**Chemical profile and effect on bovine serum albumin denaturation of extracts of *Tamarindus indica* L.(Fabaceae) an analgesic plant harvested into Kindia prefecture (Guinea)**

**ABSTRACT**

**Aims**: Pain caused by inflammation is a real public health problem. This work aims to contribute to the valorization of Guinean flora by studying the phytochemistry and in vitro anti-inflammatory activity of Tamarindus indica crude extracts.

**Study Design**: the work falls within the field of the application of organic chemistry to explore sustainable solutions to the basic health of the population in the Republic of Guinea

**Place and duration of study**: Organic chemistry laboratory, Department of chemistry, Faculty of Science, University of Kindia,. December 2024 to March 2025

**Methodology**: Phytochemical screening is carried out using the classical method based on staining and precipitation reactions. Total phenols and flavonoids were determined by the colorimetric method using gallic acid and quercetin respectively as standards. Anti-inflammatory efficacy was determined in vitro using the UV-Visible spectrophometric method, measuring the concentration of the extract that inhibits the denaturation of bovine serum albumin.

**Results**: Extraction yields were 12.12% for the aqueous fruit extract and 7.16% for the hydroethanol extract of T. indica leaves. Qualitative phytochemical screening showed the presence of alkaloids, tannins, flavonoids, leuco-anthocyanins, reducing compounds, cardiotonic heterosides and coumarins in both prepared extracts. In addition to these secondary metabolites, the hydroethanol extract of *T. indica* leaves contains anthocyanins, mucilages, steroids and saponosides. Determination of major compounds showed that the total phenolic compound content of the hydroethanol leaf extract was 134.40 ± 5.22 µg/mg gallic acid equivalent (GAE), while that of the aqueous T. indica fruit extract was 11.80 ± 0.75 µg/mg GAE. The total flavonoid content of the hydroethanolic leaf extract is very high (57.55 ± 2.00 µg eqQ/mg), compared with the aqueous extract of T. indica fruits, which is 1.73 ± 0.77 EAG. In terms of anti-inflammatory activity, the IC50 inhibitory concentration of aspirin was 456.14±4.71µg/ml, lower than that of the aqueous fruit extract (873.95±2.63µg/ml) and the hydroethanol leaf extract (664.14±8.35µg/ml).

**Conclusion**: This study confirms the use of Tamarindus indica leaves and fruit in the treatment of pain

**Key words**: Guinean flora, Tamarindus indica, phytochemical screening, anti-inflammatory activity.

1. **INTRODUCTION**

Since times, the world has used plants to treat illnesses, without knowing what their beneficial effects. Today, herbal treatments are back in the limelight, as the efficacy of conventional medicines declines over time. It is therefore necessary to have a thorough understanding of plant therapeutics. This leads researchers to conduct in-depth studies into the chemical composition of plant secondary metabolites and their therapeutic actions. Anti-inflammatory therapy is generally carried out using synthetic molecules of the non-steroidal or steroidal anti-inflammatory type (corticoids), whose side effects are sometimes serious and represent a major problem in their clinical use. Therefore, to overcome their toxicity, the development of new anti-inflammatory drugs is still needed and natural product such as medicinal plants could potentially serve as a precursor in the production of new drugs to treat inflammation with reduced or zero side effects (Lachkar et al., 2016). Although conventional medicine has undergone spectacular advances, 90% of the world's population relies on traditional medicine today (Jiofack et al., 2010). This return to plants is justified when we consider concerns about the harmful effects of chemical drugs, the quest for healthy living, and the scarcity and high cost of healthcare. The proven efficacy of plants in the treatment of a number of pathologies has led researchers to investigate the pharmacological properties of these plants. In Guinea, several plants are used in the pharmacopoeia, such as Tamarindus indica, whose maceration of powdered *Tamarindus indica* fruit is used to treat pain and inflammation (Barry et al; 2006). There is a need to evaluate the biological properties of this species with a view to its efficient use in pharmacopoeia. It is with this in mind that this work was initiated, with the aim of assessing the anti-inflammatory potential and identifying the main chemical groups with analgesic potential of Tamarindus indica L.(Fabaceaeae) harvested in the Kindia prefecture in Guinea.

1. **MATERIALS AND METHODS**
	1. **Plant material**

The plant material used in this work consists of Tamarindus indica fruits and leaves harvested at Foulayah in Kindia prefecture, Guinea. They were then dried at a constant temperature in the laboratory (18℃). After two weeks of drying, the fruits were pounded with a mortar and the leaves were ground with a Moulinex.

**2.2. Methods**

**2.2.1**. **Preparation of extracts**

Two types of extracts were prepared (aqueous and hydroethanolic extracts) in accordance with the traditional use of the plant. The method used is based on that employed by Houngbeme et al (2015); Deguenon et al (2023); and Houngbeme et al (2025). The aqueous extract was obtained by macerating 50g of crushed fruit in 500 ml of distilled water, left to stir continuously for 48 hours. The resulting homogenate was successively filtered three times on absorbent cotton. The filtrate was dried in an oven at 40°C until the dry residue was obtained. The same procedure was followed for the hydroethanol extract of Tamarindus indica leaves. The maceration solvent used was a binary water/ethanol mixture (4:6, V/V). The extracts obtained were weighed to determine the yield using the classic formula below:

Yield (%) = (Dry extract mass)/(Initial powder mass) X 100

**2.2.2. Identification of secondary metabolites**

Secondary metabolites were identified in prepared extracts by coloration and precipitation reactions as described in the work of Houngbèmè et al. (2014); Ombouma et al. (2021); Agbodjogbé et al. (2022) and Koudjina et al. (2023). Mayer’s and Dragendorff’s tests for alkaloids, Fehling’s test for free reducing sugars, Fehling’s test for glycosides, Liebermann-Burchard’s test for triterpenoids, Liebermann-Burchard’s test for steroids, frothy test for saponins, Shinoda’s and sodium hydroxide tests for flavonoids, ferric chloride test for tannins, Guignard’s test for free cyanogenetics derived and Borntrager’s test for free anthraquinones.

**2.2.3. Spectrophotometric determination of chemical groups**

**Determination of total phenols**

The content of phenolic compounds in extracts was determined by the Folin-ciocalteu method (Maiga et al., 2020; Agbodjogbe et al., 2022). 125 µl of extract at 2 mg/mL were added to 625 µl of Folin- ciocalteu reagent diluted 10-fold. After 5 minutes, the reaction was neutralized with 500 µl of saturated sodium carbonate (75 g/L). The mixture was then incubated in the dark for 2 hours at room temperature, and its absorbance measured at 760 nm using a UV-visible spectrophotometer. Gallic acid (45-500 µg/mL) was used as a standard for the calibration curve. Assays were performed in triplicate. Total phenol content was expressed as µg gallic acid equivalents (GAE)/mg dry extract.

**Quantification of Flavonoid assay**

The flavonoid content of extracts was determined by the aluminum chloride (AlCl3) method (Maiga et al, 2020; Deguenon et al., 2023). 500 µl of extract (2 g/mL) were added to equal volumes of a 2% aqueous solution of AlCl3. The mixture was shaken and absorbance read at 420 nm after incubation in the dark at room temperature for 10 minutes. Quercetin (15 to 500 µg/mL) was used as a standard for the calibration curve. Assays were performed in triplicate. Flavonoid contents were expressed as µg quercetin equivalent (RE)/mg dry extract.

**2.2.4. In vitro anti-inflammatory test**

Protein denaturation is one of the well-documented causes of inflammation and leads to various inflammatory diseases. Therefore, the ability of a substance to inhibit protein denaturation signifies potential anti-inflammatory activity (Rahman et al., 2015). The in vitro anti-inflammatory effect of tamarindus indica extracts is determined using the BSA denaturation inhibition assay according to the method of Kandikattu in 2013 with slight modifications. Thus, we prepared a range of concentrations of each extract, from 0 to 10 mg/ml. 1ml of each dilution is added to 1ml of the 0.2% BSA solution prepared in Tris-HCl (0.05 M at pH 6.6). The mixture is then incubated at 37°C for 15 min, then at 72°C for 5 min. At the end of incubation, after homogenizing with a vortex, the mixture is rapidly cooled and the turbidity measured at 660 nm using a spectrophotometer. In this test, aspirin was used as the reference anti-inflammatory. The test was carried out under the same operating conditions as those applied to the samples. The percentage inhibition of bovine serum albumin (BSA) denaturation was determined using the following formula:

% inhibition= [(absorbance control-absorbance test) / absorbance control] × 100

**3. RESULTS**

**3.1. Extraction yield**

The calculated extraction yield was 12.12% for the aqueous fruit extract and 7.16% for the hydroethanol extract of T. indica leaves. The fruits contain a greater quantity of polar compounds with a higher affinity for water than the leaves. This result may also be explained by the different nature of the two extracts, given that the molecules have different affinities with aqueous and organic solvents.

**3.2. Phytochemical groups of extracts**

Table 1 shows the results of phytochemical analysis of the hydroethanol extract of *Tamarindus indica* leaves and the aqueous extract of *Tamarindus indica* fruits.

Table 1: results for chemical groups identified

|  |  |  |
| --- | --- | --- |
| Chemical groups  | Hydroethanol leaf extract  | Aqueous fruit extract |
| Alkaloids | + | + |
| Tannins | + | + |
| Gallic tannins | + | + |
| Catechic tannins | + | - |
| Flavonoids | + | + |
| Anthocyanins | + | - |
| Leuco-anthocyanins | + | + |
| Quinone derivatives | - | - |
| Mucilages | + | - |
| Triterpenoids | - | - |
| Steroids | + | - |
| Cyanogenic derivatives | - | - |
| Saponosides  | + | - |
| Cardiotonic heterosides | + | + |
| Reducing compounds | + | + |
| Coumarins | + | + |
| O-heterosides  | - | - |
| C-heterosides | - | - |
| Free anthracenics | - | - |

(+): present; (-): absent

The analysis of the phytochemical screening results shows that the hydroethanol extract of *Tamarindus indica* leaves contains secondary metabolites such as: alkaloids, catechic and gallic tannins, flavonoids, anthocyanins, leuco-anthocyanins, mucilages, steroids, reducing compounds, saponosides, cardiotonic heterosides and coumarins. We deduce that *T. indica* leaves are very rich in chemical compounds with proven pharmacological properties. Free anthracenics, O-heterosides, C-heterosides, triterpenoids, quinone derivatives and cyanogenic derivatives are also absent. The absence of toxic chemical groups, namely cyanogenic, cardiotonic and quinonic derivatives (Houngbeme et al., 2014; Koudjina et al., 2023), goes some way to explaining why this plant is so widely consumed. We also note that the aqueous extract of *Tamarindus indica* fruits contains 7 secondary metabolites in common with the hydroethanol extract: alkaloids, gall tannins, flavonoids, leuco-anthocyanins, reducing compounds, cardiotonic heterosides and coumarins. It can be concluded that these chemical groups are the most common and give this plant the pharmacological properties it enjoys in traditional medicine.

**3.3. Phenolic compound content**

The estimated total phenolic compound content is shown in Table 2. It is calculated from the equation of the regression line given in figure 1.

**Figure 1**: Standard curve for the determination of total phenols

**Table 2**: Total polyphenol content of extracts

|  |  |
| --- | --- |
| Extracts | Polyphenols (µg/mg) EAG |
| Aqueous fruit extract  | 11,80 ± 0,75 |
| Hydroethanol leaf extract | 134,40 ± 5,22 |

Phenolic content varies from one extract to another. The phenolic content of the hydroethanol extract of *Tamarindus indica* leaves (134.40 ± 5.22 µg/mg) is 10 times higher than that of the aqueous extract of the fruit (11.80 ± 0.75 µg/mg GAE). The leaves are therefore richer in phenols than the fruit. The mixture of water and ethanol enabled the majority of phenolic compounds to be extracted from the plant.

**3.4. Flavonoid content**

Figure 2 shows the calibration curve for quercetin, whose equation is used to calculate the quercetin equivalent values given in Table 3.



 Figure 2: Standard curve for flavonoid determination

Table 3: Total flavonoid content

|  |  |
| --- | --- |
| Extracts | Total flavonoid (µg/mg) EQ |
| Aqueous fruit extract  | 1,73 ± 0,77 |
| Hydroethanol leaf extract | 57,55 ± 2,00 |

These results show that the hydroethanol extract of *Tamarindus indica* leaves has a high flavonoid content equal to 57.55 ± 2.00 µg/mg EQ, compared with the aqueous extract of *Tamarindus indica* fruits, which is 1.73 ± 0.77 EQ. In both assays, the hydroethanol extract of the leaves is richer in total phenols and flavonoids. It could therefore be said that the leaves are more active in terms of the plant's antioxidant and anti-inflammatory properties.

**3.5. Anti-inflammatory activity**

The percentage inhibition of BSA denaturation of each of the extracts and the reference (aspirin) at various increasing concentrations was calculated, and Figure 3 below shows the evolution or variation of this parameter as a function of the concentrations of the diluted solutions.



 Figure 3: Evolution of BSA denaturation inhibition by extracts and aspirin

The analysis of this figure clearly shows that the percentage of BSA inhibition increases proportionally with the concentration of extracts in solution. Inhibition is much more remarkable for the reference represented by aspirin, after which comes the hydroethanol extract of leaves, and then the aqueous extract of fruit.

To appreciate well the effect of extracts on BSA, we determined the inhibitory half-concentrations of the different extracts evaluated. Table 4 summarizes these IC50 values.

Table 4 : Inhibitory concentration of extracts and aspirin

|  |  |
| --- | --- |
| Extracts / Standard   | IC50 (µg/ml) |
|  Average ± standard deviation (n=3) |
| Aspirin |  456,14±4,71 |
| Fruit |  873,95±2,63 |
| leaves |  664,14±8,35 |

This table shows that *T. indica* fruits and leaves effectively inhibit the denaturation of bovine serum albumin (BSA). The mean values of the inhibitory half-concentrations of the extracts tested are higher than those of the reference (aspirin), which means that the extracts have a weaker anti-inflammatory effect than aspirin. This shows that the extracts have a lower inhibitory activity than aspirin.

**Discussion**

The two extracts prepared showed different yields. The highest yield was recorded for the aqueous fruit extract. This difference in yield may be linked to the polarity of the solvents used for extraction.

Qualitative phytochemical screening revealed the presence of alkaloids, tannins, flavonoids, leuco-anthocyanins, reducing compounds, cardiotonic heterosides and coumarins in both extracts. In addition to these secondary metabolites, the hydroethanol extract of T. indica leaves contains anthocyanins, mucilages, steroids and saponosides. These results are similar to those of Piba et al, (2021) who observed polyphenols, tannins and flavonoids in the aqueous extract of the stem bark/Tamarindus indica leaf mixture in the treatment of stroke sequelae and in the secondary prevention of risk factors. In addition, the work of Dongock et al (2018), in Chad, indicates the richness of tannins, flavonoids, alkaloids and saponins in plants used in the treatment of cardiovascular disease. Furthermore, catechic tannins, free flavonoids and anthraquinones were reported by Ahodegnon et al., (2018) as secondary metabolites predominantly present in the pulp of *T. indica* leaves. These results are also in line with Paula et al (2009), De Caluwé et al (2010) and Bhadoriya et al (2012), who showed that *Tamarindus indica* fruit pulp contains tannins, coumarins, saponosides and mucilages. These secondary metabolites have highly recognized properties in the treatment and prevention of cardiovascular disease (Piba et al., 2021). Indeed, studies by Kee et al. (2008) and Praveen and Kumud (2012) have shown that tannins have antithrombotic and anticoagulant properties. Work by Chen et al. (2009) and O'Leary et al. (2004) indicates that flavonoids have anti-inflammatory, antihypertensive (antiallergic) and antiplatelet activities. Researchers Proteggente et al. (2011) and Luna-Vázquez et al. (2013) have found that polyphenols have vasodilatory and antihypertensive properties, in addition to antithrombotic and anti-inflammatory properties (Manach et al., 2005). Furthermore, recent work by Bonin et al. en al, 2023, on the putative compounds identified in the ethyl acetate fraction of *Tamarindus indica* seeds has clearly shown that this fraction is rich in flavonoids and polyphenols, which are key secondary metabolites with antioxidant potential. Tamarind pulp, seeds and leaves contain phenolic compounds with antioxidant activity, 25% of which are present in the seeds (Villacís-Chiriboga et al., 2020).

However, our results differ from those of other authors. Firstly, it should be noted that secondary metabolites such as free anthracenics, O-heterosides, C-heterosides, triterpenoids, quinone derivatives and cyanogenic derivatives, are not present in our extracts. Indeed, the work of Ouédrago et al (2010) on *T. indica* fruits revealed sterols and/or triterpenes and anthracenosides in the dichloromethane extract, pectins in the hydroalcoholic extract, and organic acids, reducing compounds, anthocyanins, carbohydrates and carotenoid-related substances in the aqueous extract of the plant drug. The presence of triterpene sterols and anthraquinones in T. indica fruit extracts reported by these authors may be explained by the variability of the solvents used. In addition to these factors, a plant's composition of secondary metabolites varies according to geographical location, the organ harvested, the time of harvest and storage conditions (Dakle, 2023).

Quantitative phytochemical screening showed that the total phenolic compound content of the hydroethanol extract of *Tamarindus indica* leaves was 134.40 ± 5.22 µg/mg gallic acid equivalent (GAE), while that of the aqueous extract of *Tamarindus indica* fruits was 11.80 ± 0.75 µg/mg GAE. This suggests that the leaf extract has a more pronounced DPPH-scavenging activity and reducing power than the fruit extract. These results fall short of those of Piba et al, (2021) who found 34747.13 ± 179 (μg EAG/g MS) ± SD as the total polyphenol content in the aqueous extract of the *T. indica* stem and leaf mixture with high significance. In terms of total flavonoids, the extracts significantly reduced DPPH radicals. The results show that the hydroethanol extract of *Tamarindus indica* leaves has a high content of total phenols equal to 57.55 ± 2.00 µg/mg, compared to the aqueous extract of *Tamarindus indica* fruits which is 1.73 ± 0.77 EAG. This evidence has been justified by several authors. Indeed, Diallo revealed in 2005 that phenolic compounds are free radical scavengers. Long before the recent studies by Bonin et al. (2023), some authors reported that T. indica seeds are an important source of antioxidants such as flavonoids and polyphenols, which are heterocyclic molecules associated with beneficial effects on human health (Doughari, 2007; Siddhu-raju, 2007). These results show that the anti-inflammatory effect linked to the antioxidant activity of this species is mainly due to the polyphenols and flavonoids it contains.

In terms of anti-inflammatory activity, aspirin's IC50 inhibitory concentration of 456.14±4.71 µg/ml is lower than that of the aqueous fruit extract (873.95±2.63 µg/ml) and the hydroethanol leaf extract (664.14±8.35 µg/ml). This suggests that both extracts have an inhibitory activity on the BSA protein, whose denaturation causes inflammation. These results are similar to those of Bonin et al (2023), who confirmed the anti-inflammatory activity of T. indica fruit extract. Extracts with anti-inflammatory potential inhibit BSA denaturation after heat shock.

1. **CONCLUSION**

The present work has made it possible to enhance the value of Tamarindus indica leaves and fruits from Guinea through their anti-inflammatory capacity, proven by the inhibition of BSA denaturation after heat shock in an in vitro model. This biological activity is correlated with the richness of leaf and fruit extracts in various secondary metabolites, the most important of which are polyphenols and flavonoids recognized for their antioxidant activities. This study revealed very interesting levels of these phytochemical compounds and demonstrated their anti-inflammatory effects, albeit at a lower level when compared with control aspirin. We plan to carry out further studies, mainly evaluating cellular toxicity and identifying new bioactive molecules, in order to standardize the use of T. indica in the Republic of Guinea, against inflammation-related pain.

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