0.45 ; 0.5 ; 0.7] O^{c2}
	1,8-dioxyanthracene^{b}: [0.09 ; 0.15 ; 0.18 ; 0.22 ; 0.35 ; 0.40 ; 0.50 ; 0.60] R^{b2}. [0.32 ; 0.45] Y^{b1} -R^{b2}. [0.65] G^{b1}-R^{b2}	Sterols^{d} : [0.18 ; 0.70] G^{d1}. [0.35] Y^{d2}. [0.5] Y^{d1}-G^{d2}
$CHCl_3$	Triterpenes^{d}: [0.06 ; 0.21] P^{d1}. [0.34] P^{d1}-V^{d2}. [0.45] B^{d2}	Terpene^{c} : [0.37 ; 0.6] O^{c2} ; [0.77] G^{d1}-P^{d2}
	Coumarins^{b,e}: [0.26 ; 0.29 ; 0.37 ; 0.45] B^{b2} ; [0.27 ; 0.42 ; 0.81 ; 0.91] Y^{b2}. [0.47] G^{e2}	7,8-Dihydroxycoumarin^{k}: [0.42; 0.47] G^{k2}
	Alkaloids^{g} : [0.5 ; 0.77 ; 0.81 ; 0.87 ; 0.95] OY^{g1}	1,8-Dioxyanthracene^{b}: [0.72 ; 0.77 ; 0.85] R^{b2}. [0.87] O^{b1}-R^{b2}
		Anthracene^{b} : [0.72] R^{b2}
		Phenolic acids^{j} : [0.42] G^{j1} NI : [0.70] R^{e2}
AcOEt	Coumarins^{eb} : [0.15] B^{e2} ; [0.22] B^{c2} ; [0.47 ; 0.81] Y^{b1}-B^{cb2}	1,8-Dioxyanthracene^{b}: [0.54 ; 0.81] R^{b2}
	NI: [0.70] B^{f1}	7,8-Dihydroxy-6-methoxycoumarin^{k} : [0.85] G^{a1}-G^{a2}-Y^{k2}
	Flavonoids: [0.15 ; 0.85] B^{h2}. [0.47 ; 0.87] Y^{h1}	Angelicin or Coumestrol: [0.89] P^{a2}-G^{k2}
	Alkaloids^{g} : [0.52 ; 0.72 ; 0.75 ; 0.89] O^{g1}	
n-BuOH	Coumarins^{be} : [0.06] B^{b2}. [0.87] Y^{b2}. [0.12 ; 0.91] G^{e2}	1,8 dioxyanthracene^{b} : [0.79] P^{e2}; [0.85] R^{e2} NI : [0.94] R^{b2}
	Flavonoids^{h} : [0.06 ; 0.12] G^{h2}. [0.91] G^{h1}. [0.69] O^{h2}	
H_2O	Coumarins^{be} : [0.11 ; 0.34 ; 0.40 ; 0.07 ; 0.65 ; 0.75] B^{b2}-G^{e2}	Alkaloids^{g} : [0.65 ; 0.75] O^{g2}

OY: orange-yellow; Y: yellow; G: green; R: red; B: blue; O: orange; P: purple; a: without developer; b: KOH; c: sulfuric vanillin; d: Liebermann Burchard; e: Basic lead acetate; g: Dragendorff ; j: $FeCl_3$; h: $AlCl_3$; k:NH_3; _NI: Not identified; 1: visible 2: UV/365.

Table 4: Antioxidant parameters of *H. rotundifolia*

Sample	CR_{50} (mg /ml)	IE	EA
H. rotundifolia	0.5867	19.56	$1.704\ 10^{-3}$
Vitamin C	0.095	3.17	$7.886\ 10^{-2}$
Rutin	0.220	7.33	$9.095\ 10^{-2}$

Table 5: Percentages of inhibition of abdominal cramps induced by the injection of acetic acid byaceclofenac and *H. rotundifolia*

Batch	Number	% inhibition
Witness	30 ± 1.69	-
Aceclofenac	14.7 ± 4.35**	55.11
(75 mg/kg of CM)	3.17 ± 2.23***	89.44
(150 mg/kg of CM)	7.17 ± 3.55***	76.11
(300 mg/kg of CM)	3.50 ± 1.75***	88.83

***P < 0.001: significant difference of the treated batches compared to that of the control batch; n = 6

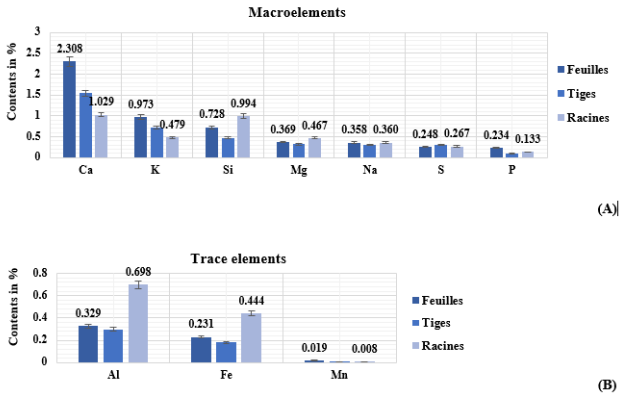

Fig. 1: Quantitative mineral composition inmacroelement (A) and trace elements (B) of *H. rotundifolia*

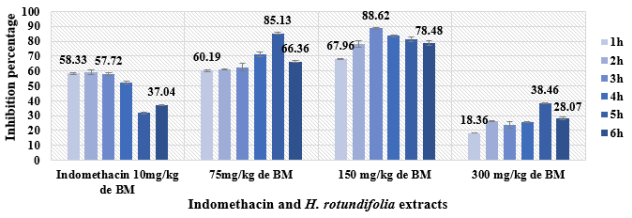

Fig. 2: Inhibition percentage of carrageenan-induced edema as a function of time by *H. rotundifolia* and indomethacin

the premises of University NANGUI ABROGOUA (UNA) located in Abidjan (5° 23′ 21″ north, 4° 01′ 09″ west). The species was authenticated at the National Floristic Center (CNF) in Abidjan in accordance with the available herbarium (*Malan 552 reference*). After harvesting and cleaning with water, the plant was dried during 5 days in an air-conditioned room (16°C). It was then reduced to powder for the tests.

2.2. Methods

2.2.1. Phytochemical tests

2.2.1.1. Determination of mineral composition. The elemental analysis of *H. rotundifolia* leaves, stems and roots of was carried out at the Analysis and Research Center of the PETROCI laboratory (Abidjan-Côte d'Ivoire) using an X-ray fluorescence spectrometer (XRF).

Using a hydraulic press, 4 g of plant powder, previously incinerated, are pressed (pressure of 10 tons) to make pellets. These pellets were introduced into the spectrometer and bombarded by photons. The primary radiation is absorbed, the atoms of the sample ionized and bridged by internal electrons emitted (beam of secondary X-rays). Electronic relaxation releases energy (X-ray fluorescence) in the form of characteristic photons for each atomic element. These photons detected by a counter make it possible to identify the atom according to its energy and the flux of photons received by the analyzer makes it possible to deduce the mass concentration. The results of the quantification of mineral elements are expressed as a percentage (%).

2.2.1.2. Identification tests by color reaction and foam index (Im). The phytochemical screening was carried out according to the methodologies of reported works.[7–9]

2.2.1.3. TLC identification test. TLC identification test was carried out on the selective extracts in accordance with the methodologies from the literature.[7–11] The aqueous macerate of the vegetable powder (10 ml) was successively exhausted with 5 ml of hexane, chloroform ($CHCl_3$), ethyl acetate (AcOEt) and n-butanol (n-BuOH). The selective fractions and the residual aqueous extract were analyzed by TLC on Silufol chromatoplate (aluminum support, silica gel 60 F_{254}, thickness 0.2 mm; Merck). For the migration of the phytocompounds, various developers (solvent systems) were used (Table 1). The molecular fingerprints of the phytocompounds

were revealed using Liebermann-Bürchard reagent and sulfuric vanillin (5%, m/v) (terpenes and sterols), methanolic KOH solution (5%, m/v) and basic lead acetate (coumarins), NH_3 vapors(coumarins, anthocyanins, flavonoids), $FeCl_3$ solution(2%, m/v) (polyphenols, tannins), $AlCl_3$ solution(1%, m/v) (flavonoids) and Dragendorff reagent (alkaloids).

2.2.2. Antioxidant evaluation: DPPH test

The evaluation of the antioxidant activity by spectrophotometry was carried out.[4,12,13] The reference antioxidants were the solutions of vitamin C and rutin prepared under the same conditions. To 1 ml of methanolic extract of the plant powder (0.0032 to 2 mg/ml) was added 2.5 ml to a methanolic solution of DPPH (0.03 mg/ml). The absorbance of the reaction mixture was read every 3 min for 30 min at 517 nm on a UV-visible spectrophotometer (Aqualytic Al800). The reduction percentage (PR); the efficiency index (IE) and the antiradical efficiency (EA) were determined from equations (1), (2) and (3):

PR (%) = (1 - ($\frac{Absorbance\ extrait}{Absorbance\ contrle}$)) × 100 (1)

IE (mg extract / mg DPPH) = $\frac{CR_{50}}{(DPPH)t=0}$ (2) EA = $\frac{1}{CR_{50}\times TCR_{50}}$ (3)

CR_{50}: Concentration that reduces 50% of the initial DPPH concentration

(DPPH) t = 0: Initial concentration of the DPPH solution

TCR_{50}: Time required reducing 50% of the initial concentration of DPPH

2.2.3. Pharmacological tests

For the pharmacological tests, we prepared samples with 20 g of vegetable powder macerated in 300 ml of distilled water under constant stirring for 24 h. After filtration, the macerate is concentrated at 80°C using a rotary evaporator (BÜCHI (Wather Bathb-480)). The concentrate obtained was maintained at 45°C in an oven for 48 hours.

2.2.3.1. Evaluation of peripheral analgesic activity: Writhing test. The methodology used is similar to that of Collier et al.[14] and Kouakou-Siransy et al.[15] The analgesic effect was achieved on five homogeneous batches each containing five Swiss albino mice (3 months), 22 g male and female, from the animal facility of the Laboratory of Animal Physiology of UNA:

1. Batch 1: control treated with distilled water before the intraperitoneal injection of acetic acid (1%);
2. Batch 2: reference treated with aceclofenac (100 mg/kg Body Mass (BM)), a non-steroidal antiinflammatory drug;
3. Batch 3: test with extract at 75 mg/kg of BM by gavage;
4. Batch 4: test treated with the extract at 150 mg/kg of BM by gavage;
5. Batch 5: test treated with the extract at 300 mg/kg of BM by gavage.

Intraperitoneal injection of acetic acid (1%) in mice causes a painful syndrome resulting in characteristic contortions (stretching movements) of the hind legs and the dorso-ventral muscle. After the administration of the samples, an intraperitoneal injection of acetic acid was made to the mice. For each dose, the percentage inhibition (% Inh) of cramps is determined according to expression:

% Inh = ((Nb control - Nb treated) / Nb control) × 100 (4)

2.2.3.2. Assessment of anti-inflammatory activity. The evaluation of the anti-inflammatory activity was carried out according to the method reported by Winter and Nuss.[16] The intraperitoneal injection of carrageenan (1%) administered to the hind paw of the rat causes the appearance of an edema, the size of which was assessed by measuring the circumference of the paw. The rats used are young Wistar albino females (6 months) weighing 100 g from the animal facility of the Normal Superior School (ENS). Inflammation was reduced by the application of anti-inflammatories. Several treatments were administered to five batches of rats:

1. batch 1: Control treated with distilled water;
2. batch 2: Reference treated with indomethacin at 10 mg/kg BM (NSAID);
3. batch 3: Test treated with the extract at 75 mg/kg of BM by gavage;
4. batch 4: Test treated with the extract at 150 mg/kg of BM by gavage;
5. batch 5: Test treated with 300 mg/kg of BM by gavage from which an inflammation was induced 1 hour before.

The percentage inhibition of inflammation (% Inh) was calculated by comparing the mean of the increase in inflammation with that of the control group according to the expression (5):

% Inh = $\frac{C-C1}{C}\times$ 100 (5)

C: average percentage increase in the circumference of the oedematous paw of the control batch.

C_1: average percentage increase in the circumference of the oedematous paw of the test group

2.3. Statistical analyzes

One-way analysis of variance (ANOVA) tests were used for analgesic and anti-inflammatory activities using Graph Pad Prism software version 5. Multiple comparisons of means by Tukey's test was made when a significant difference is noted. The significance of the test is determined by comparing the probability (P) associated with the test statistic to the theoretical value α = 0.05. If P ≥ 0.05, the

difference between the means is insignificant. If $P < 0.05$, the difference is significant.

3. Results and Discussions

3.1. Mineral composition

The results of the quantitative elemental analysis of the leaves, stems and roots of *H. rotundifolia* highlighted the variable contents of macroelements (Ca, K, Si, Mg, Na, S, P) and trace elements (Al, Fe, Ti, Mn) (Figure 1). Minerals have variable and diverse actions. They are essential to our body, in particular to the constitution of the skeleton, the teeth and the hair, and enter into many cellular reactions. Calcium (Ca) is the most abundant mineral element. Ca and phosphorus (P) are involved in building and maintaining tissues. The minerals sodium (Na), potassium (K), P, magnesium (Mg), iron (Fe) and manganese (Mn) are useful in regulating metabolism.[17] K is present in the intracellular medium. In addition, it is also essential for maintaining muscle and nerve cell function.[18] Among other main trace elements for plants, Fe is present in the analysed plant organs. This trace element plays a major role in the manufacture and functioning of haemoglobin and myoglobin.[19] Ca and Mg contribute to good muscle function and are involved in cell division and differentiation processes.[20] Aluminum (Al) is a non-essential trace element, but classified as a nutrient. It seems to be the most prevalent in the environment, but plays no physiological role in metabolic processes.[21] However, the accumulation of Al is a principal primitive characteristic of certain tropical families including the Melastomataceae.[22]

3.2. Composition of secondary metabolites

The secondary metabolite composition of *H. rotundifolia* was determined (Table 2). It reveals the presence of seven phytochemical families: polyphenols, flavonoids, coumarins, tannins, reducing compounds and saponins. Shinoda test (Mg in concentrated HCl) demonstrated the presence of flavanones and dihydroflavonols among the flavonoids present.[23] Furthermore, another source from the bibliography[24] indicates that Shinoda test also shows the presence of flavones. Indeed, their presence in a sample was confirmed by their reduction to anthocyanidins. The conjugated system in the flavonoids produces a yellow color, while the conjugation in the anthocyanidin gives a red coloration. The color variation is the indicator of the presence of flavones. The moss index (Im = 130) confirms the detection of saponins. Polyphenols, flavonoids and coumarins have various biological properties[25], and are known for their inhibitory action on enzymes responsible for the production of free radicals.[26] Tannins also have the ability to inhibit the growth of many microorganisms,[27] and saponins have antimicrobial, antibacterial and analgesic activities.[28] Thus, there is every reason to believe that *H. rotundifolia* use as a traditional medicinal treatment is governed by the co-presence of the bioactive phytoconstituents contained in the plant.

Phytochemical screening by TLC of the selective fractions was carried out (Table 3), on the one hand, in order to have a more extensive overview of the phytochemical composition of *H. rotundifolia*, and on the other hand, to isolate the suspected constituents responsible for its therapeutic properties. The results obtained confirm those obtained by colored reactions, unlike the absence of alkaloids and sterols and polyterpenes (Table 2).

Thanks to TLC screening, the presence of lupane, oleanane and ursanne type triterpenes,[23,29] anthracene derivatives (anthracene; 1,8-dioxyanthracene),[30] phenolic acids,[29] coumarin derivatives (angelicin or coumestrol; 7, 8-dihydroxycoumarin; 7,8-dihydroxy-6-methoxycoumarin)[31] and alkaloids[32] was confirmed with regard to the specificity of the molecular fingerprint differences. *H. rotundifolia* species from Nigeria does not contain alkaloids.[33] Polyphenols and flavonoids were demonstrated by quantification in the species from Benin.[34]

3.3. Antioxidant activity

H. rotundifolia antioxidant potential against the DPPH radical was evaluated by comparison with vitamin C and rutin used as reference antioxidants. The results were shown in Table 4. The reduction percentages of DPPH (Table 4) made it possible to graphically determine the median concentrations (CR_{50}), reducing 50% of the DPPH. This parameter reflects the efficiency of the sample.[35] The lower its value, the more significant the antioxidant activity. The efficacy indices (IE) and the antiradical efficacy (EA) were also determined. The species *H. rotundifolia* presents an antioxidant potential with respect to the DPPH radical with a CR_{50} and an IE of values 0.5867 mg/ml and 19.56 respectively. Considering the method for EA determining and according to Sanchez-Moreno et al.[36] classification, *H. rotundifolia* has a slower DPPH-reducing action ($1.704\ 10^{-3}$) than vitamin C ($7.886\ 10^{-2}$) and rutin ($9.095\ 10^{-2}$). This seems to depend on the ability to quickly give up hydrogen.[37,38] We recall that the DPPH test makes it possible to determine the reducing capacity of an extract by hydrogen transfer.[37,39] The overt antioxidant activity of *H. rotundifolia* appears to be confirmed by phytochemical screening, which reveals the presence of antioxidant phytochemical families. Published works[34,40] have shown that *H. rotundifolia* species is antioxidant.

3.4. Analgesic activity

The analgesic activity of the plant extract at different concentrations (75; 150 and 300 mg/kg) was evaluated. The results obtained are recorded in Table 5. The pain inhibition by extract was compared to that induced by

aceclofenac widely used and/or associated in the treatment of a number of serious cases of clinical hepatotoxicity.[41] The percentage inhibition of abdominal cramps induced by acetic acid injection of all doses received by mice (from 76.11% to 89.44%) is higher and more significant than that of aceclofenac (55.11%). The analgesic activity of *H. rotundifolia* could be explained by the presence of bioactive phytocompounds possessing analgesic properties among which flavonoids[42] and coumarins.[43] It should also be noted that studies carried out on the chemical composition of the essential oils of the leaves of *H. rotundifolia* revealed that the leaves contain bioactive compounds. Studies[6] showed that neophytadiene, a major constituent (35.8%) of the essential oil extracted from the leaves of *H. rotundifolia,* has antipyretic, anti-inflammatory, analgesic, antimicrobial properties and antioxidant.

3.5. Anti-inflammatory activity

The results of the evaluation of the anti-inflammatory activity of the aqueous extract of *H. rotundifolia* are recorded in Figure 2. After administration of carrageenan (1%) by intraperitoneal injection to the right hind paw of the rat, edema and inflammation was observed which increase with time. Indeed, the injection of carrageenan causes an inflammatory process accompanied by an edema evolving in 2 phases: phase 1 (1h) synthesis and release of chemical mediators (histamine and serotonin which maintain inflammation) and phase 2 (3h) formation of prostaglandins and leukotrienes due to activation of cyclooxygenase and lipoxygenase.[44] It is during the second phase that the inflammatory phenomena can be antagonized by natural anti-inflammatories, steroidal or not.[45,46]

After injection of indomethacin, a gradual inhibition of edema was observed from 58.89% (2h) to 31.70% (5h). The plant extract also induces a significant inhibition at the concentrations 75 mg/kg (85.13% at 5 hours) and 150 mg/kg (88.62% at 3 hours). However, this inhibition varies slightly at 300 mg/kg (from 18.36% to 38.46%). This anti-inflammatory activity is comparable to that of indomethacin. At 150 mg/kg of BM, there is a significant difference (at the threshold $P < 0.01$) at 3h (88.62 ± 0.67**), 4h (83.76 ± 0.63**) and 5h (81.15 ± 1.63**). This inhibition is greater than that of indomethacin at all times. In view of the results obtained, it is well founded to affirm that *H. rotundifolia* is more active at doses of 75 and 150 mg/kg of BM. This seems to justify the use of the species in traditional medicine for the treatment of hemorrhoids[47] and measles.[48] There is no doubt that the anti-inflammatory activity of *H. rotundifolia* depends on its phytochemical composition. Indeed, phenols, saponins, tannins, triterpenes and flavonoids also possess anti-inflammatory properties.[42,49,50]

4. Conclusion

The presence of mineral elements (macro and trace elements) essential for humans, as well as families of bioactive phytocompounds with antioxidant, analgesic and anti-inflammatory properties, was highlighted in *Heterotis rotundifolia.* The results obtained can justify the use of the plant in medicinal practices in Côte d'Ivoire.

5. Source of Funding

None.

6. Conflict of Interest

None.

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Author biography

Sylvestre Dossou Etekpo, Ph D Student https://orcid.org/0000-000-5598-2011

Kohue Christelle Chantal N'Gaman-Kouassi, Assistant Master https://orcid.org/0000-0002-2552-4471

Treya Rodrigue Yao, - https://orcid.org/0000-0002-5165-3680

Janat Akhanovna Mamyrbekova-Bekro, Professor https://orcid.org/0000-0003-1598-6020

Yves-Alain Bekro, Professor https://orcid.org/0000-0002-6267-3791

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