QUALITATIVE AND QUANTITATIVE PHYTOCHEMICAL CHARACTERIZATION OF LEAF EXTRACTS OF *Mimosa pudica* (Mimosaceae).

Abstract

Mimosa pudica is a plant of the Mimosaceae family which is a family composed of several species. It has many pharmacological properties described in Ayurvedic medicine, including the hepatoprotective property which is the subject of this study. The objective of this study was to qualitatively and quantitatively evaluate the secondary metabolites presents in *M. pudica*leaf extracts. After performing an extraction according to the methods described by Fonmboh et al, we carried out a qualitative analysis in each extract according to the methods described by Shaik et al. The quantitative analysis consisted of a determination of each metabolite in comparison with references.

This study was able to show the presence of metabolites such as alkaloids, flavonoids, polyphenols in leaves extracts. The quantification of these metabolites showed a much higher concentration of alkaloids in the extract obtained by hydro-ethanolic maceration of 698.33 ± 8.82µg/ml, in the extract obtained by decoction a higher concentration of polyphenols and flavonoids respectively of 193.87 ± 12.66 µg/ml and 72.90 ± 2.45 µg/ml. This study demonstrated a promising richness in secondary metabolites in *M.pudia* leaf extracts qualitatively and quantitatively.

Keywords: *Mimosa pudica*, secondary metabolites, phytochemical screening

Introduction

The process of developing new therapeutic molecules is a long and expensive process involving the identification of the molecule with pharmacological properties whose origin can be synthetic or natural, including plants [1]. Herbal medicine represents a special and important form of traditional medicine in which the traditional practitioner specializes in theuse of plants to treat different ailments [2]. The WHO estimates that 70 to 95% of the population in developing countries resort to traditional medicine, more precisely phytotherapy, to solve their health problems [3].

An improved traditional medicine is a concept that brings together all the medicines designed by a traditional healer or a research laboratory, based on knowledge or information from traditional medicine and pharmacopoeia [4]. Plants contain molecules called secondary metabolites which represent an important source of substances for the pharmaceutical, food and materials industries [5]. Many metabolites have already been isolated from plants and have demonstrated pharmacological properties. This is the case of morphine isolated from P*apaver somniferum* acting on the central nervous system, nicotine extracted from certain species of the nightshade family which has anti-inflammatory properties, quinine extracted from different species of Cinchona having anti-malarial properties, antipyretic [6]. Among these secondary metabolites are found the alkaloids, coumarins, stilbenes, flavonoids [5].

The genus Mimosa belongs to the Mimosaceae family, composed of about 400 species of plants distributed across the Asian, American and African continents [7]. It has been described in Ayurvedic medicine as a therapy against leprosy, dysentery, vaginal and uterine conditions,

inflammation, asthma, fatigue [8]. The objective of this study was to explore the qualitative and quantitative phytochemical composition of *Mimosa pudica* in order to better understand its pharmacological properties.

Methodology

Plant material

The collection of samples was done within the campus of the Faculty of Medicine and Biomedical Sciences of the University of Yaoundé I from November 2021 to march 2022. The plant material consisted of leaves of *Mimosa pudica*. This species was identified at the National Herbarium of Cameroon in comparison with herbarium specimen N 57673/HNC. The harvested leaves were dried under shade at room temperature in a shade because certain compounds get denatured in sunlight. The dry leaves were coarsely powdered using a grinding mill.

Preparation of extracts

The leaf extracts were prepared using three extraction methods [9]:

- Maceration: here, two different solvents were used: distilled water and hydroethanol (50%). 100g of *M.pudica* leaf powder was weighed . 300ml of solvent was addedto the mixture and was left at room temperature for 48 hourswhile stirringevery mornig. After 48 hours, the mixture was filtered to separate the residues using Whatman filter paper No 1.

- Infusion: 100g of *M.pudica* leaf powder was added to 300ml of boiled distilled water

- The decoction: in this method the water-leaf powder mixture was heated for 30min in a water bath boiling water and cooled. Once cooled, the waste was separated and the concentrated extract filtered.

Phytochemical screening

Qualitative phytochemical screening

The qualitative phytochemical screening was carried out according to the methods specific to each family of compound sought and described by Shaikh et al [10]:

- **Test for Alkaloidsidentification:**
- Hager's test: Some mL of 50mg of extract (free of solvent) was mixed with a few mL of diluted and filtered HCl and 1-2 mL of Hager's reagent. The reaction was positive when a creamy white precipitate appeared.
- Mayer/Bertrand/Valser test: Some mL of 50mg of extract (free of solvent) was mixed with a few mL of diluted and filtered HCl and 1-2 drops of Mayer's reagent. The reaction was positive when a creamy white/yellow precipitate appeared.
- Wagner's test: Some mL of 50mg of extract (free of solvent) was mixed with a few mL of diluted and filtered HCl and 1-2 drops of Wagner's reagent. The reaction was positive when a red/brown precipitate appeared.
- Tannic acid test: Acidified extract +and10% tannic acid solution was mixed.The reaction was positive when a buff-colored precipitate appeared.

Test for flavonoids identification:

- 1mL of extract and 2mL of a 2% NaOH solution (+ a few drops of diluted HCl). An intense yellow fluorescence which disappeared on adding dilute acid/Plant extract and 10% ammonium hydroxide.

Tests for phenolic compounds:

- Ferric chloride test: Aqueous solution of plant extract and a few drops of 5% ferric chloride solution. A greenish-black/bluish-black color appeared.
- Lead acetate test: The plant extract was dissolved in 5mL of distilled water and 3mL of 10% lead acetate solution. Test was positive with the appearance of a white precipitate.

Test for Tannin:

- Gelatin test: The plant extract was dissolved in 5mL of distilled water and 1% gelatin solution with 10% NaCl. The positive reaction results in the appearance of a white precipitate.
- Braymer test: 1mL of filtrate of 3mg of extract powder boiled in 50mL of distilled water for 3 minutes and 3mL of distilled water with 3 drops of 10% ferric chloride solution. The positive reaction resulted in the appearance of a blue-green color.
- 10% NaOH test: 0.4mL of plant extract and 4mL of 10% NaOH were mixed and shaken. There formation of an emulsion (Hydrolysable tannins) indicated a positive reaction.
- Bromine water test: 10 ml of bromine water was mixed with 0.5mg of plant extract. The positive reaction resulted in a discoloration of the bromine (reddish yellow).
- Test with lead acetate: 1mL of a filtrate of a small quantity of extract boiled in 5mL of 45% ethanol for 5 min and cooled then3 drops of a solution of lead acetate was added. A gelatinous creamy precipitate was formed.

Test for cardiotonic glycosides:

1mL filtrate of the extract was mixed with 1.5mL of glacial acetic acid, 1 drop of ferric chloride and concentrated H_2SO_4. A blue colored solution was observed.

Test for mucilage:

100mg extract was dissolved in 10mL of distilled water and 25mL of absolute alcohol (while shaking). A white or fluffy precipitate was formed.

Quinone identification test:

- To the plant extract was added concentrated HCl. A green color was observed

Terpenoid identification test:

2ml chloroform and 5mL of plant (evaporated in a water bath) + 3mL concentrated H2SO4 (boiled then evaporated in a water bath) + 3mL concentrated H2SO4 (boiled in a water bath). The observed result was the appearance of a grey colored solution.

Quantitative phytochemistry screening

Quantitative analysis of carbohydrates: the reaction of picric acid with glucose producing picramic acid of orange or brown color whose wavelength was measured at 570 nm made it possible to quantify the carbohydrates in the extracts [11].

Quantitative analysis of total proteins: The Lowry method complementary to that of Biuret was used. Indeed, the protein first reacts with an alkaline cupric reagent (Gornall's reagent of the biuret method) then a second reagent, called phosphotungstomolybdic (Folin-Cioccalteu's reagent), was added. It was composed of a mixture of sodium tungstate and sodium molybdate in solution in phosphoric acid and hydrochloric acid. This reagent allowed the reduction of aromatic amino acids (tyrosine and tryptophan) leading to the formation of a dark blue colored complex whose absorbance was measured between 650 and 750 nm [12].

Quantitative analysis of total polyphenols: The determination of total polyphenols by the Folin-Ciocalteu reagent was described in 1965 by Singleton and Rossi. The reagent consists of a mixture of phosphotungstic acid (H3PW12OU4O) and phosphomolybdic acid (H3PMo12O4O). It is reduced during the oxidation of phenols to a mixture of blue oxides of tungsten (W8O23) and molybdenum (Mo8O23). The color produced, the maximum absorption of which is between 725 and 760 nm, was proportional to the quantity of polyphenols present in the plant extracts [13].

Quantitative analysis of total flavonoids: Aluminum chloride forms stable acid complexes with the C-4 ketone group and with the C-3 or C-5 carbon hydroxyl group of flavones and flavonols. Also, aluminum chloride forms labile acid complexes with the orthodihydroxyl groups in the A or B ring of flavonoids, resulting in the formation of a pink color, and it was measured at 510 nm [14].

Quantitative analysis of total flavonols: Sample containing flavonols results in the formation of a green color when reacted with aluminum chloride and sodium acetate, and samples were read at 440 nm in a UV spectrophotometer -Screw [15].

Quantitative analysis of total tannins: The technique for assaying condensed tannins by the Folin-Ciocalteu method is based on the reduction of phosphomolybdic and tungstic acid in an alkaline medium, in the presence of tannins, to give a blue color whose intensity was measured between 640 and 760 nm[16]

Quantitative analysis of total alkaloids: The alkaloid, in contact with concentrated sulfuric acid and potassium dichromate, develops a violet line which turns blue then green, therefore the maximum absorption proportional to the intensity of the color developed was 650nm[17]

Statistical analysis

The software used was excel 2016 for mean±variance.

Results

Extraction yield

different yields were obtained using different solvents.The highest extraction yield was observed for the hydro ethanolic maceration compared to while the lowest yield was obtained by infusion. Table 1 summarizes these different yields depending on the solvent and the method used

Table 1: Extraction yield from different extraction solvents

Méthodes d'extraction	Mass of leaf powder (g)	Mass of extract (g)	Extraction Yield %
Aqueousmaceration	40	4,21	10,5
Hydroethanolicmaceration	40	5,03	12,5
Infusion	40	3,09	7,72
Decoction	40	4,21	10,5

Qualitative phytochemical screening

Qualitative phytochemical screening allowed the detection of secondary metabolites. In the various extracts, the presence of polyphenols, alkaloids, coumarins, flavonoids and also the absence of oxalate, resins, steroids and chalcone were observed. Table 2 summarizes the compounds highlighted in each plant extract.

Table 2: secondary metabolites present in*Mimosa pudica*extracts

Metabolites	Methods	Infusion	Decoction	Aqueous Maceration	Hydro ethanolic Maceration
	$FeCl_3$	+	+	+	+
Polyphenols	Acétate de plomb	+	+	+	+
	Wagner	+	+	+	+
	Hager	+	+	+	+
Alkaloids	Valse-mayer	+	+	+	+
	Acidetannique	+	+	+	+
Mucilage	Ethanol	+	+	+	-
Saponosides	Mousses	+	+	+	-
Coumarins	$FeCl_3$ + HNO_3	+	+	+	+
Flavonoids	NaOH	+	+	+	+
Oxalates	Acideacétiqueglacial e	-	-	-	-
Resins	Acideacétique + H_2SO_4	-	-	-	-
Cardiotonic Glucosids	Keller-killani	-	-	-	+
Steroids	Liberman	-	-	-	-
Total Tannins		+	+	+	+

Catechic Tannins		-	+	-	+
Gallic Tannins		+	+	+	+
Quinones	HCl	+	+	+	-
Chalcones	Ammonia	-	-	-	-
Flavonols		+	+	+	+

Legend: Presence (+), Absence (-).

Quantitative phytochemical screening

The dosage of metabolites in plant extracts led to their quantification.

The distribution of carbohydrates in the different extracts of the leaves of the plant shows that the decoction presents the highest concentration, followed by the hydro-ethanolic extract, the aqueous extract, the infusion presenting the weakest concentration. The figure 1 and 2 respectively illustration the calibration curve and the concentration of carbohydrate obtain in different plant extracts

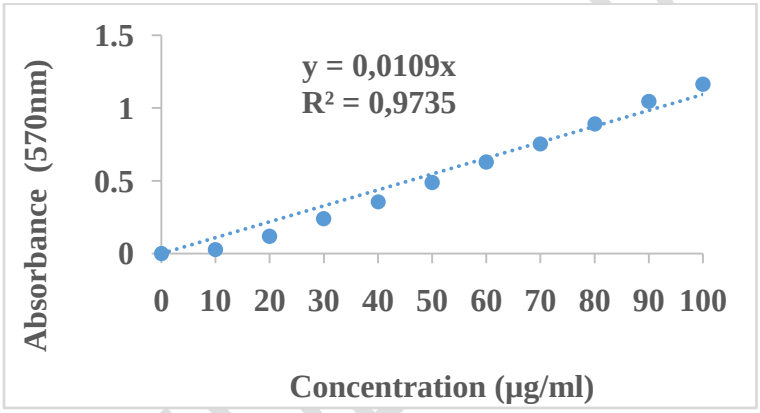

Figure 1: glucose calibration curve

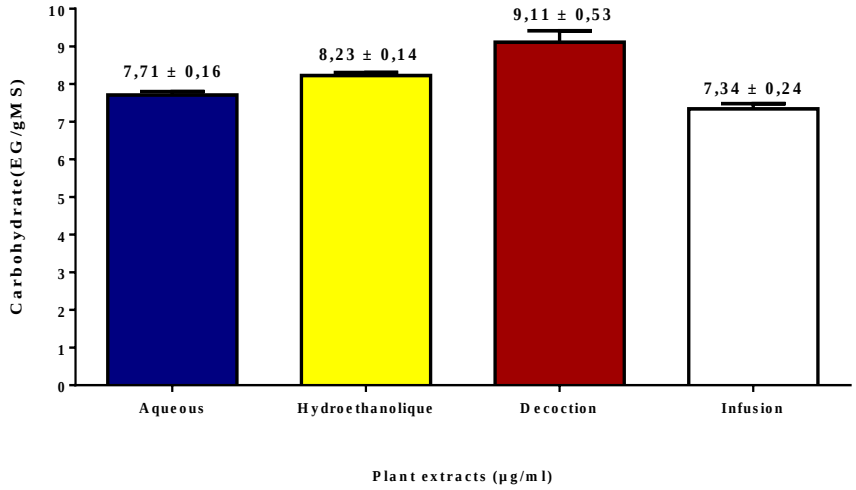

Figure 2: concentration of carbohydrate in plant extracts

The distribution of total proteins in the different extracts of the leaves of the plant shows that the decoction presents the greatest concentration, followed by the hydroethanolic extract, the aqueous extract, the infusion presenting the weakest concentration. The figure 3 and 4 respectively illustration the calibration curve and the concentration of total proteins obtain in different plant extracts

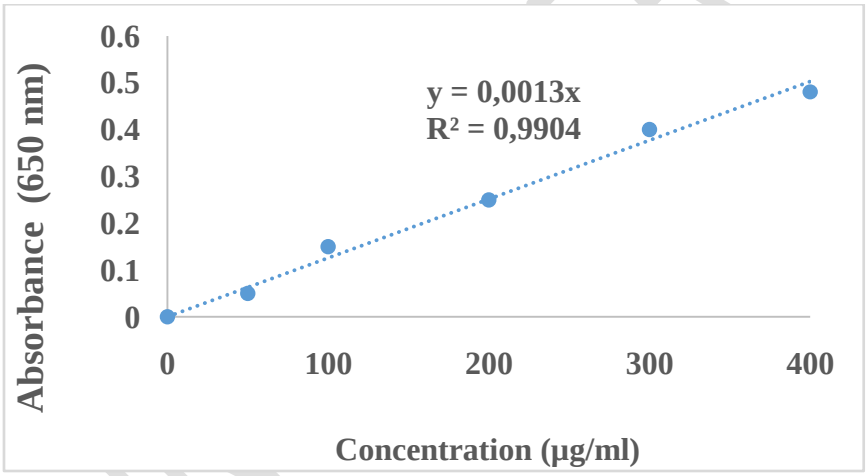

Figure 3: calibration curve of bovine albumin

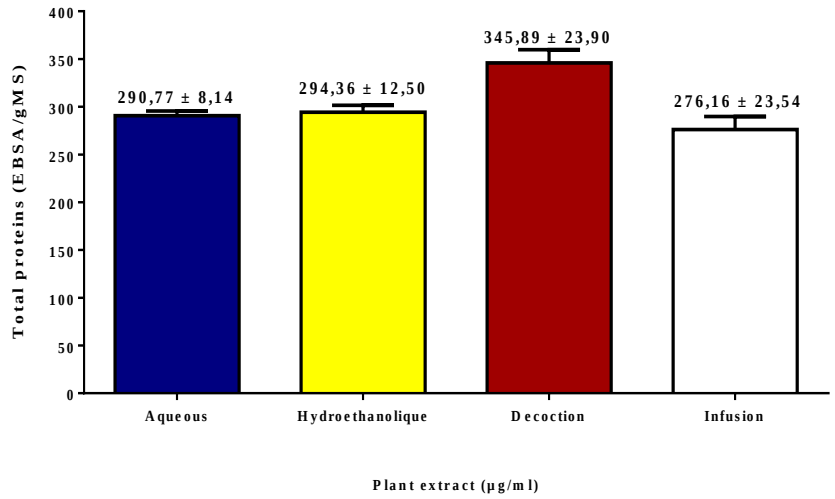

Figure 4: concentration of Proteins in plant extracts

The concentrations of total polyphenols obtained from the calibration curve show that the total quantity of polyphenols is preponderant in the decoction, the hydro-ethanolic extract, of the decoction, the aqueous extract having the lowest concentration. The figure 5 and 6 respectively illustration the calibration curve and the concentration of total polyphenols obtain in different plant extracts

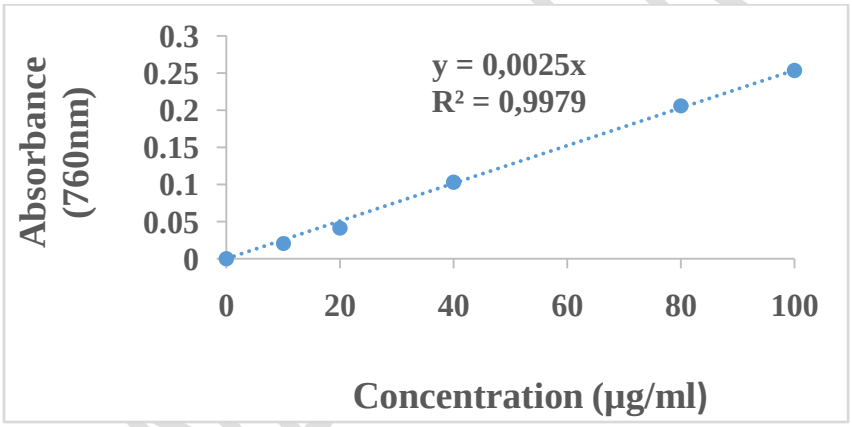

Figure 5: Calibration curve of gallic acid

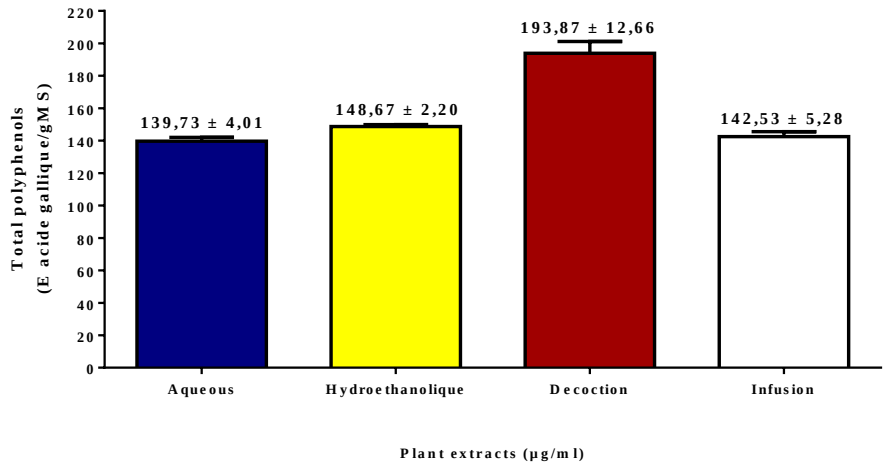

Figure 6: Concentration of total polyphenols in plant extracts

The concentrations of total flavonoids obtained from the calibration curve show that the total quantity of total flavonoids is preponderant in the decoction, followed by the hydro-ethanolic extract, the aqueous extract, the decoction. The figure 7 and 8 respectively illustration the calibration curve and the concentration of total flavonoids obtain in different plant extracts

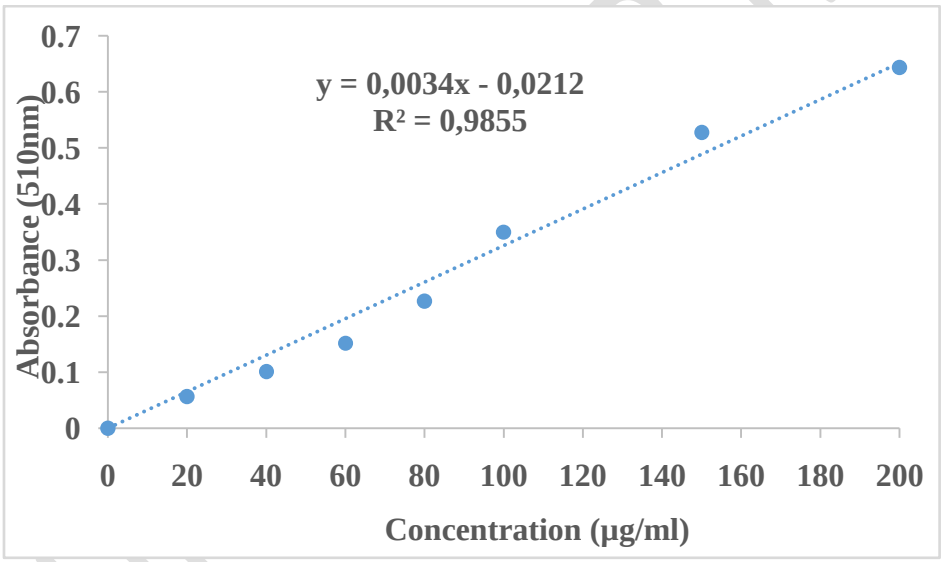

Figure 7: calibration curve of quercetin

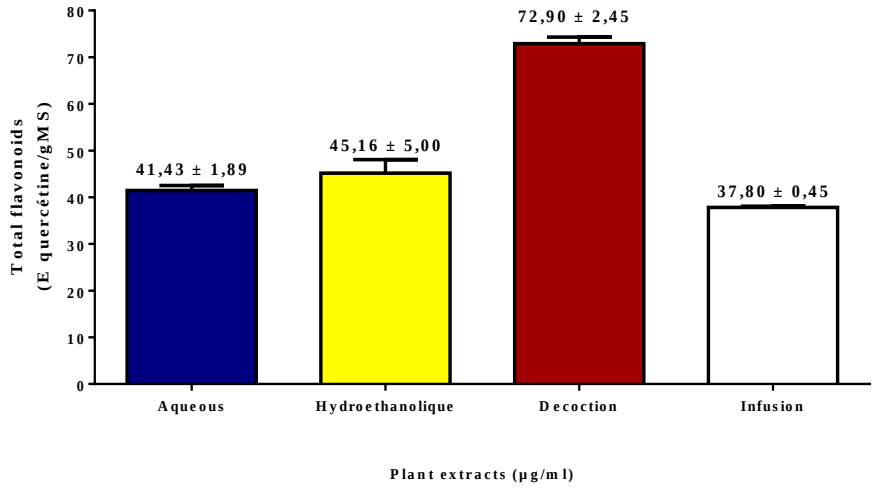

Figure 8: Concentration of total flavonoids

The total flavonol concentrations obtained from the calibration curve show that the total quantity of total flavonols is preponderant in the decoction followed by the infusion, the aqueous extract and the decoction having similar concentrations.The figure 9 and 10 respectively illustration the calibration curve and the concentration of total flavonols obtain in different plant extracts

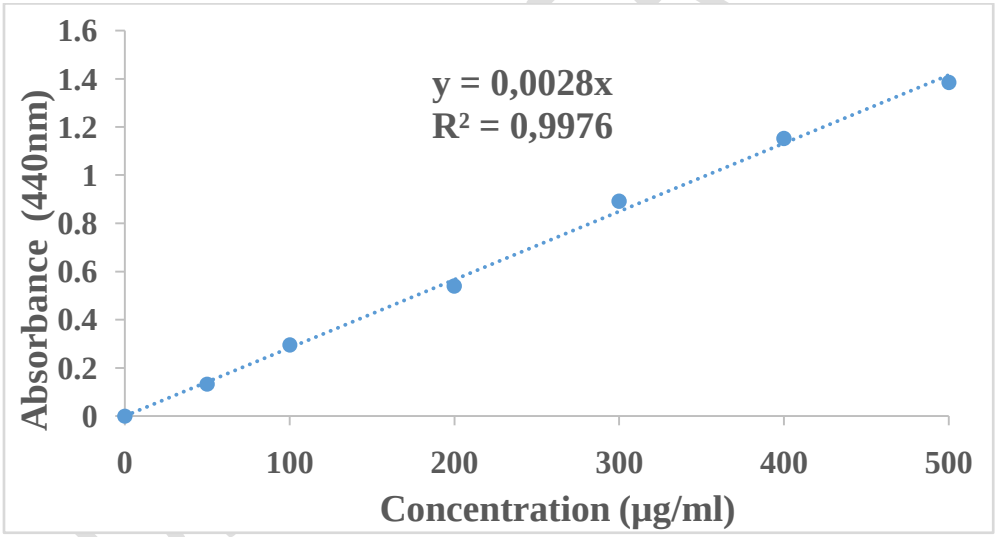

Figure 9: calibration curve of quercetin

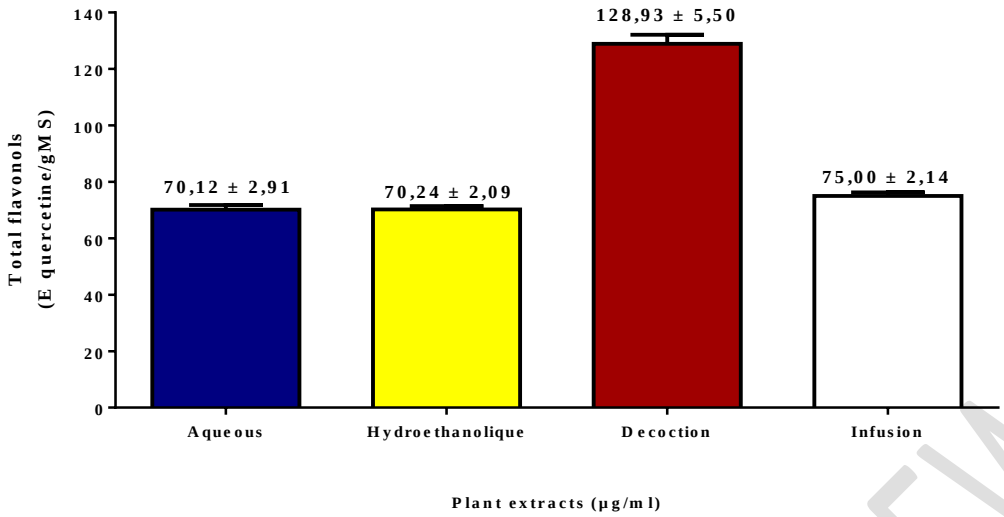

Figure 10: concentration of flavonol in plant extracts

The concentrations of total flavonoids obtained from the calibration curve, showsthat the total quantity of total tannins is preponderant in the hydro-ethanolic extract, followedaqueous extract, decoction, infusion, having the smallest quantity. The figure 11 and 12 respectively illustration the calibration curve and the concentration of Tannins obtain in different plant extracts

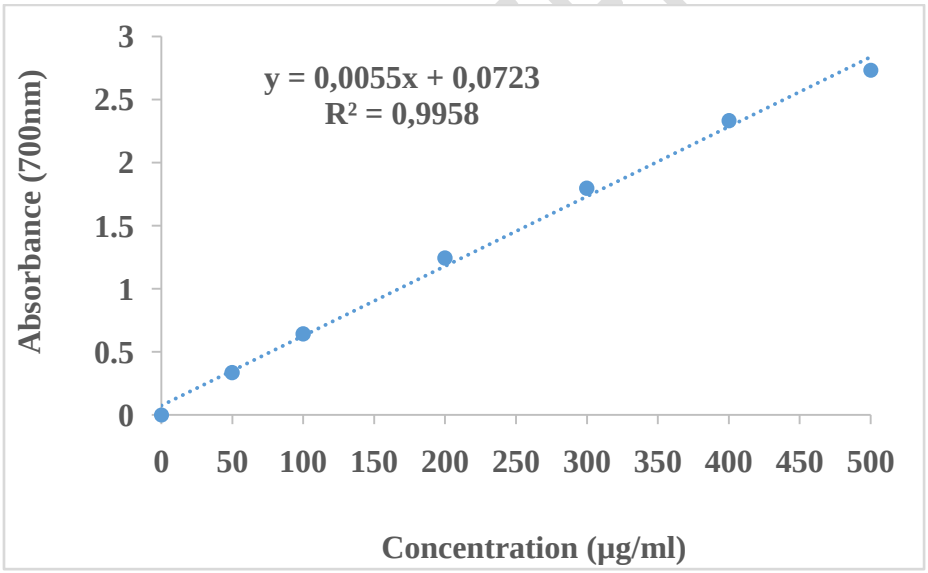

Figure 11: Calibration cur of total Flavonoids

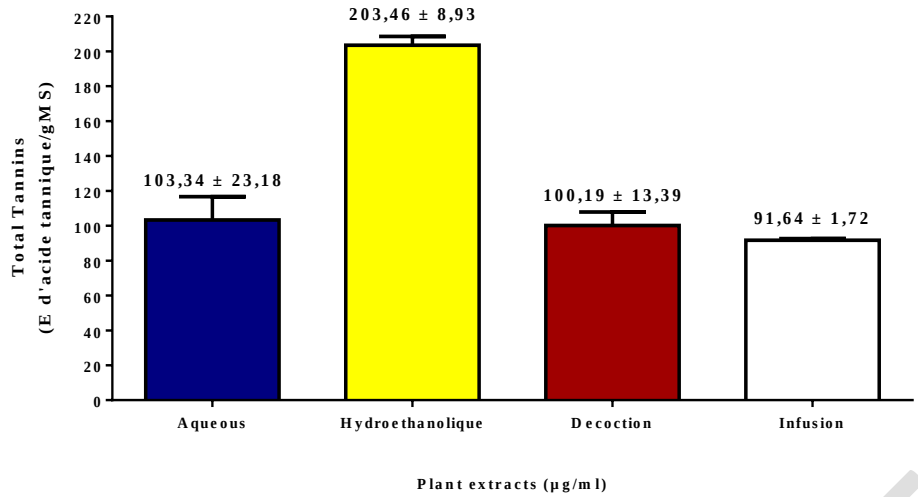

Figure 12: Concentration of Tannin in plant extracts

The concentrations of total flavonoids obtained from the calibration curve show that the total quantity of total tannins is preponderant in the hydro-ethanolic extract followed by the infusion, the decoction, the aqueous extract having the lowest quantity. The figure 13 and 14 respectively illustration the calibration curve and the concentration of Alkaloids obtain in different plant extracts

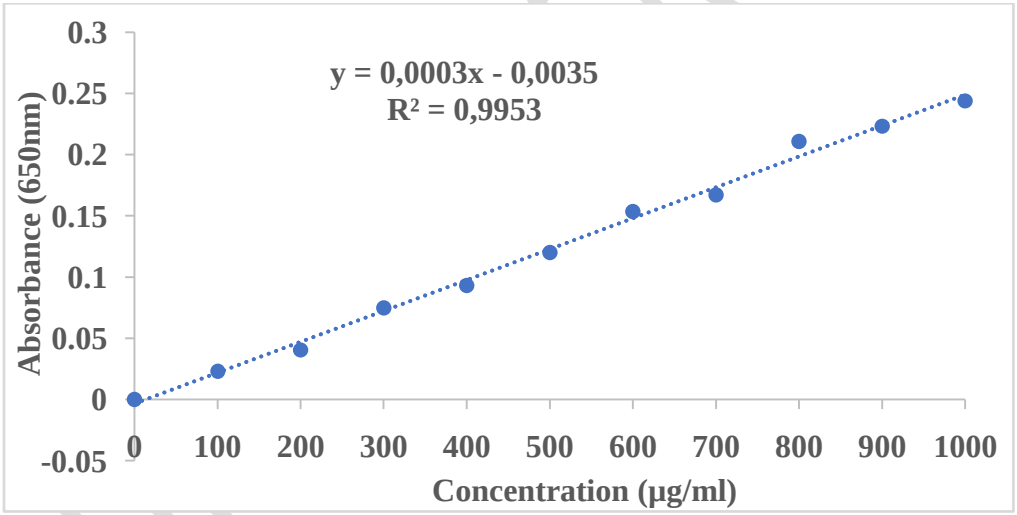

Figure 13: calibration curve of hydrochloride quinine

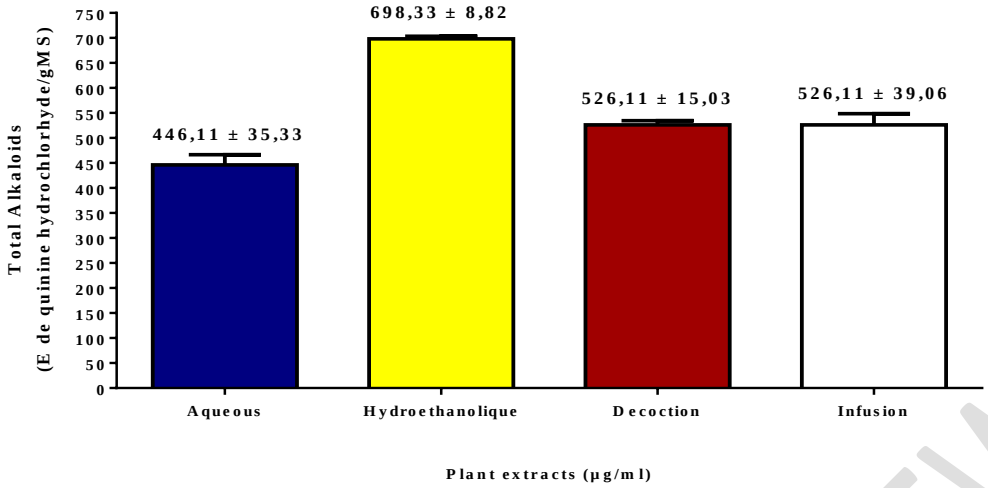

Figure 14: Concentration of alkaloids in plant extracts

The table 3 below summaries the concentrations obtain from the quantification of metabolites in different extracts.

Table 3 summarizes the quantity of metabolite evaluated in each type of extract

Table 3: Concentration of primary and secondary metabolites contained in the leaves

Metabolites	Extracts of the plant (µg/ml)			
	Aqueous	**Ethanol**	**Decoction**	**Infusion**
Carbohydrates	7,71 ± 0,16	8,23 ± 0,14	9,11 ± 0,53	7,34 ± 0,24
Total Proteins	290,77 ± 8,14	294,36 ± 12,50	345,89 ± 23,90	276,16 ± 23,54
Polyphenols	139,73 ± 4,01	148,67 ± 2,20	193,87 ± 12,66	142,53 ± 5,28
Total Flavonoids	41,43 ± 1,89	45,16 ± 5,00	72,90 ± 2,45	37,80 ± 0,45
Total Flavonols	70,12 ± 2,91	70,24 ± 2,09	128,93 ± 5,50	75,00 ± 2,14
Tannins	103,34 ± 23,18	203,46 ± 8,93	100,19 ± 13,39	91,64 ± 1,72
Alkaloids	446,11 ± 35,33	698,33 ± 8,82	526,11 ± 15,03	526,11 ± 39,06

Discussion

The chemical constituents of a plant are the basis of their pharmacological activities. These constituents are classified into primary metabolites which are involved in basic plant processes and functions and secondary metabolites are responsible for various biological responses leading to their use in therapy. Among these compounds, we distinguish phenolic compounds (tannins, coumarins, flavonoid,stilben, lignans), alkaloids, saponins, terpenes [18]. These secondary metabolites have many pharmacological properties including antimicrobial, endocrine, anticancer, healing [19].

This study investigated the phytochemical composition of *Mimosa pudica* leaf extracts. A qualitative exploration of the phytochemical composition allowed the highlighting in the four extracts of compound such as polyphenols, alkaloids, tannins, flavonoids. An evaluation of

the quantitative composition made it possible to quantify primary and secondary metabolites.; in the extract obtained by decoction flavonoids, polyphenols, total flavonolsconcentrations were 128.93 µg/ml 193.87 µg/ ml; 72.90 mcg/ml respectively. Phenolic compounds are very common in the plant kingdom and are part of the group of secondary metabolites among which we have flavonoids, phenolic acids, tannins. many plants that are part of the human diet contain significant amounts and by their complex chemical structure plays an important pharmacological role [20]. Free radicals are produced during biological processes and are highly reactive compounds that react with macromolecules such as proteins and DNA molecules. the phenolic compounds will therefore play a role of scavenger of these free radicals thus preventing an alteration of the cells [21]

With regard to tannins, a much higher concentration of 203.46 µg/ml, was obtained in the hydroethanolic plant maceration, which is lower than the results obtained by Durgadevi et al [22] in 2018 in their study on the phytochemical screening of *Mimosa pudica*. This difference could be explained by the difference in extraction method and the geographical and climatic conditions subjected to the plant. Tannins represent an important class of secondary metabolites and are of great use in the food industry, wood and animal husbandry. As far as the medical and pharmaceutical field is concerned, tannins represent an important source of drugs with antioxidant, antifungal, immune-regulating, antibacterial properties [23]. The richness in tannins of the extract obtained by hydro ethanolic maceration could justify important pharmacological properties of this extract such as antioxidant, antimicrobial, cardioprotective, antidiabetic [24].

For the quantification of the alkaloids, 698.33 µg/ml was the hydro ethanolic maceration which differs from the results obtained by Henry et alin their study on *Mimosainvisa* Mart. leaves and stems [25]. This difference can be explained by the use of an extraction solvent including methanol in their study and also by another kind of Mimosa species. Due to their chemical structure, alkaloids almost always have a physiological activity on the organisms giving rise to the pharmacological properties. These important pharmacological properties would justify the fact that 40% of drugs of natural origin would be alkaloids [26]. Plants Alkaloids has demonstrated important pharmacological activity such as Artemisinin from *Artemisia annua*reveals anti-malarial, Colchicin from *Colchicum autumnale* kills cancer cells in vitro, Quinine, quinidine, and cinchonine from *Cinchona officinali*s are active against malaria and cardiovascular diseases [27]. However, the main pharmacological activity of alkaloids is anticancer. Those plants alkaloids can act by inhibition of primary tumors, inhibition of invasion and metastasis, chemoprevention of carcinogenesis [27]

Conclusion

This study, the object of which was the phytochemical exploration of *Mimosa pudica* leaf extracts, made it possible to show its richness in potentially active secondary metabolites on the pharmacological level and a potential candidate for the development of drugs for various pathologies including polyphenols, alkaloids, tannins, flavonoids, flavonols with concentrations of 193.87 µg/ml; 698.33 µg/ml; 203.46 µg/ml; 72.90 µg/ml; 128.93 mcg/ml respectively. Further studies on the isolation, determination of chemical structures and pharmacological activity of these different phytoconstituents should be carried out.

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