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Antioxidant, Anti-Inflammatory Efficacy and HPLC Analysis of *Annona muricata* Leaves Extracts from Republic of Benin

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Abstract

Annona muricata L. (Soursop or Graviola) is a naturally occurring plant seen in Southern part of Africa, traditionally used in Benin to treat various diseases. The present study aimed to investigate phytochemical composition and antioxidant, anti-inflammatory activities of *A. muricata* leaves extracts. The secondary metabolites of ethanolic and hemi-ethanolic extracts were analysed by HPLC method. The DPPH and FRAP methods were used to evaluate the antioxidant activity. Inhibition of albumin denaturation method was used to evaluate anti-inflammatory activity of the tested extracts of which larval cytotoxicity was studied. The major identified compounds were gallic acid, chlorogenic acid, cafeic acid, tannic acid, ferrulic acid, Rutin. Ascorbic acid exhibited the highest inhibition percentage (83.33% ± 0.50%) of DPPH radical with the lowest IC_{50} (45.1 ± 0.28 µg/ml). The inhibition of the ferric ion Fe^{3+} varied (p = 0.0013) according to the extracts type. IC_{50} values of ferric ion inhibition range from 119.5 ± 3.10 to 250.8 ± 2.13 µg/ml respectively for *A. muricata* leaves ethanol and hemi-ethanolic extracts. The hemi-ethanolic extract exhibited the highest anti-inflammatory activity (96.66% ± 1.17%). The presence of phenolic compound confers to *A. muricata* leaves, through the ethanolic and the hemi-ethanolic extracts, the antioxidant and anti-inflammatory activities.

Keywords

A. muricata, Secondary Metabolites Extracts, Screening, Biological Activities,

Benin

1. Introduction

Plants are known to be rich sources of bioactive compound [1]. Generally, plant bioactive compounds differ greatly in terms of their quality and quantity, depending on the plant or on the various constituent parts of the plant [2] and they have been widely assessed for their biological properties [3]. In Africa, the population used various plants to treat all kinds of chronic diseases among which due to oxidative stress and that linked to metabolism [4]. Oxidative stress has been implicated in the pathology of numerous conditions, including aging, inflammatory disorders, diabetes, cancer, muscle wasting and muscular dystrophies [5]. Reactive oxygen species (ROS) cause cellular damage by directly and irreversibly altering macromolecules such as proteins, membrane lipids and DNA [6], but another (less studied) major cellular consequence of ROS exposure is the reversible modification of protein thiol side chains that may affect many aspects of molecular function [5]. However, medicinal plants contained the secondary metabolites that scientific research has proved their therapeutic effects over time [7]. Recent research supports the role of these types of secondary metabolites in the prevention of degenerative diseases, especially cancer, cardiovascular diseases, and neurodegenerative diseases [8]. Polyphenols and flavonoids are strong antioxidants that complete and add to the functionalities of vitamins and antioxidant enzymes with the purpose of defense against oxidative stress caused by the excessive presence of reactive oxygen species (ROS) [9]. Reactive oxygen species (ROS) are key signaling molecules that play an important role in the progression of inflammatory disorders [10].

The free radicals especially, the reactive oxygen species (ROS) create oxidative stress in the cells leading to inflammatory and infectious condition. Phagocytic cells including polymorphonuclear leukocytes (neutrophils, eosinophils) and mononuclear cells (macrophages and lymphocytes) produce excessive amount of ROS which play an important role in the host defense mechanism. Besides their defensive effects these excessively produced ROS which deregulate the cellular functions causing cellular and tissue damage, which in turn increases the state of inflammation [11]. Non-steroidal anti-inflammatory drugs (NSAIDs) represent one of the most common classes of medications used worldwide for inflammation and related disorders [12]. Unfortunately, besides the excellent anti-inflammatory potential of the NSAIDs, the severe side effects such as gastrointestinal (GI) ulceration, perforation, obstruction, and bleeding have limited the therapeutic usage of NSAID [13].

Natural antioxidants from plants play an important role in maintaining general health [14]. Several studies report that medicinal plants are used as solution to many diseases and are the source of news compounds. Furthermore, novel antioxidant, anti-inflammatory drugs or adjuvant that lower the quantities of

synthetics drugs to achieve eradication of diseases linked to the ROS with lower toxicity are urgently required [15]. Since traditional medicine involves the use of plant extracts which contains an extensive diversity of compounds, often with indefinite biological effects there is need to determine the toxicity of medicinal plants. The one approach to evaluate cytotoxicity is the study using Brine-Shrimp lethality assay [16]. The brine shrimp lethality test is used to predict compounds or extracts with toxicity or that may have anticancer activity [17].

Annona muricata L. (AML), family Annonaceae has been used as a natural solution for the treatment of many diseases in Benin. Previous reports over the years have demonstrated that roots, stem, bark, leaf, fruit and seed extracts of *A. muricata* are anti-bacterial [18]. Its leaf extract was also found to possess anti-oxidant [18] and molluscicidal properties [19]. Recently, it has also been reported to exhibit anti-inflammatory and analgesic effects [20]. Among the chemical constituents found in the leaf of *A. muricata* are alkaloids [21], essential oils [22] and acetogenins [23].

The aim of this study is to evaluate biological activities (anti-oxidant, anti-inflammatory and cytotoxicity) of phytochemical compounds contained in *A. muricata* leaves extracts.

2. Material and Methods

2.1. Plant Material and Extracts Preparation

A. muricata leaves were collected in Tchaada village (Southern Benin) and air dried at 25˚C - 30˚C (laboratory conditions) for two weeks, grinded and sieved into a bark powder. The extracts were obtained according to the method describe by Dah-Nouvlessounon *et al.* [24]. Briefly, the powder (50 g) of leaf powder was macerated into 500 ml of each solvent: Ethanol and the mixture water/ethanol (30/70v/v), under agitator for 72 h at room temperature. Each homogenate was then filtered two times on absorbent cotton and once on Whatman N˚1 paper (125 mm ø, Cat No 1001 125). The filtrate was concentrated in vacuum using a Rotary evaporator (Heidolph Instruments GmbH & Co. KG No: 591-28000-00-1). The concentrated was dried in the oven at 40˚C; the obtained powder is considered as the total extract ready to use for the biological activities. All extracts were stored in labeled sterile bottles and kept at −20˚C until further use.

2.2. Preliminary Phytochemical Screening

The leaves powder was subjected to the qualitative phytochemical investigation to identify the major components (nitrogenous, polyphenolic and terpenic compounds, and glycosides) who was done according to previous report [25].

2.3. HPLC Analysis

Phenolic compounds were identified using standard molecules and a High Per-

formance Liquid Chromatography analytical system (U-HPLC-DAD) equiped with a degasser, a binary gradient pump, a UV detector at multiple wavelengths (DAD-3000 RS and MWD-3000 RS) and a reverse phase column (C18; 150 × 4.6 mm, 5 µm; Hypersil BDS). The samples were prepared at 1 mg/ml. The mobile phases consisted of acidified distilled water 1) with 0.1% formic acid and acidified acetonitrile 2) with 0.1% formic acid. The elution gradient is as follow: 0 - 20 min, 20% - 50% B; 20 - 25 min. 50% - 70% B; 25 - 30 min, 70% - 80% B; 30 - 35 min, 80% - 20% B, 35 - 40 min, 20% B, with a flow rate of 1 ml/min and an injection volume of 20 µl. Data analysis was performed using Chromleon v.6.80 software (Dionex, Thermo Fisher Scientific).

2.4. 1,1-Diphenyl-2-Picrylhydrazyl (DPPH) Radical Scavenging Activity

The DPPH method was conducted by adaptation as described by Scherer and Godoy [26]. Equal volumes (1000 µl) of DPPH (50 µM) and plant extracts (2000 µg/ml) were mixed and allowed to stand in darkness for 20 - 30 min at room temperature. Then, the absorbance was read at 517 nm and the blank was a mixture of methanol and DPPH (v:v). The inhibitory percentage of DPPH radical indicating the antioxidant activity of extracts and BHA, ascorbic acid was obtain using the formula establish by Schmeda-Hirschmann *et al.* [27].

The Concentration providing 50% inhibition (IC_{50}) was determined graphically using a calibration curve in the linear range by plotting the extract concentration and the corresponding scavenging effect.

2.5. Ferric Reducing/Antioxidant Power (FRAP) Assay

The ferric reducing power of *A. muricata* extracts was determined according to the method described in literature [28]. Thus, 0.4 ml of sample at different concentrations was mixed with 1 ml of phosphate buffer (0.2 M, pH = 6.6) and 1 ml of 1% potassium hexacyanoferrate [K3Fe (CN) 6]. After incubating at 50˚C for 30 minutes, 1 ml of 10% trichloroacetic acid was added, tubes were centrifuged at 3000 rpm for 10 minutes. Then 1 ml of supernatant was mixed with 0.2 ml of 0.1% FeCl 3 solution and suspended in the dark for 30 minutes before measuring the absorbances at 700 nm.

2.6. Anti-Inflammatory Activity Essay

In vitro anti-inflammatory activity of *A. muricata* leaves extracts was evaluated with inhibition of albumin denaturation method [29]. Briefly, the 5 ml of reaction mixture was comprised of 0.2 ml of eggs albumin, 2.8 ml of phosphate buffered saline (PBS, pH 6.4) and 2 ml of varying concentration of extracts (1000; 500; 250; 125; 62.5; 31.5 µg/ml). Similar volume of double distilled water served a control. Then the mixture was incubated at 37˚C in incubator for about 15 mins and then heated at 70˚C for 5 mins. After cooling, their absorbance was measured at 660 nm by using pure blank. Diclofenac sodium inj.

Pb./Drugs/1804-BManufactured for Pharma plus (standard drug) at the concentration range from 6.25 to 25 mg/ml was used as reference drug and treated as such for determination of absorbance. The percentage inhibition of protein denaturation was calculated by the formula mentioned below.

$$\% \text{ inhibition} = \frac{\text{Abs Control} - \text{Abs sample}}{\text{Abs Control}}$$

2.7. Cytotoxicity Essay

The cytotoxic effect of the extracts was evaluated according to an adaptation of the method described by Kawsar *et al.* [30]. The tests are carried out twice on 72 h larvae of *Artemia salina*. Briefly, a test was constituted of 16 *A. salina* larvae in a 2 ml solution containing 1 ml of the extract tested concentration and 1 ml of sea. The number of surviving larvae is counted after incubation (24 h) and the DL_{50} was calculated using the regression line obtained from the surviving larvae in function of the extracts concentration representation.

2.8. Statistical Analysis

Biological activities experimentation was done in triplicate and data thus obtained reported as a mean ± standard deviation (SD). The data were analyzed using Graph Pad Prism 7 software. Differences of $p < 0.05$ were considered significant.

3. Results

3.1. Preliminary Phytochemical Screening

The secondary metabolites detected in *A. muricata* leaves extracts are summarized in Table 1. It was noted an uneven distribution of these metabolites from one extract to another. Indeed, 64.70% of the studied secondary metabolites were present in the hemi-ethanolic extract against 29.41% in the ethanolic extract.

3.2. HPLC Analysis

The HPLC analysis of ethanolic and hemi-ethanolic extracts of *A. muricata* leaves extracts has shown the presence of various (60) polyphenolic compounds. The major identified compounds were gallic acid, chlorogenic acid, cafeic acid, tanic acid, ferrulic acid, Rutin, Ellargic acid (Table 2). Quantitatively, tannic acid was the major identified components (4.534 µg/ml with ethanolic extract and 3.586 µg/ml with hemi-ethanolic extract). Beside, for ethanolic extract, ferrulic acid recorded the lowest amount (0.001 µg/ml) while gallic acid has the lowest compound (0.005 µg/ml) in hemi-ethanolic extract.

3.3. DPPH Radical Scavenging Activity

The DPPH radical scavenging activity of the extracts and reference molecules are

presented in Figure 1(a) and Figure 1(b) respectively. The data showed, a dose response activity according to the extracts and reference molecules. Indeed, the percentages of free radical inhibition increased as concentration increased in the both products (extracts and reference molecules). Considering the extracts, the highest inhibition percentage (80.59% ± 0.58%) was obtained with the hemi-ethanolic extracts at 1000 µg/ml. However, with the reference molecules, ascorbic acid exhibited the highest inhibition percentage (83.33% ± 0.50%) at 1000 µg/ml. Besides, the lowest IC_{50} (45.1 ± 0.28 µg/ml) was obtained with ascorbic acid followed by the ethanolic extract (IC_{50}= 50.01 ± 0.16 µg/ml), followed by the hemi-ethanolic extract (IC_{50} = 80.50 ± 0.48 µg/ml) and at the end of the BHA (IC_{50} = 104.01 ± 0.13 µg/ml).

The multivariate analysis of variance (Table 3) shows that the inhibition percentage the free radical DPPH vary on the one hand according to the extracts ($p < 0.0001$) and their concentrations ($p < 0.0001$) and on the other hand according to the reference molecules ($p < 0.0001$).

Table 1. Phytochemical constituents of *A. muricata* extracts.

Group of compounds	Class	*A. muricata* extracts	
		EtOH	EtOH/H_2O
Nitrogenous compounds	Alkaloids	+	+
Poly-phenolic compounds	Tannins	-	+
	Catechic tanins	-	+
	Gallic tanins	-	+
	Flavonoids	+	+
	Anthocyans	+	+
	Leuco-anthocyanes	+	+
	Coumarin	-	-
	Quinonics derivate	-	+
Terpenic compounds	Triterpenoids	-	-
	Steroids	-	-
Heterosides	Reducing compounds	+	+
	Free anthracenics	-	-
	O-heterosides	-	+
	O-heterosides at GR	-	-
	C-heterosides	-	-
	Mucilags	-	+

(+): Presence of secondary metabolite. (-): Absence of secondary metabolite.

Table 2. Major phenolic compounds identified in ethanolic and hemi-ethanolic extracts of *A. muricata* by HPLC.

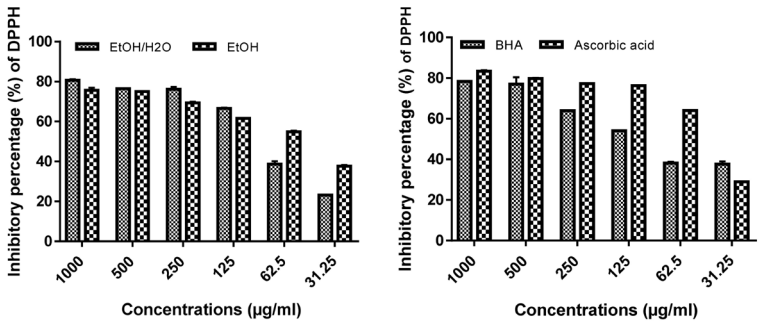
No	Compounds	T_R min	Rel. Area (%)		Amount µg/ml	
			EtOH	EtOH/H_2O	EtOH	EtOH/H_2O
1	Gallic acid	3.30	0.75	0.12	0.013	0.005
2	Chlorogenic acid	6.92	2.59	2.97	0.130	0.358
3	Cafeic acid	8.32	0.12	0.42	0.002	0.021
4	Tannic acid	10.71	0.55	0.29	3.586	4.534
5	Ferrulic acid	11.95	0.02	0.10	0.001	0.010
6	Rutin	17.61	4.76	2.42	0.086	0.106
7	Ellagic acid	18.18	7.27	5.77	0.085	0.163

EtOH: ethanolic extract; EtOH/H_2O: hemi-ethanolic extract.

Table 3. Summary of ANOVA Multivariate analysis of DPPH radical scavenging activity.

Variables	Summary (p -value)					
	1000 µg/ml	500 µg/ml	250 µg/ml	125 µg/ml	62.5 µg/ml	31.25 µg/ml
EtOH/H_2O ***vs.*** EtOH	<0.0001	ns	<0.0001	<0.0001	<0.0001	<0.0001
EtOH/H_2O ***vs.*** BHA	0.0219	ns	<0.0001	<0.0001	ns	<0.0001
EtOH/H_2O ***vs.*** Asc.ac	0.0065	0.0011	ns	<0.0001	<0.0001	<0.0001
EtOH ***vs.*** BHA	0.0087	0.0403	<0.0001	<0.0001	<0.0001	ns
EtOH ***vs.*** Asc. ac	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
BHA ***vs.*** Asc. ac	<0.0001	0.0061	<0.0001	<0.0001	<0.0001	<0.0001

Asc. ac: Ascorbic acid; ns: not significant ($p > 0.05$); EtOH: ethanolic extract; EtOH/H_2O: hemi-ethanolic extract; BHA: Buthylhydroxyanisol.



(a) extracts (b) Reference molecules

Figure 1. DPPH inhibition percentage of *A. muricata* leaves extracts (a) and reference molecules (b). Values are expressed in mean ± S.E.M. of triplicate measurement (n = 3).

The antioxidant activity index (AAI), expressing the anti-free radical power of the extracts and reference molecules was presented in **Figure 2**. It appears through this figure that ascorbic acid has the strongest antioxidant power (AAI = 1.10 ± 0.007) while BHA had the lowest antioxidant power (AAI = 0.48 ± 0.001). Furthermore, the results show that the two extracts of *A. muricata* have antioxidant powers superior to those of reference molecule (BHA). However, *A.*

muricata leaves ethanolic extract has a higher antioxidant power than that of the leaves hemi-ethanol extract. As summarized, assay shows that, in this system, the radical-scavenging activities of differents products (extracts and reference molecules) are in the order: ascobic acid > Ethnol extract > water-ethanol extract > BHA.

3.4. Ferric Ion Reducing Power

Ferric Reducing/antioxydant Power by *A. muricata* extracts is presented in Figure 3. A dose-dependent activity was observed between the *A. muricata* leaves ethanolic and hemi-ethanolic extracts. The highest percentages were obtained at 1000 µg/ml. In addition, the inhibition percentages of the ferric ion Fe^{3+} vary from 55.03% ± 0.04% (ethanolic extract) to 11.94% ± 0.12% obtained with the hemi-ethanolic extract at 31.25 µg/ml. However, the inhibition gradient of extracts concentrations made it possible to determine the IC_{50}'s which are 250.8 ± 2.13 µg/ml and 119.5 ± 3.10 µg/ml respectively for *A. muricata* leaves ethanolic and hemi-ethanolic extracts. The analysis of variance shows that the interaction between the inhibiting power of the extracts (ethanolic and hemi-ethanolic) varies according to their concentrations. Indeed, the inhibition percentage of ethanolic and hemi-ethanolic extracts varied significantly ($p = 0.0013$) at 1000 µg/ml. This variation is highly significant ($p = 0.0005$) at 250 µg/ml and very highly significant ($p < 0.0001$) at the concentrations of 62.5 and 31.25 µg/ml. Furthermore, no variation ($p > 0.05$) was observed for 500 and 125 µg/ml.

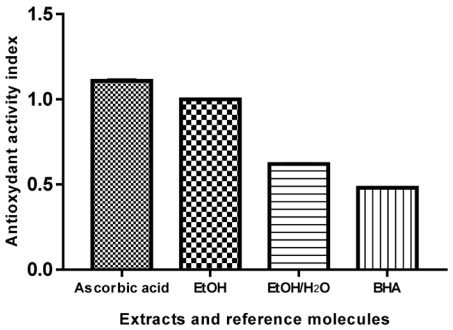


Figure 2. Antioxydant activity index (AAI) of *A. muricata* extracts and reference molecules.

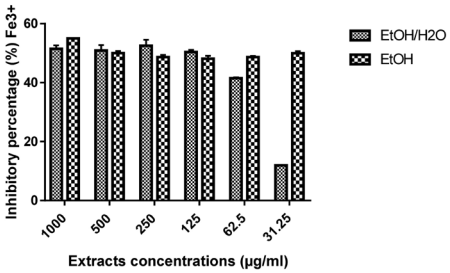


Figure 3. Ferric ion reducing power of *A. muricata* ethanolic and hemi-ethanolic extracts. Values are expressed in mean ± S.E.M. of triplicate measurement (n = 3).

3.5. Anti-Inflammatory Activity

In vitro anti-inflammatory effect of *A. muricata* extracts are summarized in the Table 4. The results showed that the extracts exhibited a concentration dependent inhibition of protein (egg albumin) denaturation throughout the concentration range from 31.25 to 1000 µg/ml. Indeed, the higher inhibition percentage obtained with hemi-ethanolic extract was 96.66% ± 1.17%, while the highest recorded with ethanolic extract was 87.12% ± 0.53%. Then, variance analysis showed the difference ($p < 0.0001$) between inhibition percentage of protein (egg albumin) denaturation considering the concentration range from 62.5 to 1000 µg/ml while there is no difference ($p > 0.05$) between the inhibition percentage at 31.25 µg/ml. However, the hemi-ethanolic extract was more active than those of ethanolic extract. This was further confirmed by comparing their IC_{50} values. Indeed, *A. muricata* hemi-ethanolic extracts display the high inhibition of protein (egg albumin) denaturation with the lowest concentration (IC_{50} = 44.3 ± 0.28 µg/ml) compared to those obtained with ethanolic extract (IC_{50} = 187.95 ± 87.75 µg/ml). Diclofenac sodium inj. (at the concentration range from 6.25 to 25 mg/ml) was used as reference drug which also exhibited concentration dependent inhibition of protein denaturation. The results showed that the high activity (94.55% ± 0.12%) of diclofenac was found at 25 mg/ml (Table 4). These results showed that *A. muricata* extracts have the good inhibition of protein denaturation comparing to reference drug (diclofenac sodium).

3.6. Cytotoxicity Activity

The bioassay to determine the lethality effect of *A. muricata* leaves extracts with *Artemia salina* model was presented in Figure 4. Indeed, the results showed the concentrations response activity. The larval mortality gradient was lowest with *A. muricata* ethanolic extract comparing to hemi-ethanolic extract. However, there is no difference ($p > 0.05$) between the LD_{50} values range from 5.21 mg/ml; $r^2 = 0.643$ (hemi-ethanolic extract) to 9.98 mg/ml; $r^2 = 0.690$ (Ethanolic extract).

Table 4. *In vitro* anti-inflammatory activity of *A. muricata* leaves extracts.

Concentrations (µg/ml)		Inhibition percentage (%)			
Extracts	**Diclofenac**	**EtOH**	**EtOH/H_2O**	**Diclofenac**	**t-value**
1000	25000	87.12 ± 0.53	96.66 ± 1.17	94.55 ± 0.12	9.23^{****}
500	12500	82.12 ± 0.53	95.00 ± 0.00	89.10 ± 0.25	12.47^{****}
250	6250	50.00 ± 0.00	92.5 ± 0.00	87.23 ± 0.37	41.15^{****}
125	-	48.75 ± 1.76	87.5 ± 0.00	-	37.52^{****}
62.5	-	16.25 ± 1.76	83.54 ± 0.29	-	65.15^{****}
31.25	-	11.25 ± 1.76	10.83 ± 1.17	-	0.40^{ns}
IC_{50} (µg/ml)		187.95 ± 87.75	44.3 ± 0.28	-	

Values are expressed in mean ± S.E.M. of triplicate measurement (n = 3).

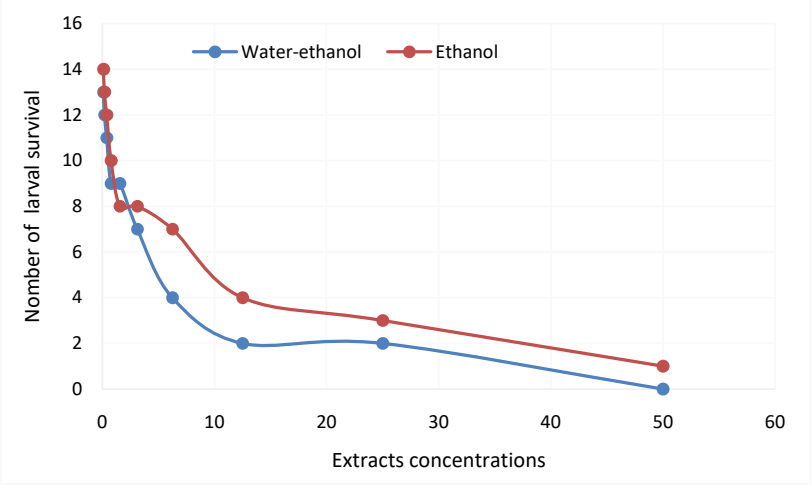


Figure 4. Variation of *Artemia salina* larval mortality according to *A. muricata* leaves extracts.

4. Discussion

The qualitative screening of *A. muricata* leaves extracts revealed the presence of various phytochemical components. Phytochemicals, such as phenolic compounds, are considered beneficial for human health, decreasing the risk of degenerative diseases by reduction of oxidative stress and inhibition of macro-molecular oxidation [31]. These compounds have been reported to be well correlated with antioxidant potential [32]. The presence of alkaloids and flavonoids (anthocyans and leuco-anthocyans) in the both extracts (ethanolic and hemi-ethanolic) indicate their potentiality to reduce *in vitro* cholesterol agents and to induce an many biological activity [33] because flavoinoids has an group of phenolics compounds involved in many biological effects [33] such as anti-inflammatory activity [34], antioxidant, hepatoprotective activities [35].

The study of the antioxidant activity in natural products has received increasing attention in recent years. Several *in vitro* methods have been used to assess the antioxidant activity in naturals products, including, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical [36], FRAP ferric reducing/ antioxidant power assay [32]. To evaluate antioxidant activity of *A. muricata* leaves extracts in this study, these two methods was used. The both extracts revealed significant scavenging of DPPH free radicals in dose-dependend manner and this may be attributed to their electron donating ability. The IC_{50} values show that *A. muricata* leaves extracts display anti-free radical activity greater than those of BHA which is a reference molecule. Baskar *et al.* [37] and George *et al.* [38] show that *A. muricata* leave extracts (methanolic and aqueous) from India have DPPH radical scavenging activity. Then these observations show that *A. muricata* extracts might prevent reactive radical species from damaging biomolecules in susceptible biological and food systems [39].

The potential of *A. muricata* leaves extracts to reduce ferric III iron was observed by FRAP method. The reducing properties of plant extracts, via hydrogen

atom donation, are generally attributed to the presence of reductones, which exert an antioxidant action by breaking the free radical chains [40]. Comparative to the DPPH method, the inhibition percentage of ferric III iron was lowest (55.03% ± 0.04%) than 80.59% ± 0.58% obtained with the DPPH method. The same observation were made by others authors [41] [42] who show that the inhibition percentage and IC_{50} values varies according to DPPH, FRAP, Nitric Oxide, Hydroxyl radical, Superoxyde radical, Lipid peroxidation methods. According to DPPH and FRAP methods used in this study *A. muricata* extracts have antioxidant activity. Ngueguim *et al.* [43] show also antioxidant capacity of *A. muricata* leaves extracts with different methods. The antioxidant activity of *A. muricata* leaves extracts observed in the present study would be link to their chemical composition. Indeed, HPLC analysis performed with the extract revealed the presence of various ploypheno lic compounds such as gallic acid, chlorogenic acid, cafeic acid, tannic acid, ferrulic acid, Rutin, Ellargic acid. Tannic acid was the major identified components, this phenolic acid was known to promote antioxydant activity [44] [45]. Others compounds identified by HPLC are reported to have antioxidant activity. It is the case of gallic acid [46] [47], caffeic acid, chlorogenic acid [48], Rutin [49], Ellagic acid [50] ferrulic acid [48] [51].

The efficacies of herbal medicines about chronic inflammatory activity have been studied in many previous cases. In the present study the protein denaturation bioassay was selected for *in vitro* assessment of anti-inflammatory activity of *A. muricata* leaves extracts. Denaturation of tissue proteins is one of the well-documented causes of inflammatory and arthritic diseases. Production of auto antigens in certain inflammatory diseases may be due to *in vivo* denaturation of proteins. Agents that can prevent protein denaturation therefore, would be worthwhile for anti-inflammatory drug development. In the present study, compared to diclofenac used as reference molecule, the experimental results show that the ethanolic and hemi-ethanolic extracts of *A. muricata* display the good anti-inflammatory activity with significant difference ($p < 0.0001$) between inhibition percentage of protein (egg albumin) denaturation. The presence of flavonoids in the both extracts could be the basis of the better activity showed by these extracts. Flavonoids have been considered to possess significant anti-inflammatory properties, both *in vitro* and *in vivo* [52] [53].

Referring to the toxicity scale established by Moshi *et al.* [17], the ethanolic and hemi-ethanolic extracts LD_{50} is greater than 0.1 mg/ml, value above which the extract is considered exhibiting no toxicity. Indeed, *A. muricata* tested extracts are not toxic at the tested doses. However, the brine shrimp (*A. salina*) mortality increased with the increasing of *A. muricata* extracts concentration.

5. Conclusion

Through the obtained results, we can conclude that *A. muricata* contains many secondary metabolites dominated by polyphenolic compounds. HPLC analysis

performed with the extract revealed the presence of various ployphenolic compounds such as gallic acid, chlorogenic acid, cafeic acid, tannic acid, ferrulic acid, Rutin. The presence of these compounds confers to *A. muricata* leaves, through the ethanolic and the hemi-ethanolic extracts, the antioxidant and anti-inflammatory activities.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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