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PHYTOCHEMICAL STUDIES, ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITIES IN-VITRO OF FICUS UMBELLATA (VAHL.) LEAVES, A PLANT USED TO PREVENT BLOOD HYPERCOAGULATION IN BENIN

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ABSTRACT

Background: *Ficus umbellata* leaves are used for a variety of medicinal applications. However, there are few in-depth studies on their phytochemical and anti-inflammatory potential. **Objective:** The present study aimed to evaluate the phytochemical potential, antioxidant and anti-inflammatory activity in-vitro of *Ficus umbellata* leaves **Methods:** The study was carried out on aqueous, ethanolic and hydro-ethanolic extracts of *Ficus umbellata* leaves. Phytochemical screening was performed using the staining method. DPPH, FRAP, ABTS and APM methods were used to assess the antioxidant activity of the three extracts. The in vitro anti-inflammatory activity of *Ficus umbellata* extracts was assessed using the albumin denaturation inhibition assay. **Results:** Phytochemical screening results demonstrated the effectiveness of pure ethanol in extracting a wide range of secondary metabolites from *Ficus umbellata* leaves. Assays of phenolic compounds, flavonoids and catechic tannins in ethanolic extracts of Ficus umbellata leaves showed significantly higher

concentrations. The results highlighted the efficacy of ethanolic extracts of *Ficus umbellata* in terms of their ability to reduce iron (FRAP), APM, DPPH and inhibition of the radical cation ABTS compared with hydro-ethanolic and aqueous extracts. Albumin denaturation inhibition values for the aqueous extract of *Ficus umbellata* remained relatively lower than for the hydro-ethanolic and ethanolic extracts. **Conclusion:** Pure ethanol proved more effective than water and diluted ethanol.

KEYWORDS: *Ficus umbellata*, Leaves, Extracts, Antioxidant, Anti-inflammatory.

1. INTRODUCTION

Under normal physiological circulation conditions, blood, which is made up of a set of potentially actionable cellular and molecular systems, circulates in a fluid state in the blood vessels, irrigating all the body's tissues.[1] Maintaining the fluid state of the blood inside the vessels is ensured by a set of mechanisms that either stop bleeding or prevent thrombosis.[2] Under certain circumstances, this mechanism can malfunction, leading to blood hypercoagulation known as thrombosis.[3] Thrombosis is a vascular disease characterized by the formation of a blood clot[4] (Thrombus) in the wall of a blood vessel, which impedes blood flow.[5] It is the cause of cardiovascular diseases such as myocardial infarction, ischemic stroke, deep vein thrombosis and pulmonary embolism.[6] In Africa, this disease is a scientific curiosity, as demonstrated by the few cases published.[7] According to Prost et al, intravascular coagulation is a severe defibrinating syndrome that accompanies well-defined clinical situations and reflects the failure of the microvascular system to maintain blood fluidity.[8]

Curative treatment relies mainly on the prescription of anticoagulants, also known as "blood thinners". These substances are not accessible to all segments of the Beninese population, who resort to traditional practices based on medicinal plants.[9] The leaves of Ficus umbellata (Vahl.) are used in Benin to treat or prevent hypercoagulation of the blood, and its effectiveness has been proven by Tchogou et al.[10] It is a highly sought-after timber and medicinal species. It belongs to the Ficus genus in the Moraceae family, and can reach heights of 6 to 10 m.[11]

The aim of this study was to evaluate the phytochemical potential, antioxidant and anti-inflammatory activities of Ficus umbellata (Vahl.) leaves in vitro.

2. MATERIALS AND METHODS

2.1.Plant material

The plant material used is the main plant species cited, namely Ficus umbellata. Ficus umbellata leaves were collected in May 2022 in the commune of Abomey Calavi (southern Benin) and identified at the Benin National Herbarium under number YH870/HNB. The leaves were dried for three weeks and finely ground.

Fig. 1: *Ficus umbellata* dried leaf powder.

2.2.Preparation of extracts

Extraction is dictated by bibliographical information on the chemistry of plant constituents. Ethanol or distilled water was used as the solvent, depending on the type of extraction (aqueous extract, ethanolic extract, hydro-ethanolic extract). Extraction was carried out in 3 stages: maceration, filtration and evaporation.

50 g of *Ficus umbellata* leaf powder were placed in 500 ml of distilled water, ethanol and a water-ethanol mixture (30:70 v/v), to obtain aqueous, ethanolic and hydro-ethanolic extracts respectively. After maceration (for 72 hours), the products were filtered with filter paper and then with absorbent cotton placed in a funnel connected to a suction pump to speed up filtration. After a few minutes, an exclusively liquid solution was obtained (the operation was repeated three times in succession). The filtrate obtained was placed in an oven at 45° C to evaporate the solvent.

The yield of extracts (R) was calculated in relation to the weight of dry plant matter according to the formula:

$$R = (Me/Mp) \times 100$$

Me: Mass of extract obtained

Mp: Mass of initial powder

2.3.Phytochemical studies

2.3.1.Chemical group detection method

The phytochemical screening carried out was based on differential precipitation and staining reactions, using the method of Houghton and Rahman.[12] This method highlighted the main chemical groups (Tannins, Flavonoids, Coumarins, Terpenes, Alkaloids, etc.) contained in the plant material.

2.3.2.Chemical group quantification method

2.3.2.1.Determination of total polyphenols

1 ml of tenfold diluted Folin-Ciocalteu reagent was mixed with 200 µl of each extract and 2 ml of H2O. The mixture was incubated at room temperature for 4 min. 0.8 ml sodium bicarbonate was added to the mixture and incubated again for 2 h at room temperature. Absorbance was measured on a Shimadzu UV-VIS spectrophotometer (λmax = 765 nm). Results were expressed as milligrams of gallic acid equivalents (GAE) per 1g of dry extract: mg GAE/1g dry extract.

2.3.2.2.Determination of total flavonoids

1 ml of AlCl3 solution (2% dissolved in methanol) was mixed with 1 ml of each sample. After 10 minutes, absorbance was measured on a Shimadzu UV-VIS spectrophotometer (λmax = 430nm). Flavonoid concentrations were deduced from the calibration curve (y = 0.06 x - 0.0002 with R^2 = 0.98) established with quercetin (0-35 µg/ml).

2.3.2.3.Determination of tannins

Condensed tannins were determined using the vanillin method described by Jessé and Ghedadba.[13-14] Vanillin reacts with free flavan-3-ols and the terminal units of proanthocyanidins, producing a red color whose intensity is proportional to the levels of flavanols present in the medium, with an absorption maximum at 500 nm wavelength. Results were expressed in milligrams of catechin equivalents (mg CE) per 1 g of dry extract (mg CE/1 g dry extract).

2.4. Activité Antioxydante

2.4.1.Activité scavenger du radical 2,2-diphényl-1-picrylhydrazyle (DPPH)

The radical-scavenging activity of Ficus umbellata leaf extracts against DPPH (2,2-Diphenyl-1-picrylhydrazyl) was evaluated according to the protocol used by Chokki et al, which we adapted to a cuvette spectrophotometer.[15]

100 µL of a 50 µM DPPH solution was combined with 100 µL of plant extracts at a concentration of 200 µg/mL. This combination was then placed in the dark and allowed to stand for 30 minutes at room temperature. Absorbance readings were taken at 517 nm using a spectrophotometer.

The efficiency of Ficus umbellata leaf extracts and standard agents (Such as ascorbic acid and BHT) in scavenging DPPH radicals was calculated using the following formula.[16]

DPPH inhibition % = [(Ac - Ae) / Ac] x 100

Ac: absorbance of control; Ae: absorbance of extracts / reference compound

2.4.2.Test de la puissance de l'antioxydant FRAP

The method used is that of Jones et al (2017), to which we have made a modification. A solution was prepared by mixing 100 ml TPTZ solution (10 mM in 40 mM HCl) with 10 ml FeCl3 (20 mM). 200 µl of this mixture was taken as working solution and mixed with 50 µL of each F. umbellata leaf extract. The mixture was then incubated at 37° C for 10 minutes. Absorbance was measured at 593 nm. The ability of the extract to reduce iron (III) to iron (II) was expressed in microgram equivalent of ascorbic acid per gram of extract (µg EAA/g sample) according to the formula below.

C = (Co. Fd)/ Ci

C: Concentration of reducing compounds in dry extract µgEAA.g-1; **Co:** Concentration of sample read; **fd:** Dilution factor of stock solution; **Ci:** Initial concentration.

2.4.3.Determination of 2,2-azinobis (3-ethylbenzthiazoline)-6-sulfonic acid (ABTS)

Evaluation of the ABTS radical scavenging potential of Ficus umbellata leaf extracts was carried out according to the methodology described by Cudalbeanu et al.[18] 5 mL of a 7.8 mM ABTS solution was mixed with 5 mL of a 140 mM potassium persulfate solution. This mixture was incubated at room temperature for 12 h in the dark. For the assay, 100 µL of freshly prepared ABTS solution was combined with 100µL of Ficus umbellata leaf extract. After allowing the mixture to incubate for 30 min, its absorbance was recorded at 734 nm.

Trolox was used as the reference for this assay. Results were expressed both in percentage inhibition of the ABTS radical and in molar equivalent of Trolox (mol ET/g) according to the formula below. All tests were performed in triplicate.

C= (C0 x fd)/ (Ci x M)

C: concentration of reducing compounds in MolET.g-1 dry extract; **Co:** concentration of sample read; **fd:** dilution factor of stock solution; **Ci:** initial concentration; M molar mass of Trolox.

2.4.4.Ammnium-phosphomolybdenum (APM) reducing activity

The determination of phosphorus and molybdenum was carried out following the protocol used by Bhatti et al.[19] We prepared a 0.1 mL aliquot dilution of the sample extract in a triplicate test tube. Each was treated with 1 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). Tubes were incubated at 95 ◦C in a water bath for 90 minutes. Samples were cooled to room temperature and absorbance recorded at 765 nm with the BioMATE 3S UV-visible spectrophotometer, Thermo. Ascorbic acid was used as a positive control to generate a standard curve ($y = 1.4831x - 0.1568$; $R^2 = 0.9957$). Antioxidant activity equivalence (AAE) was derived from the standard cure and expressed using the following equation:

AAE =(X×Df) / (Cm×1000) ×Ey

X = neutralization activity (mM/mL); **Df** = dilution factor; **Cm** = initial extract solution concentration (mg/mL); **Ey** = extraction yield (g/kg plant matter).

2.5. Evaluation of the in-vitro anti-inflammatory activity of Ficus umbellata leaves

In-vitro anti-inflammatory activity will be assessed by the heat-induced ovalbumin denaturation inhibition method of Chandra et al.[20]

The reaction involved mixing 0.2 ml of freshly pipped ovalbumin, 2.8 ml of phosphate-buffered saline (PBS, pH 6.4) and 2 ml of extract at different concentrations (0.625 to 5 mg/ml). A similar volume of distilled water was used as a control. The mixture was incubated at (37±2)°C in the oven for 15 minutes, then heated to 70°C for 5 minutes. Absorbances were read at 660 nm with a spectrophotometer after cooling to room temperature against water. Diclofenac sodium in the half-dilution concentration range (1.25 to 10 g/ml) was used as the reference molecule. The test was carried out in duplicate for each sample, and the percentage inhibition of denaturation was calculated using the following formula:

%inhibition=100x (Vt/Vc-1)

Vt = absorbance of sample, Vc = absorbance of control.

The concentration of extract or reference molecule for 50% inhibition (IC50) was determined by fitting the percentage inhibition versus concentration curve.

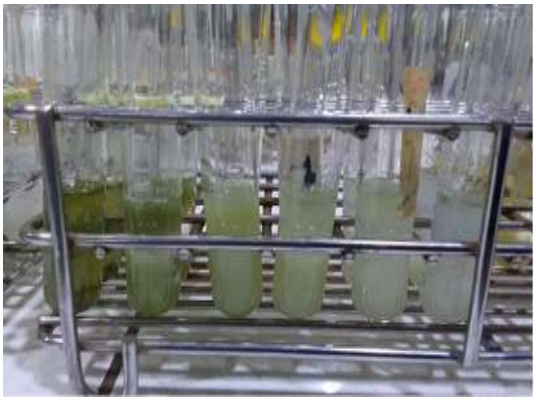

Fig. 2: In-vitro anti-inflammatory activity.

3. RESULTS

3.1.Phytochemical studies

3.1.1. Extract yields

The yield results and characteristics of the various extracts (aqueous, ethanolic and hydro-ethanolic) of Ficus umbellata leaves are shown in Table 1. The highest yield was found for the ethanolic extract (20.36 ±0.36%).

Table 1: Yield and characteristics of various *Ficus umbellata* leaf extracts.

Plant material weight (g)	Extracted types	Aspect	Color	Weight of extracts (g)	Yield(%)
50g	Aqueous	Powder	Black	4,74±0,63	9,49±1,25
	Ethanolic			10,18±0,24	20,36±0,36
	Hydro-ethanolic			7,36±0,81	14,72±1,63

3.1.2.Phytochemical screening

Phytochemical screening enabled us to identify the various secondary metabolites contained in the aqueous and ethanolic extracts of F. umbellata dry leaves. The qualitative results of this phytochemical screening test are summarized in Table 2.

We detected more metabolites in the ethanolic extract than in the hydro-ethanolic and aqueous extracts.

Table 2: Phytochemical screening of F. umbellata dry leaf extracts.

		EA	EE	EH
Compound group	**Metabolites**			
Polyphenolic compounds	Flavonoids	+	+	+
	Gallic tannins	-	-	-
	Catechin tannins	+	+	+
	Coumarin	-	-	-
	Anthocyanins	-	+	-
	Leuco-anthocyane	-	+	+
Nitrogen compound	Alkaloids	-	+	+
Heterosides	Saponosides	-	+	-
	D. cyanogenic	-	-	-
	Reducing compounds	-	+	+
	Anthraquinones	+	+	+
	Mucilages	-	+	-
Terpene compound	Steroids	-	+	+
	Triterpenoids	-	+	+

EA: Aqueous extract**; EE:** Ethanolic extract**; EH:** Hydroethanolic extract

3.1.3. Quantitative characterization of *Ficus umbellata* leaf extracts

3.1.3.1. Total polyphenols

The highest concentration of phenols was measured in the ethanolic extract. The results obtained show that the concentration of phenols in the ethanol extract was 125.65 ± 2.1 mg EAG/g dry extract, compared with those in the aqueous ($P<0.0001$) and hydro-ethanolic ($P<0.0001$) extracts, which were 58.06 ± 1.6 and 45.11 ± 1.88 mg EAG/g dry extract respectively (Fig. 3).

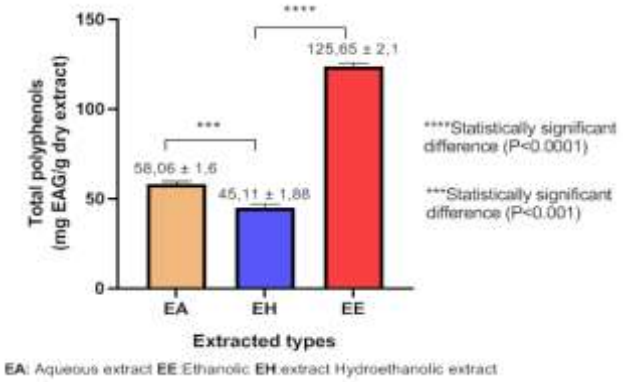

Fig. 3: Total polyphenol content.

3.1.3.2. Flavonoids

The highest concentration of flavonoids was measured in the ethanolic extract. The results obtained show that the flavonoid concentration in the ethanol extract was 28.80 ± 2.55 mg

EQ/g dry extract compared with those in the aqueous (P<0.05) and hydroethanolic (P<0.05) extracts, which were 7.51 ± 1.97 and 11.61 ± 1.37 mg EQ/g extract respectively (Fig. 4).

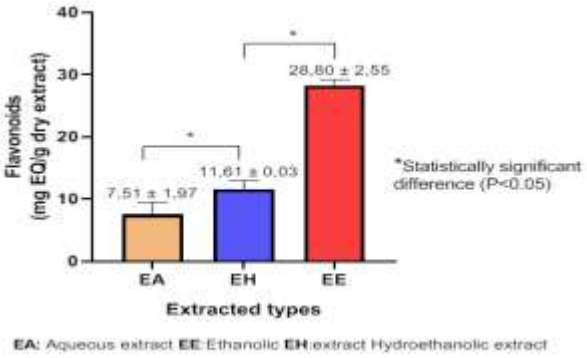

Fig. 4: Flavonoid content.

3.1.3.3.Condensed tannins

The ethanolic extract contains the highest tannin content (22.01 ± 0.03 mg EC/g Ext Sec) compared to the aqueous (7.30 ± 0.89 mg EC/g Ext Sec) (P<0.001) and hydro-ethanolic (17.77 ± 1.57 mg EC/g Ext Sec) extracts (Fig.5).

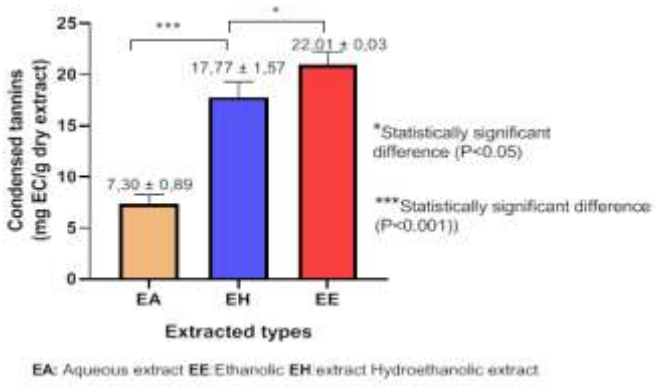

Fig. 5: Condensed tannin content.

3.2.Antioxidant activity

3.2.1.Antioxidant activity obtained by the DPPH method

3.2.1.1.Percentage of free radical inhibition

According to the results shown in fig.6, the percentage of free radical inhibition increases with increasing concentration.

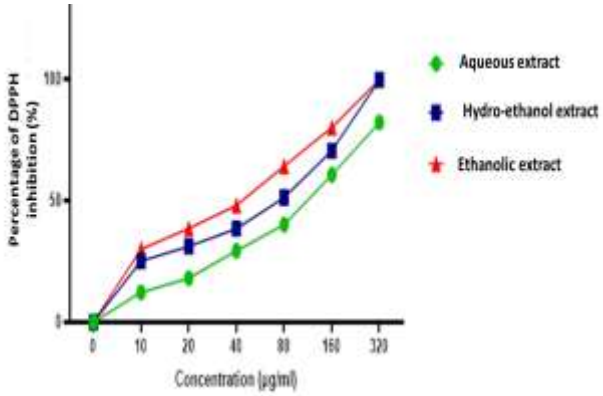

Fig. 6: Percentage of free radical inhibition. Each value represents a mean ± SE (n = 3).

3.2.1.2.Determination of IC50

The antioxidant capacity of extracts was determined from the IC50, i.e. the concentration required to reduce 50% of the DPPH radical. The lower the IC50 value, the greater the antioxidant activity of a compound. For each extract, we determined the concentration required to reduce 50% of the DPPH free radical, or IC50, from the linear regression equations of the graphs.

According to the results presented in Fig.7, the IC50 obtained for ascorbic acid (0.005 ±0.003 mg/ml), BHT (0.011 ±0.084 mg/ml) used as reference molecules, is lower than that of the extracts and therefore, a very high antioxidant activity. The ethanolic extract has an IC50 of the order of 0.08 ±0.09 mg /ml, lower than that of the hydro-ethanolic (0.11±0.073 mg /ml) and aqueous (0.15 ±0.05 mg /ml) extracts. The ethanolic extract exhibited high antioxidant activity compared with the hydro-ethanolic (P<0.001) and aqueous (P<0.001) extracts.

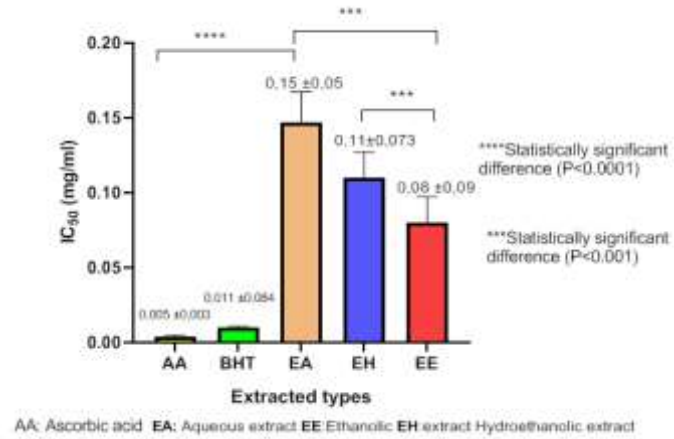

Fig. 7: Determination of IC50.

3.2.2.Antioxidant activity obtained by FRAP method

The highest ferric reduction capacity was found in the ethanolic extract (41.19±2.65µg AAE g-1), followed by the hydro-ethanolic extract (35.54±1.57µg AAE g-1) and the aqueous extract (32.93±1.49µg AAE g-1) (Fig. 8).

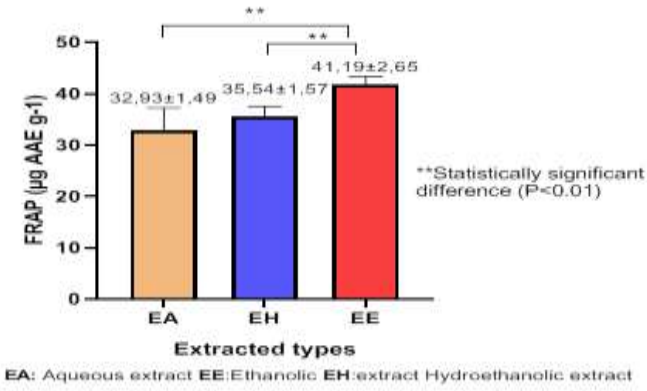

Fig. 8: Antioxidant activity obtained using the FRAP method.

3.2.3.Determination of 2,2-azinobis (3-ethylbenzthiazoline)-6-sulfonic acid (ABTS).

The hydroethanolic extract showed a higher reducing power (0.45±0.11mol ET/g) compared with the ethanolic (0.41±0.13mol ET/g) and aqueous (0.23±0.04 mol ET/g) extracts, in contrast to the results obtained with the FRAP method (Fig.10).

The percentages of inhibition by the extracts are in the following order: Aqueous extract (39.74±1.05%) < Ethanolic extract (71.24±1.05%) < Hydro-ethanolic (78.26±1.57%). (Fig. 9)

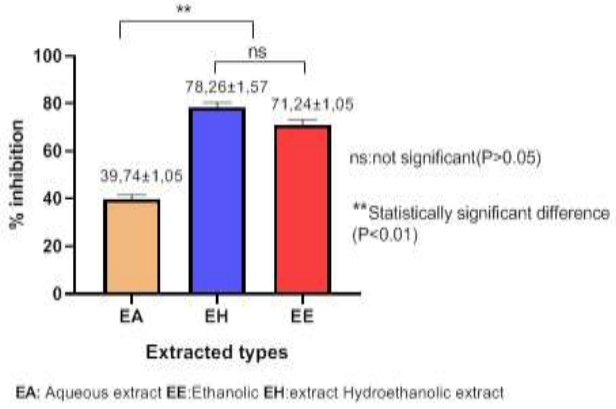

Fig. 9: Percentage inhibition of the radical cation.

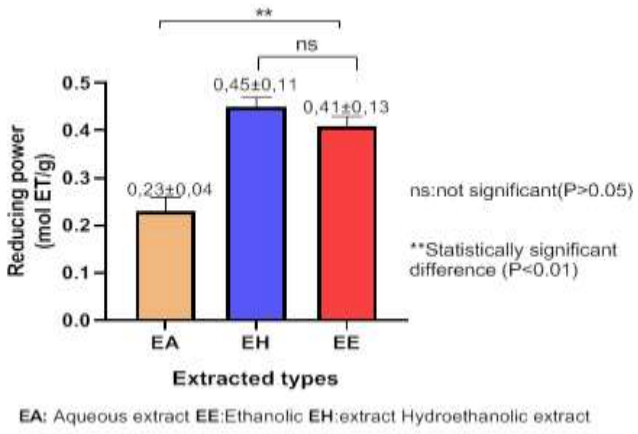

Fig. 10: Reducing power of the ABTS radical.

3.2.4.Ammnium-phosphomolybdenum (APM) reducing activity

The ethanolic extract showed a higher reducing activity (7.77±0.14 mMol AAE/g) than the hydro-ethanolic (6.5±0.17 mMol AAE/g) and aqueous (5.7±0.59 mMol AAE/g) extracts. (Fig. 11).

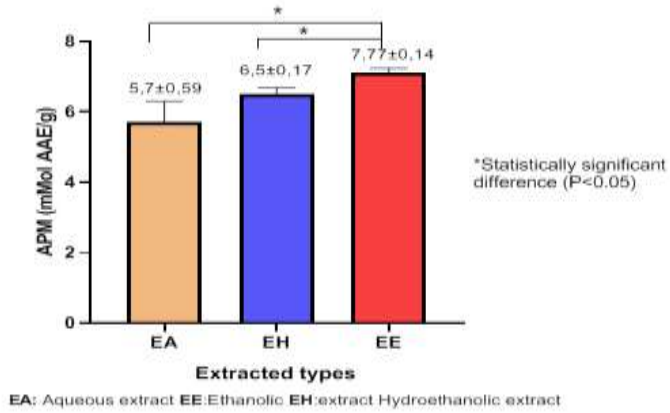

Fig. 11: Ammnium-phosphomolybdenum assay.

3.3.Evaluation of the in-vitro anti-inflammatory activity of *Ficus umbellata* leaves

3.3.1.Rate of inhibition of albumin denaturation

According to the results shown in fig.12, the percentage inhibition of albumin denaturation increases with increasing concentration. Of the three extracts tested, the ethanolic extract had the highest inhibition rate, followed by the hydro-ethanolic and aqueous extracts, and behaved like the reference molecule diclofenac.

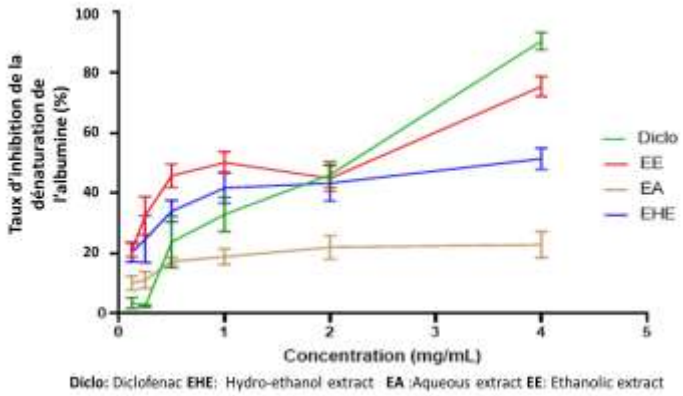

Fig. 12: Rate of inhibition of albumin denaturation.

3.3.2.Determination of IC50

In-vitro anti-inflammatory activity was determined from the IC50. The lower the IC_{50} value, the greater the anti-inflammatory activity of a compound. According to the results presented in Fig. 13, the IC_{50} obtained for Diclofenac (5.2 - 0.94 mg/ml) used as a reference molecule, is lower than that of the extracts and therefore has a very high anti-inflammatory activity. The hydroethanolic extract has an IC50 of 8.46 ± 6.27 mg /ml, lower than that of the ethanolic (8.66 ±3.04 mg /ml) and aqueous (29.75 ±15.49 mg /ml) extracts. Analysis of variance revealed no significant difference between the IC_{50} obtained for the hydro-ethanolic and ethanolic extracts (P>0.05). Both ethanolic and hydro-ethanolic extracts showed high anti-inflammatory activity compared with aqueous extracts.

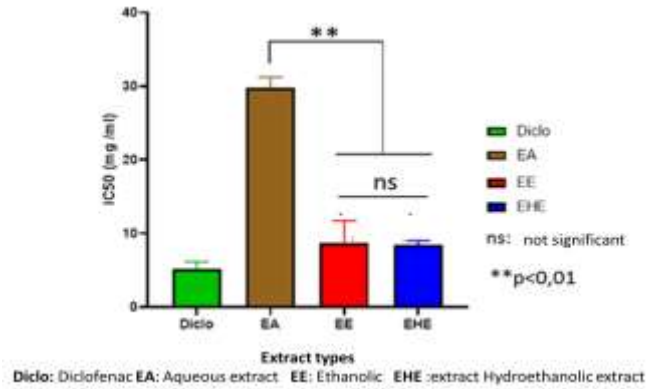

Fig. 13: In-vitro anti-inflammatory activity determined from IC_{50}.

4. DISCUSSION

The results obtained show that the extraction yield is higher in the ethanolic medium than in the hydro-ethanolic and aqueous media. Our results are in line with those of Abeysinghe et al[21] who state that different solvents have different polarities and will therefore extract different types of compounds depending on their solubility. According to Nga et al[22], the

solvent used to prepare extracts is a factor influencing extraction and the content of metabolites present in the leaves.

The results of the phytochemical screening show the effectiveness of pure ethanol in extracting a wide range of secondary metabolites from Ficus umbellata leaves. Pure ethanol, with its intermediate polarity, is able to solubilize both polar and non-polar compounds, which explains why it extracted almost all the secondary metabolites present in the leaves.

Solvents such as water and dilute ethanol are less effective at extracting certain compounds. Water, being a very polar solvent, will not be able to extract non-polar compounds efficiently. Diluted ethanol, although a good compromise, cannot be as effective as pure ethanol due to the presence of water, which reduces its ability to solubilize certain metabolisms.

Comparison with the study by Soumahoro et al[23] reveals some interesting similarities. Although this study also used ethanol for extraction, it did not detect quinones and anthraquinones, which could be explained by differences in extraction methodologies, experimental conditions, or even natural variation in the chemical composition of the plants studied.

Assays of phenolic compounds, flavonoids and catechic tannins in ethanolic extracts of Ficus umbellata leaves show a significantly higher concentration compared to hydro-ethanolic and aqueous extracts. These results confirm the effectiveness of pure ethanol as a solvent for extracting these secondary metabolites, which can be attributed to ethanol's ability to solubilize both polar and non-polar compounds. According to the study by Soumahoro et al,[23] the polyphenolic profile of plant extracts can vary under the influence of various factors including temperature, extraction solvent and geographical location.

Evaluation of the antioxidant activity of Ficus umbellata leaves through aqueous, ethanolic and hydro-ethanolic extracts revealed that ethanolic extracts exhibit greater free radical inhibition capacity, measured by DPPH inhibition, compared to aqueous and hydro-ethanolic extracts. Determination of antioxidant activity from IC50 showed that ethanolic extracts have a lower IC50 value, signifying stronger antioxidant activity. These results are in agreement with the observations of Gongbo et al, who state that a lower IC50 value corresponds to higher antioxidant activity.[24] Flavonoids and tannins, present in greater quantities in

ethanolic extracts, are known for their ability to give up hydrogen atoms, enabling them to reduce and decolorize DPPH, as demonstrated by Bougandoura et al.[25] The results highlight the superior efficacy of ethanolic extracts of Ficus umbellata in terms of their ability to reduce iron (FRAP) and inhibit the radical cation ABTS, compared with hydro-ethanolic and aqueous extracts. This confirms the idea that ethanol is a highly effective solvent for extracting the bioactive compounds responsible for these antioxidant activities. Bentabet et al[26] report that there is a direct relationship between antioxidant activity and the reducing power of plant components. The polyphenolic compounds contained in our extracts are probably responsible for the antioxidant activity. Indeed, Trabsa et al[27] have documented the efficacy of flavonoids in various studies, highlighting their ability to reduce oxidation and protect against oxidative stress. Tannins, thanks to their phenolic cores, possess high antioxidant capacity, as demonstrated by Amlan et al.[28] These phenolic compounds are particularly effective at neutralizing free radicals and inhibiting oxidative reactions, giving them an important role in protection.

The ethanolic extract of *Ficus umbellata* leaves showed higher ammonium-phosphomolybdenum (APM) reducing activity compared to hydro-ethanolic and aqueous extracts. This observation reinforces the efficacy of pure ethanol as a solvent for extracting bioactive compounds responsible for this antioxidant activity.

The in vitro anti-inflammatory activity of Ficus umbellata extracts was assessed using the albumin denaturation inhibition assay.[29] The results showed that the ethanolic extract of *Ficus umbellata* had inhibition percentages comparable to those of diclofenac, a reference molecule commonly used for its anti-inflammatory properties. Albumin denaturation inhibition values for the aqueous extract of *Ficus umbellata* remained relatively lower than for the hydro-ethanolic and ethanolic extracts, the latter offering superior protection. Comparing these results with those of Habibur et al.[30] who observed protection in the order of 29.63%, it appears that the ethanolic extract of F. umbellata leaves offers better protection, exceeding 60%.

5. CONCLUSION

The results of this study highlight the importance of solvent selection for the extraction of bioactive metabolites from *Ficus umbellata* leaves. Pure ethanol proved more effective than water and diluted ethanol, not only for the extraction of secondary metabolites, but also for maximizing the antioxidant and anti-inflammatory activity of the extracts. These results

confirm the therapeutic potential of *Ficus umbellata*, particularly when using pure ethanol as an extraction solvent.

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