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Antioxidant and Antiinflammatory Activities of *Imperata cylindrica* (L.) P. Beauv. (Poaceae) Extracts in Relation to Extraction Methods and Plant Organ

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Abstract: The involvement of oxidative stress/inflammation couple in the pathophysiology of many diseases increasingly emphasized. Treatments controlling the inflammatory process and the production or elimination of free radicals may be useful in limiting their harmful effects. Imperata cylindrica is a widely used medicinal plant in Côte d'Ivoire due to its numerous biological properties. This study investigated the antioxidant and anti-inflammatory activity of *Imperata cylindrica* organs in relation to different extraction methods. Leaves and roots of I. cylindrica collected from Côte d'Ivoire were washed, shade-dried and pulverized for storage. Phytochemical screening of water, alcohol and acetone raw extracts from roots and leaves was performed using conventional methods. Aqueous, alcoholic and acetonic decoctates and macerates from the leaves and roots of the plant prepared according to methodologies used in the literature were used to measure antioxidant and antiinflammatory activities by methods of inhibition of lipoperoxidation and denaturation respectively. The comparative study showed a difference in the extraction rate of bioactive compounds as well as the biological properties. The most suitable parameter for extracting antioxidant and anti-inflammatory compounds turned out to be the trio: roots / water / boiling. The richness of bioactive substances would justify the antioxidant and anti-inflammatory activities of the plant.

Keywords: Antioxidant, Antiinflammatory, Extraction, Leaves, Roots, *Imperata cylindrica*

1. Introduction

Inflammation results from the stimulation of the innate immune system by endogenous factors (IL33, IL-α, HMGB1, alarms) and exogenous factors (bacteria, viruses, allergens, toxic compounds, foreign bodies) in order to defend, restore and maintain cellular balance through a series of cellular and molecular reactions [1-3]. An inappropriate inflammatory response is the cause of loss of function, associated with numerous pathologies (cardiovascular diseases, cancer, atherosclerosis, diabetes, hypertension and other degenerative diseases) [4-6]. Protein denaturation associated with inflammation is thought to occur when proteins have lost their structures and functions [8]. This denaturation causes conversion of phospholipids into eicosanoids and a mixture of oxidized phospholipids which can therefore be pro- and anti-inflammatory inducers causing an aggravation of the inflammatory process [8, 9, 11].

Free radicals are atoms or molecules resulting from metabolism and redox reactions that take place at multiple cellular levels [12]. Their excessive production, associated with the activity of proteolytic enzymes and hyperactivated leukocytes, is thought to be the cause of tissue and cellular damage [7]. Indeed, free radicals interact with proteins and lipids [14].

In order to avoid their harmful effects, the body has put in place defences (vitamins and enzymes) to neutralise them [13]. When these means are exceeded and an accumulation of free radicals is observed, a cascade of radical reactions is triggered leading to oxidative stress [14].

Llipoperoxidation is known to be the main consequence resulting of this state [15]. Membrane phospholipids and polyunsaturated fatty acids (mainly linoleic, linolenic, arachidonic and eicosapentaenoic acids) constituted the preferred targets of oxygenated free radicals and other reactive oxygen species [15-17]. Lipoperoxidation products are known to play a major role in the deregulation of key pathways in the pathophysiology of many diseases [18, 19].

Treatments able to controlling the inflammatory process and/or oxidative stress would be an alternative to limit the consequences of oxygenated free radicals [5, 13, 20].

Metabolites of medicinal plants are known to regulate and modulate various functions and processes, including inflammatory response and oxidative stress [21]. Their extractions constitute an important step in the discovery of bioactive components. Thus, biological activities of extracts have often presented significant differences depending on the extraction methods [22].

Imperata cylindrica is a *Poaceae* (*Monocotyledon, cyperales*) from tropical, subtropical or warm temperate regions [23]. In Côte d'Ivoire, it is found throughout the territory where it is used in the treatment of anemia and sinusitis. Furthermore, the plant is used alone or in combination with other plants, in decoction or maceration, for its antihypertensive, neuroprotective, antibacterial, antihelmintic, astringent, anti-inflammatory, digestive diuretic, emollient, febrifuge, hemostatic, antihyperglycemic, antidiarrheal and antioxidant properties. [25, 26, 28].

Phytochemical studies of the plant have highlighted presence of alkaloids, phenolic acids, flavonoids, tannins, glycosides, saponins, phenylpropanoids, sesquiterpenes [28-30].

This study investigated antioxidant and anti-inflammatory activity in relation to different methods of extraction of roots and leaves of Imperata cylindrica.

2. Materials and Methods

2.1. Material

Leaves and roots of *I. cylindrica* collected in central Côte d'Ivoire, were identified under number 051 of the Africa Rice herbarium (Rice Center for Africa, Bouaké). They were washed, then shade-dried for 7 days and then pulverized for Storage, protected from humidity.

2.2. Méthodes

2.2.1. Preparations of Extracts

The extracts were prepared according to methodologies used in the literature [31, 32].

Decoctates (5%) of the various solvents were obtained by extraction of leaves and roots three times under reflux for successive periods of 2h-1h30-1h. For each organ the 3 solutions are mixed.

Macerates (5%) are obtained after 24 hours. leaves and roots were macerated in different solvents (water – ethanol 70% – acetone 30%).

The extraction yield ρ (%) was expressed as follows:

$$\rho = (QA/QA0) * 100 \quad (1)$$

With QA: Amount of material extracted by the solvent

QA0: initial amount of plant material used

2.2.2. Phytochemical Screening

Phytochemical screening was carried out using the stepwise solvent method. The targeted compounds were alkaloids (with Bouchardat and Dragendorff methods), polyphenols (with FeCl3 test), flavonoids (with cyanidin reaction), tannins (with Stiasny reaction followed by that of ferric chloride), saponins (by index of moss), cardiac glycosides (with Keller-kiliani methods), quinones (with Borntraëger reaction), sterols (with Liebermann-Buchard reaction) [33].

2.2.3. Inhibition of Protein déNaturation Using Egg Albumin Method

The anti-inflammatory potential of the aqueous, alcoholic and acetonic extracts of *Imperata cylindrica* was determined by the inhibition of the egg albumin denaturation method. The ability of *I. cylindrica* extracts to inhibit protein denaturation was determined by methods described in the literature with some modifications [34-36].

The reaction mixture consisted of 0.2 mL of egg albumin (1%), 4.78 mL PBS (pH 6.4) and 0.02 mL of /diclofenac extract (1-2, 5-5-10 mg/mL). It was then incubated at 37°C ± 2 for 15 min and then heated to 70°C for 5 min. After cooling, the reading was taken with a spectrophotometer at 660nm against the blank consisting of PBS (pH 6.4). Distilled water and diclofenac served as negative and positive controls, respectively. The percentage of denaturation inhibition was calculated according to equation (2). The results of the extracts were compared to those of diclofenac.

$$\%\text{Anti-denat} = [100*(\text{Abs extracts} / \text{Abs Contrôle}) -1] \quad (2)$$

2.2.4. Inhibition of Lipid Peroxidation: TBARS Method

The reaction mixture was prepared with 10 µl of extracts of *I. cylindrica* (10 mg/mL), 50 µL of egg yolk solution (10% v/v in 1.15% KCl), 150 µL of 20% acetic acid solution (pH 3.5) and 150 µL of a solution of thiobarbituric acid (0.8% in 1.1% SDS). The volume of the reaction mixture is then adjusted to 400 µL with distilled water. The whole is mixed

for 5 seconds by vortex then incubated at 95°C for 1 hour. At the end of the incubation time, 1mL of butanol is added. The mixture is stirred for 5 s and centrifuged at 1500 g for 5 min. The butanolic fraction is extracted and its absorbance is read at 532nm. Distilled water and ascorbic acid are used as negative (distilled water) and positive (0.2 mg/mL ascorbic acid) controls, respectively. The percentage of inhibition is calculated according to the following relationship [37].

$$\%Inh = 100*[1-(Abs\ contrôle/\ Abs\ extract)] \quad (3)$$

2.3. Statistical Analyses

Data are presented as mean ± standard deviation (SD), and experiments were performed three times. The software (Microsoft Excel 2013) was used for measurements (means and standard deviations). Statistica 7.1 software was used for analysis of variance (ANOVA). Comparison of the means was obtained by the LSD test (significance threshold = 0.05). Determination of IC50 was made using the Graphpad software.

3. Results

Table 1. *Extraction yield (%).*

	Solvent	Leaves	Roots
Maceration	Water	3,86 ± 0,35 ^{h}	6,89 ± 0,26 ^{f}
	Alcohol	4,95 ± 0,30 ^{g}	14,10 ± 0,50 ^{c}
	Acetone	5,43 ± 0,27 ^{g}	8,83 ± 0,57 ^{e}
Decoction	Water	8,68 ± 0,59 ^{e}	12,67 ± 0,60 ^{d}
	Alcohol	7,91 ± 0,28 ^{e}	18,33 ± 0,76 ^{a}
	Acetone	8,58 ± 0,36 ^{e}	15,27 ± 0,52 ^{b}

On each column and each row, the yield values assigned different alphabetical letters are statistically different at p < 0,05 (ANOVA, LSD).

Table 2. *Phytochemical profile of the different extracts of Imperata cylindrica.*

	Roots			Leaves		
	Ethanol 70%	Eau	Acétone 30%	Ethanol 70%	Eau	Acétone 30%
Alkaloids	+	+	+	-	+	-
Polyphenols	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+
Polyterpenoids	-	-	-	+	+	+
Galic Tannins	+	+	+	+	+	+
Catechical Tannins	-	-	-	-	-	-
Saponins	-	+	+	-	+	+
Quinones	-	-	-	-	-	-

+: Presence, - Absence

Table 3. *IC_{50} of Imperata cylindrica concentration-dependent extracts.*

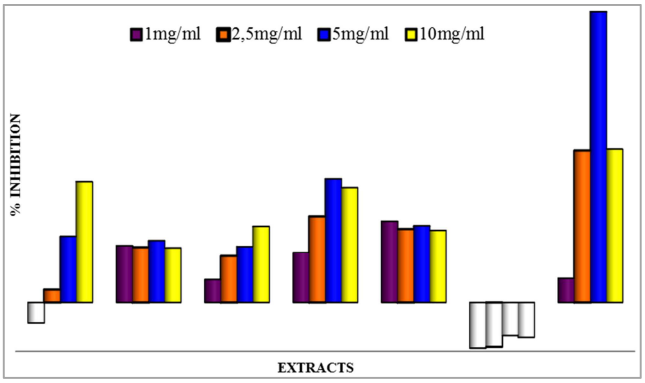
EXTRACTS	IC_{50} mg/mL
FMO	5,76
FMA	13,64
FDA	8,94
FDC	6,48
RMO	12,47
RMC	11,08
RDO	5,94
Diclo	3,63

On each column and each row, the yield values assigned different alphabetical letters are statistically different at p < 0,05 (ANOVA, LSD). Diclo: diclofenac F: leaves; M: macération; D: decoction; R: roots

Table 4. *Percentage of lipid peroxidation inhibition of Imperata cylindrica different extracts at 10 mg/mL and ascorbic acid at 0.2 mg/mL using egg yolk homogenate as medium.*

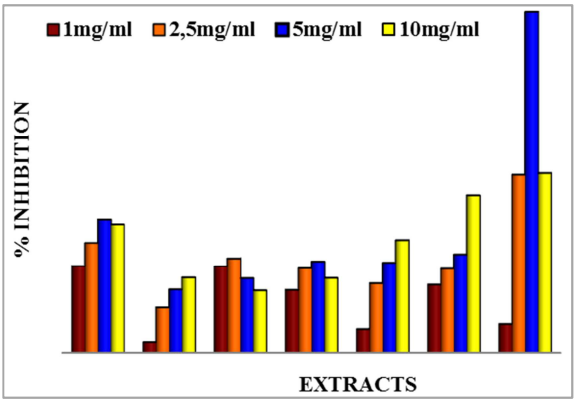
EXTRAITS	% Inh
FMO	32,96^{a}± 0,65
FMA	32,43^{a}± 5,06
FMC	51,84^{c}± 2,85
FDO	39,33^{ab} ± 0,39
FDA	69,81^{de} ± 3,21
FDC	51,76^{c} ± 2,26
RDO	76,33^{ef} ± 6,14
RDA	76,55^{ef} ± 6,73
RDC	74,01^{ef} ± 8,80
RMO	62,77^{d} ± 5,27
RMA	43,52^{b} ± 2,47
RMC	81,05^{f} ± 3,16
AA (0,2mg/mL)	41,72^{b} ± 4,63

The values expressed are the averages of the inhibition percentages, n = 3 replications, significant difference between the extracts at p < 0,001; Diclo: diclofenac F: leaves; M: macération; D: decoction; R: roots



Diclo: diclofenac F: leaves; M: macération; D: decoction; R: roots

Figure 1. *Inhibition of protein (albumin) denaturation by differents concentrations of various Imperata cylindrica roots extracts.*



Diclo: diclofenac F: leaves; M: macération; D: decoction; R: roots

Figure 2. *Inhibition of protein (albumin) denaturation by differents concentrations of various Imperata cylindrica leaves extracts.*

4. Discussion

The work explored extracts of *Imperata cylindrica* leaves and roots obtained from two methods (decoction and maceration) using three solvents (distilled water, ethanol 70%, acetone 30%) (Table 1). The extraction rate of leaves generally doubled with increasing temperature from 3.9% to 8% when moving from maceration to decoction. Solvent and temperature impacted extraction of the roots both in maceration and in decoction (Table 1).

The highest values are obtained by 70% ethanol (14.10 ± 0.50% and 18.33 ± 0.76%) followed by acetone (8.83 ± 0.57% and 15.27 ± 0.52%) and finally distilled water (6.89 ± 0.26% and 12.67 ± 0.60%). The increase in temperature probably impacted the solubility and diffusivity of the solvent and the breaking of bonds in plant cells for better extraction of molecules as mentioned in other works [38, 39]. Furthermore, it has been admitted that under the same extraction conditions the solvent is one of the most important factors that can impact the extraction yield [40].

Phytochemical screening of crude aqueous, ethanolic and acetoneic extracts of roots and leaves highlighted presence of several secondary metabolites (alkaloids, saponins, polyphenolic compounds) which are known to posses numerous medicinal properties in other studies [41-44]. Alkaloids are thought to have anti-inflammatory, antioxidant, antimicrobial and neuro-stimulating activities [45, 46]. Saponins are said to have anti-inflammatory and anti-diarrheal properties [47-49]. Other works has also shown preventive and/or therapeutic effects of polyphenols on metabolic diseases, neurodegenerative diseases, cell proliferation, inflammatory conditions and many other diseases [50, 51].

It is accepted that composition in secondary metabolites of medicinal plants is influenced by parameters as environment, period of harvest of the material, type of soil. Likewise extraction conditions (part of the plant material, particle size), extraction method, type and concentration of extraction solvents, solvent-plant ratio, extraction temperature and time, affect the rate and the quality of the extracted metabolites and therefore their biological properties [22].

In vitro anti-inflammatory activity of *I. cylindrica* extracts was evaluated through the protective activity of the extract against albumin denaturation. It has been noted that this mode of action, in this case the inhibition of the denaturation of bovine serum albumin at pH 6.2-6.4, is one of the mechanisms followed by several non-steroidal anti-inflammatory drugs [10]. The higher the degree of inhibition of albumin denaturation, the greater its anti-inflammatory potential [11, 52-54]. In this test, the egg albumin was denatured by heat; the inhibitory effect of the different extracts is presented in Figure 1 and Figure 2.

The percentage of inhibition varies from 0% to 55.16% for the extracts and from 10.01% to 119.30% for Diclofenac®. An extract is considered to have anti-inflammatory power when it produces a percentage of inhibition > 20% [54]. The extracts in the study could be classified into three classes: extracts without effect (RDC), extracts exerting their maximum at low concentration (FDO, RDA, RMA, FMC) and extracts having a concentration-dependent anti-inflammatory power at 1mg/mL.

The RDA, RMA, FMC extracts with an inhibition percentage between (21.96% and 33.56%) would exert maximum anti-inflammatory activity. Indeed, work had noted that algae extracts (*Undaria pinnatifida, Fucus vesiculosus, Macrocystis pyrifera, Ascophyllum nodosum, and Laminaria japonica*) rich in fucoidanes reduced levels of pro-inflammatory markers in vitro both in PBMC and in THP-1 cells with maximum activity at low concentrations [55]. This effect has been attributed to the low molecular weight of the extracts, better absorption across the cell surface as well as a rapid mode of action. Most plant extracts, however, have anti-inflammatory activity concentration-dependent. In this case, determining the IC50 makes it possible to classify them. The smaller the IC50, the greater its inhibitory potential. On the basis of the IC50 it was thus possible to estimate that the aqueous root decoction (RDO) and aqueous leaf macerate (FMO) with respectively 5.94 mg/mL and 5.76 mg/mL were the most effective extracts active in inhibiting denaturation of egg albumin. Their potential is, however, lower than that of our reference substance (Diclofenac) (3.63 mg/mL). The anti-inflammatory potential could be attributed to a phenolic derivative salicin structurally similar to Aspirin® and already isolated from flowers [56]. These results could justify the use of extracts of I. cylindrica against feverish conditions and malaria in a traditional environment [57].

During the work, the lipid supplier was egg yolk solution which contains between 31% and 35% lipids, mainly made up of linoleic and linolenic acids. The percentage of inhibition of the extracts is greater than 30% with a maximum activity (81.05% ± 3.16) at the level of the acetone macerate of the roots (RMC) which is however not statistically different from the values of the aqueous decoctions. (76.33%), alcoholic (76.55%) and acetone (74.33%) of the roots. The study also revealed that extracts from the root have a more interesting activity (43.52b ± 2.47

to 81.05 ± 3.16) % than leaf extracts (32.43a± 5.06 to 69.81 of ± 3.21) %. It should be noted that most extracts have greater activity than ascorbic acid at 0.2 mg/mL. The results of the study suggest that *Imperata cylindrica* extracts could have a cytoprotective property by inhibiting or reducing lipoperoxidation of non-enzymatic origin in a lipid medium. Polysaccharides isolated from *I. cylindrica* have been shown to be effective in protecting against kidney damage by inhibiting lipid peroxidation, increasing antioxidant defenses and inhibiting the secretion of pro-inflammatory factors, regulating purine metabolism, PI3K-Akt, NF signaling pathways -κB, mTOR and MAPK [58].

5. Conclusion

The present study on the anti-inflammatory and antioxidant activity of extracts of leaves and roots of *I. cylindrica* using different solvents and two extraction methods noted that the plant is rich in bioactive compounds. The study also identified alcohol as the best solvent for extracting these bioactive compounds. Furthermore, the study found that water was the suitable solvent to investigate the antilipoperoxidation and antidenaturation activities of proteins.

The optimal extraction parameter of antioxidant and anti-inflammatory compounds was: roots / water / decoction.

Author Contributions

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Conflicts of Interest

The authors declare no conflicts of interest.

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