

## Evaluation of Anti-inflammatory, Antipyretic, Antioxidant Effects, total polyphenol and flavonoid contents and phytochemical screening of *Maranthes glabra*'s leaf Extracts. (Oliv.) Prance leaf extracts (Chrysobalanaceae)

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**Abstract: Purpose :** The objective of the present study was to evaluate the anti-inflammatory, antipyretic, antioxidant properties of the aqueous extract, the content of polyphenols and total flavonoids (extracts: aqueous, hydroethanolic, ethanolic) and the chemical composition of the different families of leaves of *Maranthes glabra*.

**Methodology:** The pharmacological activities of the aqueous extract of *Maranthes glabra*, were evaluated using the appropriate methods including: inflammation induced by carrageenan 1%, fever caused by the suspension of yeast (*Saccharomyces cerevisiae*) 20% and the production of oxygenated derivatives by the quantitative method of DPPH with gallic acid as the reference molecule. The determination of the different chemical families was carried out by the assays of the total polyphenols by the "Folin-Ciocalteu" reagent; flavonoids by the aluminum trichloride method (AlCl<sub>3</sub>.10%) and tests of conventional coloring reactions in tubes were also used to reveal the different chemical compounds.

**Results:** The aqueous extract of the leaves of *Maranthes glabra*, at doses of 250 and 500 mg / kg significantly reduce the inflammation caused by carrageenan 1% and it is admitted that these leaves have anti-inflammatory properties. The evaluation of the antipyretic activity showed that the same aqueous extract at the same doses, significantly reduces the fever induced by a 20% suspension of beer yeast in the rats and also has antipyretic properties. The antioxidant power of three (3) types of extracts was evaluated by the quantitative method of DPPH: aqueous (0.234 mg / mL), hydroethanol (0.273 mg / mL) and ethanolic (IC<sub>50</sub> = 0.195 mg / mL), the reference molecule, gallic acid (0.234 mg / mL). The determination of the total polyphenols and flavonoids revealed that it is the aqueous extract which has the highest contents with respective contents of: 8.25 mg E AG / g MS, and: 16.20 ± 0.126 mg EQ / gMS compared to hydroethanol and ethanolic extracts. These pharmacological properties would be attributed to chemical compounds envoyed of these leaves of *Maranthes glabra*: The tests of the tube staining reactions of the phytochemical profile of the extracts of *Maranthes glabra* leaves revealed the presence of six (6) chemical families: alkaloids, flavonoids, saponosides, cardiotonic heterosides, tannins and quinones free.

**Conclusion :** The leaves of *Maranthes glabra* are frequently used in traditional medicine in the treatment of several pathologies including inflammation, fever ... .. The objective of this study was to evaluate the anti-inflammatory, antipyretic, antioxidant, total polyphenol and flavonoid content of *Maranthes glabra* leaves, The results obtained showed that the aqueous extract of the leaves of *Maranthes glabra*.opposes the evolution of the volume of rat paw edema induced by carrageen 1%. This extract therefore has anti-inflammatory properties; Significantly reduced hyperthermia caused by 20% yeast suspension in Wistar rats. This extract therefore has antipyretic effects. Like the reference molecule (gallic acid), the aqueous extract has an antioxidant capacity that can be used for the fight against free radicals because of the best anti-radical activity.

*The richness of this extract polyphenols and total flavonoids justifies the anti-inflammatory, antipyretic and antioxidant effects observed because these compounds are recognized and are at the origin of these properties.*

**Keywords:** *Maranthes glabra; anti-inflammatory, antipyretic, antioxidant, total polyphenols, total flavonoids*

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## I. Introduction

*Maranthes glabra* is a plant found in Africa. Previous work has shown that the leaves of *Maranthes glabra* have not yet been the subject of a scientific study on the anti-inflammatory, antipyretic, antioxidant properties, and the evaluation of polyphenol contents, total flavonoids and chemical screening (Bouquet 1969, Adjanohoum et al., 1983, Abena et al., 2003, Agbonon et al., 2002, Akpan and Etuk 2012, Elion et al., 2017, Epa 2015). In Congo *Maranthes glabra* leaves are frequently used by traditional healers in the treatment of inflammatory diseases, fever and oxygen-related diseases (Bouquet 1969, Adjanohoum et al., 1983, Bahorun et al., 1996, Ayoola et al. al., 2011; Epa et al., 2018; Epa et al., 2019). The traditional use of these, justifies in the present study the evaluation of the anti-inflammatory, antipyretic, antioxidant effects, polyphenol contents, total flavonoids and chemical screening of the leaves.

## II. Material And Methods

### Plant material

Samples of dry *Maranthes glabra* leaves were used. These samples were harvested, in the north of Congo Brazzaville, in the “Cuvette-ouest” at the geographical references of 0505581 of Latitude and 9839554UTM of Longitude. They were identified after harvest by Botanists-Taxonomists. A sample of this plant has been deposited at the herbarium of the Institute of Research of Exact and Natural Sciences (IRSEN) of Congo. These leaves were dried at room temperature away from the sun ( $28 \pm 1$  °C) in the laboratory of “Sciences de la Vie et de la Terre” of the Ecole Normale Supérieure for two weeks and then reduced to powder with the help of a grinder type mill (Blender Model: YF-1737). The powder obtained was stored for pharmacological studies.

### Animal material.

Male albino rats of wistar strain weighing between 180 and 300 g were chosen for animal tests excluding gravid or lactating females. These rats were raised at the animal house of the Faculty of Science and Technology, with adequate ventilation and at room temperature. They were fed regularly and had access to tap water at will.

### Methods

#### Preparation of *Maranthes glabra* leaf extracts

The aqueous extracts of 25 or 50 g, hydroethanol and ethanolic *Maranthes glabra* leaves were prepared in the same way: 50 g of vegetable powder in 500 ml (aqueous extract); 300 mL water / 200 mL ethanol 900 (hydroethanolic extract) and 500 mL ethanol 900 (ethanolic extract), with magnetic stirring for 72 hours. The macerates filtered on hydrophilic cotton were evaporated to dryness at a temperature of 70 ° C. (aqueous extract), 40 ° C. ethanolic extract) and 52 ° C. (hydroethanol extract). The concentrates obtained served as extracts to make the different dosages. The doses of 200 and 500 mg / kg were used to evaluate the antidiabetic properties.

The evaluation of the anti-inflammatory, antipyretic and antioxidant properties was carried out with the aqueous extract of the leaves in accordance with the therapeutic use of traditional medicine, the extract closest to the population.

#### Evaluation of the anti-inflammatory effect of the aqueous extract of the leaves of *Maranthes glabra*.

This method was used by: Winter et al., 1962; Sawadogo et al., 2006; Sajeli et al., 2010; Elion Itou et al., 2014; Epa, 2015; Elion et al., 2017; Epa et al., 2018 to cause inflammation in the rat with carrageenan 1%. The animals used for this pharmacological test are Wistar rats left on an empty stomach 24 hours before the experiment. These are divided into 4 lots of 3 rats each as follows:

- Lot 1 (control): distilled water (0.5ml / 100g). (p.c.);
- Lot 2: diclofenac (50 mg / kg). (p.c.);
- Lots 3 and 4: aqueous extract (250 and 500 mg / kg). (P.c.).

One (1) hour after administration of the extract, 1% carrageenin is injected under the footpad at a dose of 0.05 ml.

The evolution of the edema was measured 30 min, 1 h, 2 h, 3 h, 4 h, 5 h and 6 h after.

The anti-inflammatory effect was evaluated by measuring the volume of the paw received carrageenan using the Plethysmometer.

The percent inhibition (% I) of the edema is calculated by the following formula:

$$\% I = ((v_t - v_0)_{\text{control}} - (v_t - v_0)_{\text{test}}) / (v_t - v_0)_{\text{control}} \times 100;$$

$v_0$ : volume of the paw before injection of carrageenan;

$v_t$ : volume of the paw at different times after administration of the test products and injection of carrageenan;

$v_t - v_0 = v$ : volume of the edema.

#### **Evaluation of the antipyretic effect of the aqueous extract of *Maranthus glabra* leaves induced by the suspension of 20% beer yeast in the rat.**

The method used has been described by: Akpan and Etuk 2012; Epa et al., 2018; Epa et al., 2019.

After the initial rectal temperature was taken, the rats received, subcutaneously in the dorsolateral region, an injection of a suspension of beer's yeast (*Saccharomyces cerevisiae*) (20%) at the standard dose of 10. ml / kg body weight (Pc). The animals were fasted for seventeen (17) hours of fever duration, then the rectal temperature was again raised and the animals which did not have fever (temperature difference < 0.5 °) were excluded from the experiment. 20 rats constituting four batches of 5 rats each were treated as follows:

- Lot 1: control (10ml / kg) (P.c.)
- Lot 2: Aspirin (500 mg / kg) (P.c.)
- Batches 3 and 4: Aqueous extract (250 and 500 mg / kg) (P.

The rectal temperature was taken at 1 h, 2 h, 3 h, 4 h and 5 h after the administration of the substances.

#### **Evaluation of antioxidant activity**

The DPPH analytical method described by Brand-Willam et al., 1995; Majhenic et al., 2007 was used to evaluate the antioxidant activity of the aqueous, hydroethanol and ethanolic extracts of *Maranthus glabra* leaves and the standard antioxidant (gallic acid).

The results are expressed as percent inhibition (% I). It was calculated by the following formula:

$$\% I = (\text{white absorbance} - \text{absorbance extract}) / (\text{white Absorbance}) \times 100$$

#### **Dosage of total polyphenols (PPT).**

The determination of the total polyphenols of leaf extracts and gallic acid was carried out by the "Folin-Ciocalteu" reagent according to the method of Singleton and Rossi (1965). The absorbance was read on a spectrophotometer at 725 nm against a blank of distilled water.

#### **Dosage of total flavonoids (FVT).**

The total flavonoid assay of the various extracts of *Maranthus glabra* leaves and of quercetin was performed according to the Aluminum Trichloride method first described by Bahorum et al. In 1996. The absorbance was read at 510 nm. .

#### **Screening phytochemical leaves of *Maranthus glabra*.**

It was carried out by tube reactions according to the protocols described by (Adjanahoum et al., 1984, Braca et al.2002, Amzal et al., 2008, Boubekri2014).

#### **Statistical analysis of the results**

The results were expressed as mean values  $\pm$  S.D. For the analysis of statistical significance ANOVA were applied, excepted when normality and equal variance were passed, it was followed by the Tukey test. Student's t test was applied to study the significance of difference between two treatment groups. In all cases,  $p < 0.05$  was considered to be significant.

### **III. Results**

#### **Anti-inflammatory effects of the aqueous extract of *Maranthus glabra* leaves on acute inflammation induced by carrageenan 1%.**

Figure 1 shows the decrease in the volume of rat paw edema by the aqueous extract and the reference molecule. FIG. 2 shows the inhibition percentages of the inflammation at 2 hours, 4 hours and 6 hours, of the molecule of diclofenac 50 mg / kg with the inhibition percentages of: 90.32; 97.69 and 98.99% and aqueous extract at doses of 250 and 500 mg / kg, respectively, at the inhibition percentages of: (89.32, 96.83 and 99.94%) and: 87.09, 94.8 and 99.98%).

### Antipyretic effects of the aqueous extract of the leaves of *Maranthes glabra* Effect of the aqueous extract of the leaves of *Maranthes glabra* on the fever induced by a suspension of the yeast of beer at 20% in the rat

Table I shows the effect of the aqueous extract of the leaves of *Maranthes glabra* sur on the hyperthermia induced by the injection of a suspension of the yeast of beer (20%).

Injection of suspension of beer's yeast has caused rectal temperature rise after 17 hours in all rats. In control rats treated with distilled water, an increase in rectal temperature was observed, which increased from  $37.7 \pm 0.12$  to  $37.82 \pm 0.36$  at the 5th hour. For rats treated with aspirin (reference molecule) at the dose of 500 mg / kg, a decrease in rectal temperature at the 2nd hour is observed with a value of  $37.18 \pm 0.10$  to a value of  $36.68 \pm 0.16$  at the 5th hour compared to the control rats. The aqueous extract of the leaves of *Maranthes glabra*. At a dose of 500 mg / kg, the rectal temperature decreases in rats from the 3rd hour with a value of  $37.2 \pm 0.18$  to a value of  $36.26 \pm 0.18$  at the 5th hour. the same is true for the aqueous extract at the dose of 250 mg / kg which also began to decrease from the third hour with a value of  $36.88 \pm 0.27$  up to a value of  $36.32 \pm 0.15$  at the 5th hour.

Table I: Effect of the aqueous extract of *Maranthes glabra* leaves on the hyperthermia induced by the injection of yeast (20%) in rats.

#### Total polyphenol containers.

The variations in the total polyphenol contents of the various extracts of *Maranthes glabra* leaves are shown in figure 4. This figure 4 shows that the aqueous extract is richer in total polyphenols (PPT) (8.25 mg EAG / gMS), followed by the hydroethanolic extract (7.4 mg EAG / gMS) and the ethanolic extract (4.22 mgEAG / gMS). Each value represents the average content with  $n = 3$ .

#### Total flavonoids content.

The different values of the total flavonoid contents of the different extracts of the *Maranthes glabra* leaves are shown in Figure 5. These results show that the aqueous extract is richer in total flavonoids ( $16.20 \pm 0.126$  mg EQ / gMS), followed by the ethanol extract ( $12.061 \pm 0.07$  mg EQ / gMS) and in the end the extract hydroethanol ( $11.792 \pm 0.08$  mg EAG / gMS). Each value represents the concentration,  $n = 3$ . It is found that the aqueous extract is richer in flavonoids than the other extracts.

#### Phytochemical Screening

The phytohimic screening of *Maranthes glabra* leaf extracts revealed six (6) chemical families and the absence of two (2) others was notified. The results of this phytochemical profile are shown in Table II

### IV. Discussion

The aim of our work was to evaluate the anti-inflammatory, antipyretic, antioxidant properties, and phenolic compound contents of the aqueous, hydroethanolic and ethanolic extracts of *Maranthes glabra* leaves.

The present study showed that the aqueous extract of *Maranthes glabra* leaves has anti-inflammatory, antipyretic, and antioxidant properties. The various extracts: aqueous, hydroethanolic and ethanolic contain the phenolic compounds.

#### Anti-inflammatory effect of the aqueous extract of *Maranthes glabra* leaves

The aqueous extract at doses of 250 and 500 mg / kg significantly reduce edema with inhibition 2 hours after injection of 1% carrageenan compared to control rats. Indeed the aqueous extract of the bark trunk of this same plant significantly inhibits edema (250 and 500 mg / kg) with maximum inhibition 3 hours after carrageenin injection compared to controls. Agbonon et al., 2002, Akçahan et al., 2004; Ayoola et al., 2011; Epa et al., 2018. Carrageenan is a mucopolysaccharide which administered at the plantar level to rats causes acute inflammation which is manifested by the appearance of edema which results from the trans-parietal diffusion, consequence of the increase of the blood pressure in the capillary and venous network thus causing the increase of the blood pressure. Carrageenan acts synergistically by the cyclooxygenase 2 pathway by stimulating prostaglandin synthesis, the thickness of the edema reaches its maximum 6 hours after the application of 1% carrageenan (Winter et al., 1962, Banerjee et al. Rousselet et al., 2005, Epa et al., 2018, Epa et al., 2019). The inflammatory effects of carrageenan are potentiated by the anti-inflammatory effects of the aqueous extract of *Maranthes glabra* leaves (Figure 1 and Figure 2). This suggests that the leaves better inhibit inflammation at a dose of 500 mg / ml. kg; this effect is due to the presence of flavonoids and saponosides, which are powerful anti-inflammatory and antioxidant agents (Ryn et al., 2010, Ribeiro et al., 2010, Dufall et al., 2003). Previous work by other authors have shown the anti-inflammatory properties of other plant extracts on carrageenan-induced inflammation in rats (Prashant et al., 2005, Okemy-Andissa et al., 2006). Inserm 2016). The pro-

inflammatory and inflammatory molecules released (eg cytokine and others) are often pyrogenic and cause fever (Stalikas 2007, Steinnan 2008;).

#### **Antipyretic effect of the aqueous extract of *Maranthes glabra* leaves**

The yeast suspension (20%) induces hyperthermia in rats which allowed to evaluate the antipyretic effect of *Maranthes glabra* leaves. The aqueous extract of *Maranthes glabra* leaves at doses of 250 and 500 mg / kg and aspirin 500 mg / kg significantly reduce the hyperthermia caused by the injection of a suspension of yeast (20%) (Table I) . The antipyretic effect of the extract could be due to phenolic compounds that would inhibit the release of cytokines and the biosynthesis of prostaglandins which are pyrogens. The antipyretic properties of plant extracts in rats have been shown by the work of: Abena et al., 1997 and 2003, Sawadogo et al. Aronoff et al., 2006; Steinnan 2008; Akpan et al., 2012, who showed antipyretic effects. Inflammation is often accompanied by free radicals that destroy healthy cells and thus aggravate inflammation (Ames et al., 1981, Freeman and O'Neil 1984, Ferrari et al., 1991, Therade-Matharan 2004, Fontaine et al., 2002. Rochette 2008).

#### **Antioxidant effect of leaves of *Maranthes glabra***

The results obtained (FIG. 3) show that the extracts of *Maranthes glabra* leaves have an antioxidant activity with respect to DPPH. Thus the antioxidant activity of these extracts is probably due to the presence of phenolic compounds. The results obtained showed that the ethanolic extract has a high antioxidant capacity with an IC50 of 0.196 mg / ml much higher than that of the gallic acid reference molecule whose value is 0.234 mg / ml. Inhibitory concentration (IC 50) is inversely proportional to the antioxidant capacity of a compound. indeed, the lower the IC50 the more the plant has a high antioxidant potential (Fontaine et al., 2002, Husebye et al., 2006). Work previously carried out on the trunk bark of the same plant also showed that they have antioxidant properties with an IC50 of 1.25 mg / ml. (Aganga et al., 2001, Afonso et al., 2007, Belaich and Boujraf 2016).

#### **Dosage of total polyphenols.**

The dosage of the total polyphenols extracted from the leaves of *Maranthes glabra* showed that The levels of phenolic compounds in these leaves vary according to the type of extract (Figure 4). A rather high content was found in the aqueous extract with a value of (8.25 mg EAG / gMS), followed by the hydroethanolic extract with a value of (7.4 mg EAG / gMS) and in the end the ethanolic extract with a value of (4.22 mgEAG / gMS). Other work previously carried out by M'hadj Moussa Ali (2012) had shown the antioxidant properties of other leaves on the  $\alpha$ -amylase, the presence of phenolic compounds revealed in these extracts would be at the origin of the capture and the neutralization of free radicals or oxygenated derivatives frequently observed during inflammations and other infections that cause fever (Bouquet, 1969, Bahorun et al., 1996, Stalikas 2007, Ameszal 2010, Boubekri 2014).

These free radicals are potentially dangerous and aggravate inflammations by attacking healthy cells (lyses of cell membranes, proteolysis, proteins, lipid peroxidation and denaturation of DNA).

Indeed, the capture of these oxygenated derivatives by phenolic compounds would protect these healthy cells against the risk of cancer and circumscribe the inflammation until healing of tissues injured by oxygenated derivatives (Duranteau 1998, Bilodeau and Hubel 2003, Boutet2009, Dupre -Crochet et al., 2013). Other studies on other plants have shown the presence of phenolic compounds in the root leaves of *Terminalia macroptera* (Stalikas 2007, Boubekri 2014).

#### **Dosage of total flavonoids**

The total flavonoid content of the aqueous, hydroethanolic, ethanolic extracts of the *Maranthes glabra* leaves shown in FIG. 5 varies significantly according to the solvent used. This content is higher in the aqueous extract with a value (16.20  $\pm$  0.126 mg EQ / gMS), followed by the ethanol extract with a value (12.061  $\pm$  0.07 mg EQ / gMS) and finally the hydroethanolic extract with a value (11.792  $\pm$  0.08 mgEAG / gMS). It is likely that these phenolic substances are more excreted in the polar solvent such as water and ethanol (Aganga et al., 2001, Hersh et al., 2008). These flavonoids present in these leaves are responsible for the anti-inflammatory, antipyretic and antioxidant properties of these extracts. They would act by capturing and destroying free radicals and blocking pyrogenic substances that cause fever. (Bouquet A., 1969, Braca et al., 2002).

#### **Phytochemical profile of *Maranthes glabra* leaf extracts**

Tests of tube staining reactions in the phytochemical profile of *Maranthes glabra* leaf extracts (Table II) revealed the presence of six (6) chemical families: alkaloids, flavonoids, saponosides, cardiotonic heterosides, free tannins and quinones, which would be substances responsible for the various anti-inflammatory, antipyretic and oxidant effects observed (Brand-William et al., 1995, Aganga et al., 2001, Amzal 2010, Boubekri2014) .The absence of Anthraquinones, Anthocyanins and Sterols and terpenes in these leaves.

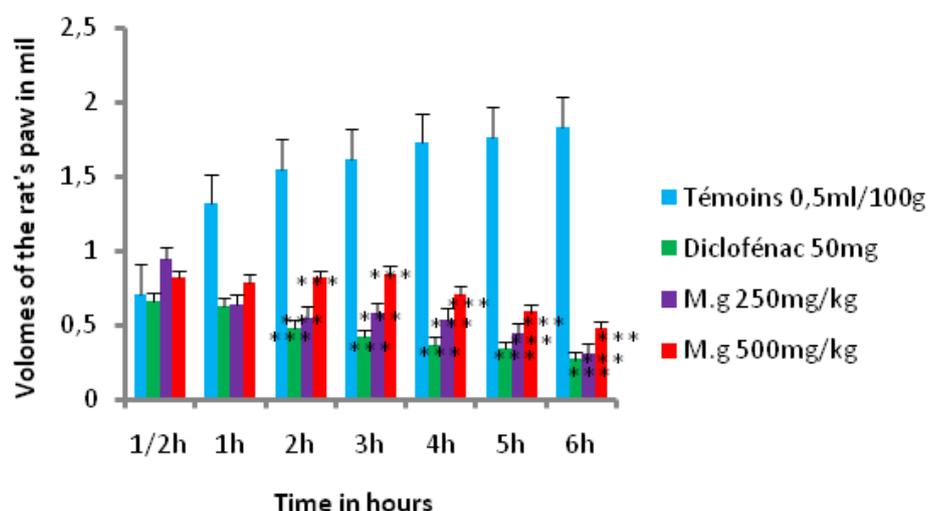
## V. Conclusion

The leaves of *Maranthes glabra* are frequently used in traditional medicine in the treatment of several pathologies including inflammation, fever. The objective of this study was to evaluate the anti-inflammatory, antipyretic, antioxidant properties, total polyphenol and flavonoid content of *Maranthes glabra* leaves. The results obtained showed that the aqueous extract of the leaves of *Maranthes glabra* opposes the evolution of the volume of rat paw edema induced by carrageenan 1%. This extract therefore has anti-inflammatory properties; Significantly reduced hyperthermia caused by 20% yeast suspension in Wistar rats. This extract therefore has antipyretic effects. Like the reference molecule (gallic acid), the aqueous extract has an antioxidant capacity that can be used for the fight against free radicals because of the best anti-radical activity. The richness of this extract polyphenols and total flavonoids justifies the anti-inflammatory, antipyretic and antioxidant effects observed because these compounds are recognized and are at the origin of these properties.

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- [53]. Figure 1: shows the decrease in the volume of rat paw edema by the aqueous extract and the reference molecule.



**Figure 1.** Effect of aqueous leaf extract of *Maranthus glabra* (M.g.) on carrageenan-induced 1% rat paw edema in rats.  $p < 0.05$ ; \*\*\*  $p < 0.001$ .

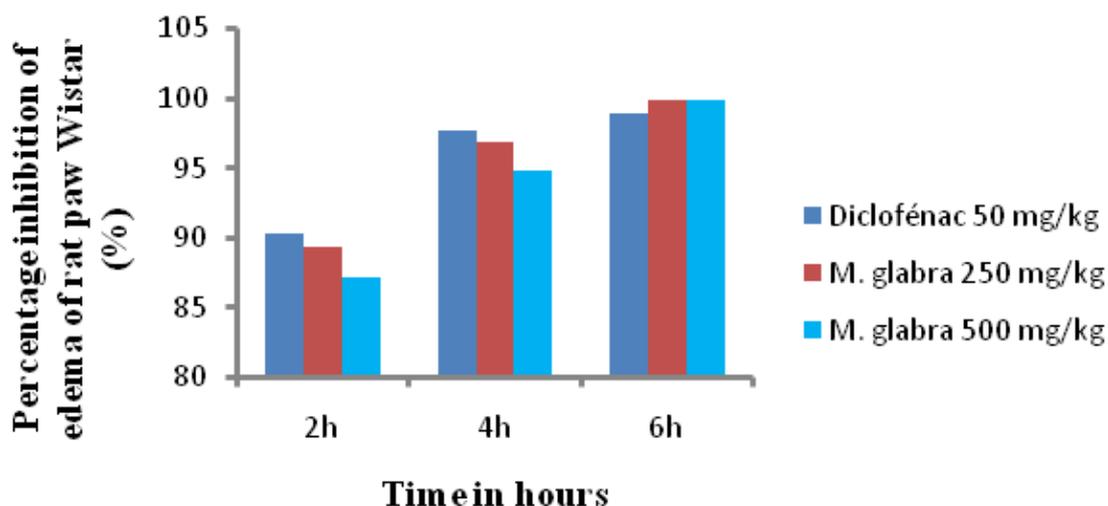


Figure 2. Inhibition of acute inflammation by diclofenac 50mg / kg and Aqueous extract at doses of 250 and 500 mg / kg of leaves of *Maranthes glabra* (M.g.).

Table I: Effect of the aqueous extract of *Maranthes glabra* leaves on the hyperthermia induced by the injection of yeast (20%) in rats

Treatment	Température (°C)						
	T <sub>initiale</sub>	T <sub>fièvre</sub>	1 h	2 h	3 h	4 h	5 h
Distilled water (10 mg/kg)	36.48 ± 0.24	37.7 ± 0.12	37.88 ± 0.18	37.82 ± 0.29	37.46 ± 0.32	37.74 ± 0.31	37.82 ± 0.36
Aspirine(500 mg/kg)	36.44 ± 0.26	37.4 ± 0.24	37.26 ± 0.18	37.18 ± 0.10 ***	36.76 ± 0.12 ***	36.74 ± 0.29 ***	36.68 ± 0.16 ***
Extract(250 mg/kg)	36.14 ± 0.26	37.3 ± 0.21	37.22 ± 0.19	36.98 ± 0.20	36.88 ± 0.27 ***	36.38 ± 0.17 ***	36.32 ± 0.15 ***
Extract(500 mg/kg)	36.38 ± 0.28	37.76 ± 0.16	37.48 ± 0.16	37.2 ± 0.18 **	37.3 ± 0.18 **	36.86 ± 0.22 **	36.26 ± 0.18 **

The values are expressed as mean ± SEM, \*\* p < 0.01; \*\*\* p < 0.001 is considered significant compared to the control, n = 5

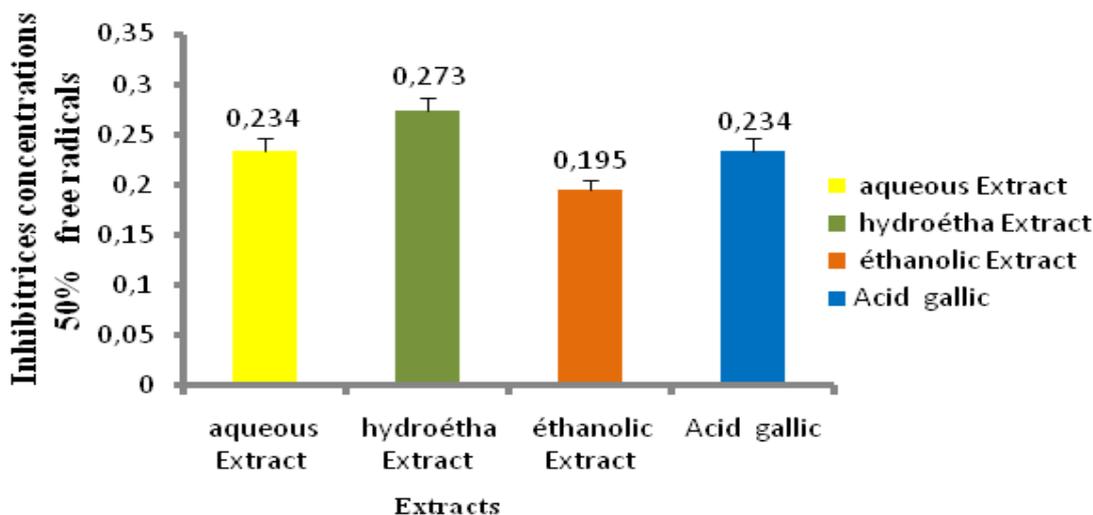


Figure 3. Free radical inhibitory concentrations of extracts: aqueous, hydroethanolic and ethanolic, compared to gallic acid (reference molecule) of *Maranthes glabra* leaves.

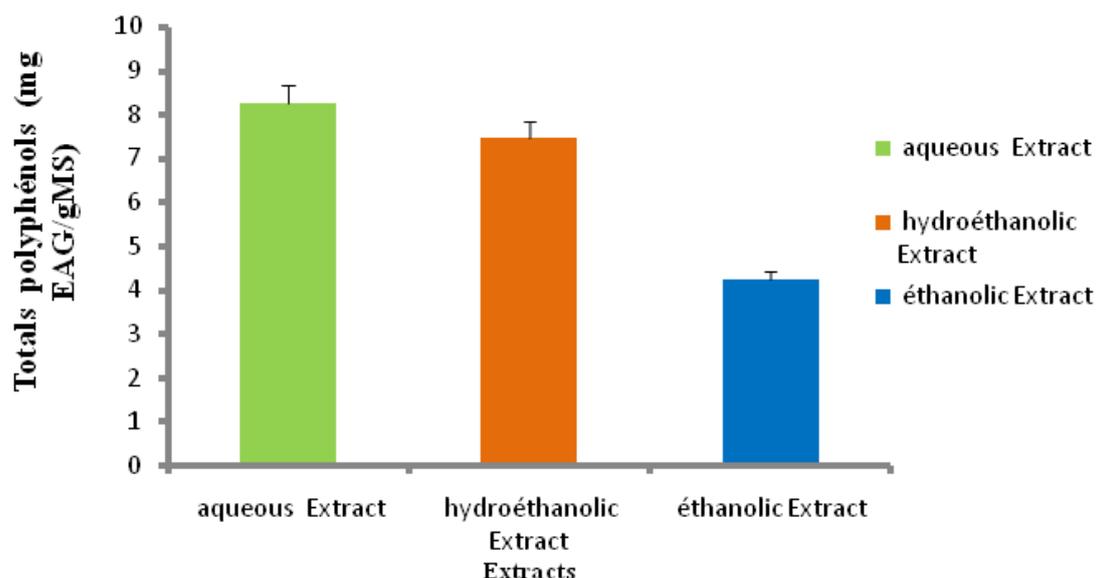


Figure 4. Variation of the total polyphenol contents in the extracts: aqueous, hydroethanolic, ethanolic leaves of *Maranthes glabra*.

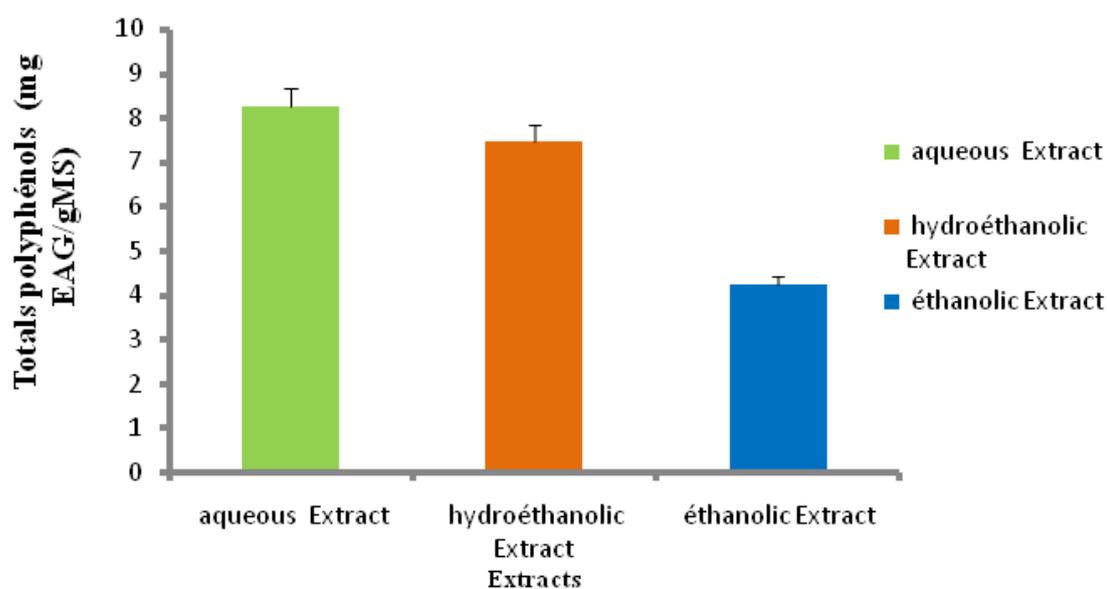


Figure 5. Variation in total flavonoid contents of the aqueous, hydroethanol and ethanolic extracts of *Maranthes glabra* leaves.

Table II. Phytochemical profile of *Maranthes glabra* leaves.

Chimical Families	Résults
Alcaloïdes	+++
Flavonoïdes	+++
Saponosides	+
Hétérosides cardiotoniques	+++
Anthraquinone	-
Tanin	++
Anthocyanes	-
Quinone libre	++
Stérols et terpenoïdes	-

+++ strong presence; ++ presence; low presence; - absence