ANTI-ARTHROTIC, ANTI-GRANULOMATOUS AND ANTI-PYRETTIC EFFECTS OF CYLICODISCUS GABUNENSIS ETHYL ACETATE EXTRACT (HARMS) IN RATS

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ABSTRACT
Cylicodiscus gabunensis is used in traditional medicine for the treatment of headache, rheumatism, malaria and inflammatory related diseases. This work aimed to evaluate the anti-arthritis, anti-granulomatous and anti-pyretic effects of the ethyl acetate extract of Cylicodiscus gabunensis (EACg) in rats. The arthritis was induced with the Complete Freund Adjuvant, while the granuloma formation was induced by a carrageenan air pouch. Fever was induced by a brewer’s yeast suspension. A High Performance Liquid Chromatography (HPLC) was done for the extract active compounds analysis. The results showed that EACg (200 and 400mg/kg) and dexamethasone (1mg/kg) administered per os significantly reduced the paw oedema, SGOT, SGPT, cholesterol, triglycerides, LDL, nitrites, MDA level, and increased the levels of HDL, glutathione, catalase and SOD. The dose 200mg/kg reduce the granuloma tissue weight, (p<0.01) the volume of the exudate (p<0.01) and the migration of the white blood cells into the exudate (p<0.01), all the same as indomethacin (3mg/kg p.o). The fever was also reduced (p<0.01) by EACg and aspirin. The EACg anti-arthritis, anti-granulomatous and anti-pyretic, properties might be due to the presence of compounds such as cylcodiscoside, gabunoside and cyclodione revealed by the HPLC.

Key words: Arthritic; Granuloma; Leukocytes; Oedema; Pyretic; Complete Freund Adjuvant.

INTRODUCTION
Rheumatoid arthritis (RA) is an inflammatory autoimmune disease, which is characterized by chronic inflammation of the synovial tissues in multiple joints. RA can lead to joint destruction through inflammatory involvement of the synovial membrane, cartilage, and subchondral bone (Ramesh et al. 2013).
Inflammatory process has two phases: acute and chronic. Acute inflammation is characterized by fever, pain, and oedema, while chronic inflammation is characterized by cellular proliferation and granuloma formation (Rousselet et al., 2005). Pyrexia is the body’s natural defence to create an environment where infectious agent or damaged tissue can’t survive (Cheng et al., 2005). Elaboration of interleukin-1 and tumour necrosis factor-α is believed to initiate the synthesis and release of the fever-causing autacoid prostaglandin E2 (PGE₂) by the endothelium and pericytes of brain capillaries (Steiner et al., 2006).

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In clinic, there are various drugs such as azathioprine, tumor necrosis factor alpha (TNFα) blockers, non-steroidal anti-inflammatory drugs (NSAIDs) that are used for the treatment of RA. Although these drugs can temporarily alleviate the symptoms, they do not halt progression of joint destruction and they are accompanied with many undesirable adverse effects, including gastrointestinal bleeding, renal or hepatic failure (Gege-Adebayo and Shafe, 2013). This makes these drugs widely unacceptable, especially in the elderly where the disease is more prevalent (Osadebe and Okeye, 2003). Furthermore, the rising costs of orthodox medicine and the scarcity of some drugs give the phytomedicinal treatment a very important place in the management of inflammatory diseases. Among plants used against fever and inflammatory diseases, Cylicodiscus gabunensis (Mimosaceae) known as Adoum bokoka, by Eton population of Cameroon, is a very big tree that grows mainly in damp equatorial forest of Central and West Africa (Adjanohoun, 1996). The stem bark extract of this plant is used in traditional medicine as remedies for headache, rheumatism, diarrhoea, (Kouitcheu et al., 2006) and malaria (Okokon et al., 2002). The Ethyl acetate extract of the stem bark of Cylicodiscus gabunensis showed antimicrobial activity against pathogenic species isolated from patients including Escherichia coli, Klebsiella pneumoniae, Shigella dysenteriae, and Morganella morganii (Kouitcheu and Etoa, 2007). The ethyl acetate fraction this plant has analgesic and anti-inflammatory properties (Keugni et al., 2014). Considering the traditional uses of Cylicodiscus gabunensis stem’s barks, the present study was undertaken to investigate its anti-arthritic, anti-pyretic and anti-granulomatous properties in rats.

METHODOLOGY

Collection and identification of the plant material

The stem barks of Cylicodiscus gabunensis were harvested in April 2012, at Mbankomo (Center Region Cameroon), and a voucher specimen was deposited at the National Herbarium of Yaoundé, under the number 2154/ SRF/ Cam, after botanical identification by Dr Nole, a plant taxonomist in the Medical Institute of plants and Medicinal research (IMPM) of Yaoundé.

Preparation of plant extract

The powder (2500g) obtained from the air dried and grounded stem barks of C. gabunensis was macerated during 72 hours in 6L of methylene chloride/methanol (1:1). After filtration using a Whatman paper N°3, filtrate was concentrated using a HEIDOPHW 2000 rotary evaporator at 40 °C to obtain 197.67 g (yield 7.91 %) of a brown powder. Thereafter, 100g of this dough was exhausted in 1.5L of ethyl acetate, and concentrated using a rotary evaporator, giving 38g (yield 38 %) of a brown ethyl acetate fraction of the methyl chloride methanol.

High Performance Liquid Chromatography of the EACg

The High Performance Liquid Chromatography (HPLC) of the EACg was carried out according to the protocol described by Longo et al., (2015).

Animals

Adults Wistar males and females rats weighting 150g to 180g were used for this work. Animals were bred in the animal house of the Faculty of Science, University of Yaoundé I, in natural light conditions (12 light and 12 dark cycles). All the animals were allowed to have free access to food (standard diet for rodent) and water. Animals were deprived of food 14 hours, but allowed free access to water before the experiment. All experiments were performed according to guidelines for the care of laboratory animals from the Cameroon National Ethical Committee (Ref. no Fw-IRB00001954).

Drugs and chemicals

The drugs used for the experimentation were obtained from the pharmaceutical factory Sigma Aldrich, except valium and ketamine obtained from the pharmaceutical factory Roche. Chemicals used were obtained from local institute store.

Experimental procedure

Freud’s Complete Adjuvant (FCA) induced arthritis in rat

Twenty female’s rats were used for arthritis induction. Arthritis was induced by a single right hind paw injection of 0.1mL of Freund’s complete adjuvant (FCA). The swelling paw was measured up to 21 days using a plethysmometer (Ugo Basile, Italy, model 7140) at the moment 0h; 2h; 4h; 24h; then 5 ; 9 ;13 ; 17 ; and 21days (Suha, 2011). On the day 9, animals were divided into six groups and received treatments via oral route, once per day, up to the days 21 as followed:

Group 1: Control non arthritic rats received DMSO 2%. 
Group 2: Arthritis induced rats received DMSO 2 %.
Group 3: Arthritis induced rats received dexamethasone 1mg/kg.
Group 4: Arthritis induced rats received EACg 200mg/kg. 
Group 5: Arthritis induced rats received EACg 400mg/kg. 
On the day 21, all rats were anesthetized by intraperitoneal injection of valium (2mg/kg) and secondary ketamine (1mg/kg), then scarified by carotid section. The blood was collected in dry tubes for determination of the liver function such as Serum Glutamate Oxaloacetate Transferase (SGOT) and Serum Glutamate Pyruvate Transferase (SGPT) and lipid metabolism (HDL; LDL; triglycerides; cholesterol). The liver and spleen were removed, homogenized in Tris-HCl
buffer (0.1M, pH7.4) for the glutathione, catalase, superoxide dismutase (SOD), malondialdehyde (MDA), and nitrite contain determination.

**Carragenan air pouch induced granuloma**

The protocol used in this test was described by Dajeong et al., (2012), with slight modifications. Rats were divided into 5 groups of 5 females’ rats each. The animals of the first four groups were shaved on their back using a sterilised scissors, after anaesthesia with valium (3mg/kg) and ketamine (10mg/kg) through intraperitoneal pathway. The fifth unshaved remaining group was used as the control group, and they received neither air nor carrageenan. Thereafter, the shaved zone was disinfected with alcohol 70% using a sterilised syringe of 10mL, a pouch of 6mL of air was created in the shaved back, and filled with 4 mL of a saline NaCl (0.9%) carrageenan solution 2%. After injection with carrageenan, animals were treated during seven days by oral route as follows: The control group and one of the shaved group received DMSO 2%, 1mL per 100g b.w. Two other groups received the plant extract at the doses of 200 and 400mg·kg⁻¹ respectively. The last group received indomethacin 3mg/kg. On the day 8, animals of all groups were sacrificed under slight anaesthesia with ether. The blood leukocytes counting, was performed by an automatic counter (Sysmex-poch 100i). Each blood sample collected in EDTA tube was introduced in the cell counter. The number of lymphocytes, monocytes, granulocytes and the total leukocytes was obtained directly from the counter on a printed sheet of paper. For each animal, the granuloma tissue was carefully removed, then dried during 48 hours in the oven at 60 °C, and weighed with an accurate scale.

The percentages of inhibition relative to the control, the granuloma’s weight, or white blood cells number in the exudate were calculated using the formula:

\[ P = \frac{1-(X_{\text{treated}}/X_{\text{negative control}})}{100} \times (X= \text{mean value of the parameter concerned}) \]

The number of white blood cells was converted after the Malassez cell counting using the formula:

\[ Q = \frac{N \times f \times d \times 10^6}{n \times V} \]

\( N \): number of white blood cell counted with Malassez cell

\( V \): volume of one rectangle of Malassez cell (0.01 mm³)

\( f \): dilution factor = 1/10 ; \( n = 4 \) (number of rectangle in which cells were counted)

\( Q \): Number of leukocytes per litre of exudate

**Yeast induced pyrexia**

The antipyretic activity of the plant extract was assessed by the method described by Parimalakrishnan et al., (2007). Male Wistar rats were fasted overnight with water ad libitum before the experiments. Pyrexia was induced by subcutaneously injecting 20% (W/V) brewer's yeast suspension (10mL/kg) into the rat's neck region. Eighteen hours after the injection, the rectal temperature of each rat was measured using a digital thermometer (SK-1250 MC, Sato Keiryoki Mfg. Co., Ltd. Japan). Only rats that showed an increase in temperature in the range 0.5-1.5°C were used for experiments and divided in four groups of five rats each. The extract 200 and 400mg/kg were administered orally and the temperature was measured at 0.5; 1; 2; 3; 4; 5; and 6 hours after drugs administration.

**Statistical analysis**

Each result was presented as mean ± S E M (Standard Error on Mean). One way ANOVA followed by Turkey tests were performed, and the P value less than 0.05 was considered as significant.

**RESULTS AND DISCUSSION**

**Effects of EACg on the paw volume**

Injections of FCA to rats induced a paw oedema of 0.4 ± 0.03mL nine days later (Fig 1). From the day 9, administration of dexamethasone and EACg significantly reduced the oedema by 71.74 % (p<0.001); 53.91% (p<0.01); 45.21% (p<0.01) on the day 21, respectively for dexamethasone, the doses 200 and 400mg/kg of EACg.

**Effects of EACg on some serum biochemical parameters**

FCA during 21 days significantly (P<0.05) increases in the negative control group, when compared to the control, the liver SGOT and SGPT by 263.75% and 119.82% respectively (Tab 1). Administration of EACg and dexamethasone significantly reduced these transaminases rate. For the EACg (200mg/kg), the percentage of reduction was 311.65% and 59.48% for the control, the liver SGOT and SGPT respectively compared to 277.83% and 136.89 % for dexamethasone. FCA in the negative control group also significantly increased the serum concentration of cholesterol (61.03%), LDL (520.69%), triglyceride (115.17%), and decreased the concentration of HDL (120.80%). Administration of EACg (200 and 400mg/kg) significantly reduced the cholesterol level (81.77%; 66.71%), LDL (690.00 %; 197.27%), triglyceride (100.37%; 61.36%) and increased the level of HDL (88.77%; 66.71%). Dexamethasone also significantly reduced the level of cholesterol (72.09%), LDL (493.87%); Triglyceride (116.19%) and increased the level of HDL (102.83%).
Effects of EACg on some tissue biochemical parameters

The dose of 200mg/kg of EACg reduced respectively in the liver and spleen, the amount of proteins (97.95%; 69.45%), MDA (262.49%; 427.30%) and nitrites (64.40%; 140.12%), compared to 92.69%; 191, 90%; 74.39% for dexamethasone in the liver and 66.67%; 403.79%; 180.63% in the spleen respectively for proteins, MDA and nitrites. In contrast, on the negative control, FCA, significantly (p<0.001) increased proteins (119.40%; 68.04%) and SOD (194.26%; 122.70%) in the liver and spleen (Fig 2A,B,C).

The EACg reduced the concentration of glutathione GLU (114.83%; 206.41%) and the activity of catalase, CAT (341.92%; 295.07%) and superoxide dismutase, SOD (234.06%; 119.32%). The EACg at the dose of 200mg/kg as well as dexamethasone was efficient than the dose 400mg/kg to improve the parameters of oxidative stress. However, the EACg (200mg/kg) significantly (P<0.001), increased in the liver and the spleen respectively, the activity of CAT (335.51%, 292.08%); SOD (194.26%; 122.70%) and the concentration of GLU (135.51%; 201.52%) (Fig 2D,E,F).

Effect of EACg on granuloma tissue weight in rat

The administration of the ethyl acetate extract of Cylicodiscus gabunensis at the doses of 200 and 400mg.kg-1 significantly (p<0.01) reduced the weight of the granuloma tissue in rat (Fig.3). In the negative control group, the carrageenan induced the formation of 4.20 ± 0.22g of granuloma tissue. In the presence of the plant extract at the doses of 200 and 400mg/kg, the weight was reduced to 2.23±0.13 (p<0.001) and 3.24±0.05 g (p<0.01) respectively, corresponding to 46.63%, and 22.69% respectively. Indomethacin also displayed a significant reduction of the granuloma tissue weight up to 53.33% (p<0.001).

Effects of EACg on the exudate volume

The EACg reduced exudate accumulation in the back air pouch, with a significant percentage of inhibition of 56.63 % at the dose of 200mg/kg (p<0.001), compared to 58.41% inhibition for indomethacin (p<0.001). In the negative control group, the carrageenan induced the accumulation of 8.32 ± 0.22mL of exudate (Fig 4).

Effects of EACg on the number of white blood cell in the exudate and in the blood

Injection of carrageenan in the rat back pouch induced accumulation of white blood cells in the exudate (Fig 5A). In the blood, the number of total white blood cells in the negative control group was significantly (p<0.001) decreased vs the control (Fig 5B). The EACg at all the doses, significantly reduced the number of white blood cells in the exudate, and increased their number in the blood. At the dose of 200mg/kg, the number of total white blood cells was increased from 7.34 ± 1.34 x10^3 (negative control) to 17.40 ± 3.31 x10^3 per micro litre of blood (p<0.001). At this same dose of 200mg/kg, the number of blood leukocytes was 4.99 ±0.28 x10^3; 9.96 ± 1.4 x10^3; 3.67 ± 0.29 x10^3 per µL of blood, respectively for granulocytes (Figure 5C); lymphocytes (Fig 5D) and monocytes (Fig 5E); Indomethacin also significantly increased the number of polymorphonuclear cells (4.18 ± 0.30 x10^3), lymphocytes (6.92 ± 1.10 x10^3) and monocytes (3.02 ± 0.36 x10^3).

Effect of the EACg on the yeast induced pyrexia in rat

All the doses of the extract showed a significant antipyretic activity similarly to that of the standard drug aspirin (p < 0.001). The subcutaneous injection of yeast suspension marked elevated rectal temperature 18 hours after injection (Tab 2).

High Performance Liquid Chromatography of the EACg

The chromatogram of the ethyl acetate extract of Cylicodiscus gabunensis stem bark showed the relative abundance of different compounds (Fig 6). The study of the chromatogram permitted to identify tree triterpenoids identified as ganosioside, clycicodiscoside and cyclodione.

Table 1. Effects of EACg on Freud’s Complete Adjuvant induced some serum biochemical parameters

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>FCA+DMSO</th>
<th>FCA+Dexa</th>
<th>FCA+Cg200</th>
<th>FCA+Cg400</th>
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<tr>
<td>TG (mg/dL)</td>
<td>54.10±5.52</td>
<td>116.41±9.20</td>
<td>53.84±4.67</td>
<td>57.43±6.50</td>
<td>72.05±7.55</td>
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<tr>
<td>HDL (mg/dL)</td>
<td>116.47±7.54</td>
<td>51.81±4.94</td>
<td>103.80±8.09</td>
<td>97.80±9.89</td>
<td>86.37±9.10</td>
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<td>LDL (mg/dL)</td>
<td>29.67±3.21</td>
<td>184.16±16.79</td>
<td>31.01±2.56</td>
<td>23.29±7.68</td>
<td>61.95±8.32</td>
</tr>
<tr>
<td>CHO (mg/dL)</td>
<td>146.15±6.35</td>
<td>235.36±18.24</td>
<td>136.83±9.70</td>
<td>129.97±6.54</td>
<td>140.53±10.87</td>
</tr>
<tr>
<td>SGPT (UI)</td>
<td>16.98±1.84</td>
<td>37.32±4.45</td>
<td>15.54±1.70</td>
<td>23.40±1.26</td>
<td>25.80±1.53</td>
</tr>
<tr>
<td>SGOT (UI)</td>
<td>30.68±1.40</td>
<td>11.58±1.90</td>
<td>29.51±1.11</td>
<td>27.09±1.60</td>
<td>34.43±4.24</td>
</tr>
</tbody>
</table>

Values are means ± SEM, n=5. a=**; p<0.001; b=***p<0.01; significant differences vs the negative control (FCA+DMSO). d=++p<0.01; f=+p<0.05 significant differences vs the normal control (DMSO). Dixa: dexamethasone; FCA: Freund’s Complete Adjuvant, Cg: Cylicodiscus gabunensis, DMSO: Dimethyl sulfoxide; EACg: Ethyl acetate extract of Cylicodiscus gabunensis.
Fig 1. Inhibition of Freud’s Complete Adjuvant-induced hind paw swelling by EACg

Curves represent oedema volume ± SEM (mL), n=5
a= ***p<0.001; b= **p<0.01; c = *p<0.05 significant differences vs the negative control (FCA+ DMSO); Cg : *Cyclicodiscus gabunensis*; Dexa : Dexamethasone; FCA : Freund’s Complete Adjuvant; DMSO : Dimethyle sulfoxide; EACg : ethyl acetate extract of *Cyclicodiscus gabunensis*. D : day; h : hour.

Fig 2. Effects of EACg on the concentration of proteins (A); GLU (B); CAT (C); SOD (D); nitrite (E); MDA (F)
Each bar represents mean ± SEM, \( n=5 \) a=***\( p<0.001 \); b=**\( p<0.01 \); c=*\( p<0.05 \): significant differences vs the negative control (FCA+DMSO). d=+++\( p<0.001 \); e=++\( p<0.01 \); f=+\( p<0.05 \): significant differences vs the control (DMSO). Dexa: dexamethasone, FCA: Freund’s Complete Adjuvant, Cg: Cylicodiscus gabunensis, DMSO: Dimethyl sulfoxide; EACg: ethyl acetate extract of Cylicodiscus gabunensis; MDA: Malondialdehyde, SOD: Superoxide dismutase; CAT: Catalase; GLU: Glutathione.

**Fig 3. Effects of EACg on granuloma tissue weight in rat**

Each bar represents mean weight of granuloma tissue ± SEM (g), \( n=5 \) a=***\( p<0.001 \); b=**\( p<0.01 \), values are significant when compared to the negative control group (carra +DMSO). Carra: carrageenan; Indo: indomethacin; Cg: Cylicodiscus gabunensis; EACg: Ethyl acetate extract of Cylicodiscus gabunensis; DMSO: dimethyl sulfoxide.

**Fig 4. Effects of EACg on the exudate volume in rat**

Each bar represents mean exudate volume ± SEM (mL), \( n=5 \) a=***\( p<0.001 \), significant difference vs the negative control group (carra+DMSO). Carra: carrageenan; Indo: indomethacin; C.g: Cylicodiscus gabunensis; EACg: Ethyl acetate extract of Cylicodiscus gabunensis; DMSO: dimethyl sulfoxide.
Figure 5. Effects of EACg on exudate white blood cells (A); total blood leukocytes (B); blood polymorphonuclears amount (C); blood lymphocytes amount (D); blood monocytes amount (E).

Each bar represents mean ± SEM, n= 5. a=***p<0.001; b=**p<0.01; c=*p<0.05: significant differences vs the negative control (carra+DMSO). d=+++p<0.001; e=++p<0.01; f=+p<0.0 significant differences vs the normal control group (Water) Carra: carrageenan; Indo: indomethacin; Cg: Cylicodiscus gabunensis; EACg: Ethyl acetate extract of Cylicodiscus gabunensis DMSO: Dimethyl sulfoxide.
Fig 6a. Spectra fingerprint HPLC of the EACg

Fig 6b. Triterpenes arising from the fingerprint HPLC of EACg

[M+H]⁺ = 827.43965: Gabunoside.

[M+H]⁺ = 473.3622: Cyclicodiscoside

[M+H]⁺ = 601.4256: Cyclodione
DISCUSSION

Arthritic animal treated with dexamethasone or the plant extract developed low oedema volume. This can be explained by the inhibition of neutrophil migration, and exudate formation by dexamethasone (Subash, 2012). Terpenes contain in the EACg may display anti-oedematous effects by the same mechanism as dexamethasone. Freund’s adjuvant induced arthritis occurs through cell-mediated autoimmunity. Inoculation of FCA activates macrophages and lymphocytes or their products such as, cytokines, and chemokine which are involved in abnormal lipid and protein metabolism (Schorlemmer and Bartlett, 1999).

FCA also induced free radicals production, which lead to membrane peroxidation with MDA level increase. Furthermore, free radicals provoke depletion of glutathione concentration, and the activity of anti-oxidant enzymes as CAT and SOD in tissues like liver, spleen, and kidney (Jung et al., 2005) then increase the spleen and liver contain in MDA and nitrite. The HDL, the glutathione and the activity of peroxidase (CAT, SOD) were also significantly reduced. Administration of the plant extract and dexamethasone to the rats, significantly prevent cellular migration into the vascular endothelium before extravasation to the injured tissues (Kobayashi and Boelte, 2007). Administration of indomethacin and the plant extract to rats significantly prevent cellular migration into the exudate. In fact, indomethacin is an anti-inflammatory drug that impairs inflammatory response by many mechanisms among which inhibition of the white blood cells migration towards the inflammatory site (Suresha et al., 2012). The EACg may act similarly on inflammation. Prostaglandins E2, has been described as an effective mediators in the pathophysiology of RA (Jennifer et al., 2002).

Prostaglandins E2 induced vasodilatation and stimulate the hypothalamus to produce fever (Steiner et al., 2006). Yeast is among factors that stimulate prostaglandins production to induce fever (Parimalakrishnan, 2007). The fever condition enhanced formation of cytokines such as interleukins, interferons and tumour necrosis factor α, inducing the synthesis of prostaglandin (Dajeong et al., 2012).

The plants extract at all the doses, and aspirin decrease the body temperature. Aspirin, a non-steroidal anti-phlogististics display antipyretic activity by inhibiting the cyclooxygenase 2, an enzyme responsible for the synthesis of prostaglandins of series E (Cheng et al., 2005). The fever condition enhanced formation of cytokines such as interleukins, interferons and tumour necrosis factor α, inducing the synthesis of prostaglandin (Dajeong et al., 2012).

The plants extract at all the doses, and aspirin decrease the body temperature. Aspirin, a non-steroidal anti-phlogististics display antipyretic activity by inhibiting the cyclooxygenase 2, an enzyme responsible for the synthesis of prostaglandins of series E (Cheng et al., 2005). Some secondary metabolites of the plant extract as characterized by Keugni et al., (2014) and Kouitchu et al., (2006) may act by the same mechanism as aspirin.
The qualitative analysis of EACg by HPLC coupled to the mass chromatography revealed the presence of three triterpenoids previously isolated in the stem bark of C. gabunensis and named as gabunoside, cylicodiscoside and cyclodione (Tane et al., 1995; Tene et al., 2010). It has been reported that triterpenoids possess various pharmacological properties including anti-inflammatory activity (Geetha, and Varalkshmi, 2001). The dose 200 mg/kg was more efficient than 400 mg/kg.

CONCLUSION
According to these results, the anti-arthritic, anti-inflammatory and anti-pyretic properties of EACg stem bark might be due to its content in triterpenoids that interact with nitric oxide, pathways, free radical neutralization. These pharmacological activities justified the traditional use of Cylicodiscus gabunensis in the management of inflammatory diseases such as malaria and rheumatism.

REFERENCES

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CONFLICT OF INTEREST
The authors declare that they have no conflict of interest.


