INTRODUCTION

Plants have long played a very important role in the evolution of humankind, they can synthesize a large number of complex organic molecules with often potential biological activities. They are wonderful plant seedlings that give us the joy of healing with a therapeutic gesture [1-2]. It is used traditionally to heal, relax, flavor food and preserve food etc.

Until today, the popular use of plants remains of great importance. According to the WHO (World Health Organization), 80% of the world’s population treat their health problems by traditional remedies, because they often do not have access to medicines prescribed by modern medicine and, secondly, because these plants are often very effective [3]. Indeed, the majority of current medicines are of origin or made from their models (synthesis or chemical semisynthesis of active ingredients). Herbal medicine has become a great science, in which we start from the plant to the active ingredient.

In West Africa, as in the rest of the continent, more than 80% of the population uses traditional medicine and medicinal plants for their primary health care. Lack of essential drugs, inadequate health care, the high cost of drugs and socio-cultural habits explain the use of traditional herbal practices [4]. In addition, the efficacy of recipes in the treatment of several pathologies makes them more interesting than modern synthetic molecules [5].

In this context, the use of medicinal plants as an alternative to modern medicine is not to be minimized. Knowing, improving and valuing the use of medicinal plants is an urgent and permanent need. In this regard, Tridaxprocumbens is one of the herbs used in the treatment of hyperthermia, typhoid fever, asthma, epilepsy, and diarrhea [6]. It is used for the treatment of bronchial catarrh, dysentery, malaria, diarrhea, hypertension, bruising [7]. Several researchers have reported that it has immunomodulatory properties [8], anti-diabetic agents [9], anti-hepatotoxic, antioxidant [10], antimicrobials [11], antibacterial [12], antiplasmodium [13], and anti-cancer [14]. The hydroethanolic extract of T. Procumbens is a good antioxidant that significantly reduces the accumulation of hepatic lipids and stimulates their oxidation in HepG2 cells [15]. Several secondary metabolites in T. Procumbens extracts. In Benin, phytochemical screening revealed the presence of catechin tannin, flavonoid, mucilage and reducing sugar [16]. Similarly, a study report revealed the presence of triterpenoids, steroids, flavonoids, mucilages and reducing compounds [17].

The present work aims to evaluate the effect of tridaxprocumbens on blood clotting in Wistar rats. For this purpose the objectives of the work are formulated as follows:

The overall objective of this study is to evaluate the effect of tridaxprocumbens on the blood clotting in Wistar rats.

Generalty

Tridax procumbens

Description botany

From the family Asteraceae (sunflowers), Tridaxprocumbens is an annual species native to tropical America, introduced in the tropical,
Animal material consisted of the phase of realization of the fractions of the leafy stems. Methods for evaluating coagulation parameters were carried out on adult male and female Wistar type rats aged from 10 to 12 weeks and weighing between 100 and 200 g. These animals were raised at the laboratory of Biomembranes and Cell Signaling from FAST to UAC under standard environmental conditions. Animals have free access to water and food.

Materials and Methods

Plant material

The plant material consists of stems and leaves of Tridax procumbens.

Animal material

The experiments were carried out on adult male and female Wistar strain weighing between 100 and 200 g. Upon receipt, the rats were randomly placed in groups of 3 in the standard cages for an acclimation period before being used in the different experiments. During this period, the animals had free access to food and water and remained in the animal laboratory of the biomembrane and cell signaling laboratory of the Department of Animal Physiology of the University of Abomey-Calavi.

Methods

The phase of realization of the fractions of the leafy stems. It consisted:

- Batch 1: 3 rats, fed with distilled water.

Tridax procumbens is a spreading herbaceous plant whose flowering ends are ascending, its root is a powerful pivot (root that sinks vertically into the ground). The opposite leaves, simple and irregularly toothed, arrow-shaped; they are thick, soft and dark green in color. The leaves are bound by a petiole 1 to 2 cm long. The lamina is ovate to lanceolate, with a base attenuated in the wedge and irregularly toothed margin (fig. 1). The stem is cylindrical, full and strongly hispid, covered with multicellular white hairs of 1 mm, tubercular at the base. The flowers are assembled in solitary heads. The flower heads at the end of long peduncles are composed of 4 to 7 cream-colored ligulate peripheral flowers and numerous yellow tubular flowers. In the center of the capitulum 10 to 30 yellow, tubular flowers that are hermaphroditic separated by membranous scales. The fruit of the plant is a hard conical achene covered with stiff hairs and surmounted feathery featheryfeathery. Tridax procumbens is propagated by seed but has a strong potential for cuttings especially in the rainy season. Although an annual species, the plant can be planted when regularly cut [18].

**Use in traditional medicine**

Tridax procumbens is a plant with many virtues from which are used in traditional medicine. Thus, it is used in the treatment of many pathologies such as bronchial catarrh, dysentery, diarrhea, hair loss, jaundice [5-19-20]. Leaf sap is also used to treat fresh wounds and stop bleeding. The entire plant is used in Guatemala for the treatment of protozoan diseases such as malaria, leishmaniasis, dysentery and in the treatment of colic and gastroenteritis [21-22]. It is also used in West Africa to treat conjunctivitis [23]. In Nigeria, it is used against yellow fever, typhoid fever, asthma, cough, epilepsy, stomachaches, and diarrhea [6-24]. In Togo, the fresh leaves of T. procumbens are crushed and used for dressing the wounds; Leaf decoction is used orally to relieve abdominal pain and fungal infections and to treat malaria [25]. In traditional veterinary medicine, T. procumbens is also mentioned in plants for pharmacological use recognized and used by breeders in Africa [26]. In Benin, the plant is also widely used by pastoralists in the diet of several crops including cattle and rabbits [27-28].

**MATERIALS AND METHODS**

**Materials**

- **Plant material**
  The plant material consists of stems and leaves of Tridax procumbens.

- **Animal material**
  The experiments were carried out on adult male and female Wistar strain weighing between 100 and 200 g. Upon receipt, the rats were randomly placed in groups of (3) in the standard cages for an acclimation period before being used in the different experiments. During this period, the animals had free access to food and water and remained in the animal laboratory of the biomembrane and cell signaling laboratory of the Department of Animal Physiology of the University of Abomey-Calavi.

- **Methods**
  The phase of realization of the fractions of the leafy stems. It consisted:

- Batch 1: 3 rats, fed with distilled water.
The blood from the rats of lots (1-3) are all collected before treatment at D = 0. The blood sample is taken according to the experimental protocol used by [29], and modified by Descat in 2000[30]. Puncture of the retro-orbital sinus is performed under anesthesia (diethyl ether). The anesthetized animal is held in one hand in the lateral decubitus, and held by the skin of the neck. The pressure of the thumb on the neck, behind the angle of the jaw, will achieve compression of the jugular vein, and thus a venous stasis to the head, favoring the filling of the retro-orbital sinus. By making a slight pull on the upper eyelid with the index finger, we will create an exophthalmia facilitating the collection of blood using a non-heparinized hematocrit tube. The end of the tube is slowly introduced into the lateral angle of the eye. The progression through the tissues is facilitated by printing a slight rotation of the tube. As soon as one reaches the venous plexus, the blood gushed in the periorbital space and rises by capillary action in rotation of the tube. As soon as one reaches the venous plexus, the blood gushed in the periorbital space and rises by capillary action in the tube. The volume of blood collected is approximately 1 to 2 ml in EDTA tubes, citrate tubes, and dry tubes. Before removal of the tube, the compression is released and the bleeding stops spontaneously when the ocular pressure is normalized. The recovered blood is used for the determination of different parameters of hemostasis.

The bleeding time was carried out according to the method of DUKE, in which two healthy rats and two rats treated with a dose of 100 and 300 mg/kg of PC were used. One rat was taken at a time, the anterior surface of his cleanly shaved tail was carefully cleaned using cotton buffered in 70% ethanol and with disposable lancets 3 mm deep a laceration was done on the shaved tail. A drop of the preparation of the extract was delivered directly into the experimental laceration and a judgment was observed. The addition was made periodically and blotted using cotton until the bleeding stopped and the time taken for the bleeding to stop was recorded as the bleeding time. Another laceration was done on the tail of another rats to serve as a control and the above procedure was repeated but this time without adding the extract and the bleeding time was recorded. Both procedures were then repeated for the other rats.

**RESULTS AND DISCUSSION**

**RESULTS**

**Realization of fractions of tridaxprocumbens**

The yield of the different fractions obtained is calculated according to the formula:

$$R = \frac{\text{Mass of the fraction}}{\text{Mass of the powder}} \times 100$$

<table>
<thead>
<tr>
<th>Solvent type</th>
<th>Extract yield in%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexane</td>
<td>0,160</td>
</tr>
<tr>
<td>Dichlorométhane</td>
<td>0,073</td>
</tr>
<tr>
<td>Ether-diéthylé</td>
<td>0,093</td>
</tr>
<tr>
<td>Acétate d'éthyle</td>
<td>0,150</td>
</tr>
</tbody>
</table>

These yields are consistent with those of Amagbegnon in 2017 [31], but very low compared to those of Koudoro et al in 2014 [32] which by this method carried out on cochlospermumplanchoni root bark obtained 0.0% and 1.5%, % for the respective fractions of diethyl ether and ethyl acetate. We believe that this difference in yield could be due to the duration of the mixing of the aqueous phase with the different solvents.

**Table 2: Results of phytochemical screening phytochemical screening**

<table>
<thead>
<tr>
<th>Chemical compounds</th>
<th>Leaf and stems of tridaxprocumbens</th>
</tr>
</thead>
<tbody>
<tr>
<td>alkaloids</td>
<td>-</td>
</tr>
<tr>
<td>Polyphenolic compounds</td>
<td>-</td>
</tr>
<tr>
<td>flavonoids</td>
<td>+++</td>
</tr>
<tr>
<td>anthocyanin</td>
<td>-</td>
</tr>
<tr>
<td>Buffy antocyane</td>
<td>-</td>
</tr>
<tr>
<td>Quinone derivatives</td>
<td>-</td>
</tr>
<tr>
<td>saponoids</td>
<td>-</td>
</tr>
<tr>
<td>triterpenoids</td>
<td>+</td>
</tr>
<tr>
<td>sterols</td>
<td>+</td>
</tr>
<tr>
<td>mucilage</td>
<td>++</td>
</tr>
<tr>
<td>coumarins</td>
<td>-</td>
</tr>
<tr>
<td>Reducing compounds</td>
<td>++</td>
</tr>
</tbody>
</table>

+: Low presence, +: Strong presence, ++: Very strong presence, -: Absence

The results obtained following the phytochemical screening of the leaves and stems of tridaxprocumbens are recorded in the table. We thus note in the leaves a very strong presence of flavonoids, a weak presence of sterol, mucilage and a weak presence of terpene.

**Blood collection method**

The results obtained following the phytochemical screening of the leaves and stems of tridaxprocumbens are recorded in the table.
and a strong presence of reducing compounds this is in accordance with the results of Koukou in 2015, and Ganjiun in 2013 [16-33]. By other research like that of Ikewuchi [ude et al, 2009 identified other phenolic compounds in Tridax Procumbens extracts such as alkaloids, tannins, carotenoids, saponosides [34].

**Evolution of the weight of rats during treatment**

We observed an increase of the weight in the control rats whereas the weight of the treated rats (100 and 300 mg/kg of PC) decreased. Tridaxprocumbens extracts could, therefore, be said to reduce slightly or prevent weight gain in rats. This may be due to the lipid-lowering effect [16] thus inhibiting the accumulation of fat in the adipose tissue.

**Fig. 2: Evolution of the weight of normal control rats and those treated with the hydroethanolic extract of the leaves and stems of tridaxprocumbens during 07 d**

We can deduce from the interpretation of this fig. that the number of platelets has increased.

**Fig. 5: Effect of tridaxprocumbens on platelet count**

**Determination of activated partial thromboplastin time (APTT)**

**Fig. 6: Effect of tridaxprocumbens on activated partial thromboplastin time**

**Fig. 7: Effect of tridaxprocumbens on the prothrombin rate**

**DISCUSSION**

Tridaxprocumbens, commonly known as French rabbit herb and Azuiman or Hladogboenfon, is a plant of the family Asteraceae. It is used in traditional medicine for various purposes. There is an important hepatoprotective and healing property, the combination of Allium Sativum/TridaxProcumbens is used to inhibit platelet aggregation.

**Fig. 3, shows the results obtained from the bleeding time experiment.**

**Determination of bleeding time**

**Fig. 3: Effect of tridaxprocumbens on bleeding time**

**Fig. 4: Effect of tridaxprocumbens on coagulation time**

**Fig. 4, shows the results obtained from the coagulation time experiment. The clotting time decreased in all the rats treated with the extract.**

**Determination of clotting time**

**Fig. 4: Effect of tridaxprocumbens on coagulation time**

**Determination of prothrombin levels (TP)**

**Fig. 4: Effect of tridaxprocumbens on coagulation time**

**Fig. 7: Effect of tridaxprocumbens on the prothrombin rate**

**Determination of platelet count**

**Fig. 5: Effect of tridaxprocumbens on platelet count**

**Determination of clotting time**

**Fig. 4: Effect of tridaxprocumbens on coagulation time**

**Determination of prothrombin levels (TP)**

**Fig. 7: Effect of tridaxprocumbens on the prothrombin rate**

**DISCUSSION**

Tridaxprocumbens, commonly known as French rabbit herb and Azuiman or Hladogboenfon, is a plant of the family Asteraceae. It is used in traditional medicine for various purposes. There is an important hepatoprotective and healing property, the combination of Allium Sativum/TridaxProcumbens is used to inhibit platelet aggregation.
From these data, we set ourselves the objective of evaluating the activity of the hydroethanolic extract of the stems and leaves of Tridax Procumbens on the coagulation of blood.

From the results of our study, the hydroethanolic extract of the stems and leaves of Tridax Procumbens gives a yield of 9.92%. We thus note in the leaves a very strong presence of flavonoids, a weak presence of sterol, mucilage and a weak presence of terpene and a strong presence of reducing compounds this is in accordance with the results of Koukouiin 2015, and Ganju in 2013. By other research like that of Ikewuchi Jude in 2009 identified other phenolic compounds in Tridax Procumbens extracts such as alkaloids, tannins, carotenoids, saponosides.

The results obtained show that the bleeding time without the extract is 2.58±0.31 min while the average bleeding time after treatment with the extract is 1.61±0.70 min at a dose of 100 mg/kg PC and 1.77±0.69 min at a dose of 300 mg/kg PC. Thus the extract significantly lowers the bleeding time. These results are comparable to those of Adogo J. L. [35]. Who found that the average bleeding time without the extract is 2.49±0.09 and that treated with the extract is 1.40±0.16 min.

Coagulation time decreased in all rats treated with the extract. The coagulation time at D0 is on average 2.85±0.13 min at D0 and 2.52±0.24 min at D7 for the control rats. For those treated with the extract, the mean clotting time is 2.99±0.16 min at D0 and 2.44±0.54 min at D7 at a dose of 100 mg/kg PC, 2.39±0.25 min at D0 and 2.24±0.29 min at D7 at a dose of 300 mg/kg PC. Thus the extract significantly reduces the coagulation time. These results are comparable to those of Kukwa D T [35], who proved that the clotting time decreased steadily as the dosage of the applied extract increased.

We can deduce from the interpretation of fig. 5 that the number of platelets increased. However, in rats treated with tridaxprocumbens extract, the increase in platelet count was considerably elevated in the rats treated with the 300 mg/kg dose of PC than in those treated with the dose of 100 mg/kg PC. These results are consistent with those of Z. C. Okeoye [35], who have demonstrated a beneficial effect of tridaxprocumbens on blood clotting.

With activated partial thromboplastin time, the results are expressed second to a control. A time inferior to that of the control has no pathological significance. TCA is the coagulation time of a plasma treated under particular conditions. Fig. 7 shows the effect of tridaxprocumbens on the prothrombin rate. In patients on anticoagulants, the treatment is considered effective when the INR is between 2, 4, and 5 is a rate of prothrombin less than 20% which is the case of our results. This implies that the intrinsic and extrinsic pathways are ignored. Indeed, the coagulation factors involved in these pathways occur in minute amounts in the blood, unlike prothrombin which occurs in large quantities. It is therefore likely that the extract exerts its procoagulant effect by rapid activation of prothrombin [35].

It is not always possible to have all these exams. In the absence of TCA, TP, TH, fibrinogen assay or platelet count, and if the planned intervention or its immediate consequences reveal a real bleeding risk, at least:

-To achieve a bleeding time at the earlobe (Duke). This test may be an interesting alternative to platelet count. It also informs more generally about primary haemostasis. However, it does not dispense with a study of the coagulation phase.

-To achieve a coagulation time that will allow to roughly quantify the haemorrhagic disorder without being able to define the cause of the disorder.

-To warn the surgeon so that the surgical haemostasis is as careful as possible.

CONCLUSION

The T. procumbens plant is considered a weed. However, it represents a "pharmacy" plant used for therapeutic purposes in both human and animal health. During this study, carried out in the laboratory of biomembranes and cellular signalling of Abomey Calavi, it was revealed the different chemical groups present in the leaves and stems of Tridax procumbens as well as its effect on the coagulation of blood. At the end of our work, we can conclude that our sample contains phenolic compounds, reducing sugars, sterols, terpenes, flavonoids, and mucilages found in the leaves and stems of the plant. The presence of these metabolites in our samples would be responsible for the procoagulant activity of tridaxprocumbens demonstrated in this study. The dose of 100 mg/kg of body weight would be more appropriate to reduce it and stop the bleeding time and also avoid an increase in activated partial thromboplastin time. The 300 mg/kg dose of PC would better for platelet counting, prothrombin activation, reduction in body weight and coagulation time.

The plant material studied in this study is, therefore, a credible alternative for an effective fight against bleeding and also promotes the coagulation of blood.

AUTHORS CONTRIBUTIONS

All the author have contributed equally

CONFLICT OF INTERESTS

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

REFERENCES


