Biochemical and Hemostatic properties of herbal plants used for the treatment of bleeding in Mali

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ABSTRACT

Malian population successfully uses medicinal plants to treat bleeding disorders. In this study, the biochemical and hemostasis properties of extracts from 12 parts of 10 plants were investigated. The chemical composition of dried and powdered material was characterized using chemical groups’ specific detection reagents and TLC. Water, dichloromethane and methanol were incubated in a proportion of 10% weight/volume of powdered macerated for 24 h in solvents. The effect of the extracts on hemostasis parameters was investigated using whole blood from healthy volunteer donors at the final concentration of 0.25 g/L. Activated Prothrombin time (aPTT) and Prothrombin Time (PT) were measured using an coagulation automaton (STA satellite®) at 0 and 30 min after incubation. Buffer was used as control. All extracts were very rich in catechic tannins, sterols and terpenes derivatives, flavonoids (mostly anthocyanins type) and soxes and holosides. *Pteleopsis suberosa, Entada africana and Erythrina senegalensis* showed a high content of coumarins. Eleven extracts had an aPTT ratio of <1.2 under basal conditions. Only extracts from *P. suberosa* (bark and trunk) were associated with aPTT ratio of >1.2. After 30 min, the aPTT ratio from all extract was lower than 1.2. The PT was not modified by any extracts. Medicinal plants traditionally used in Mali to treat bleeding events contained substances with known pro-coagulant and anticoagulant activities. Some extract from herbal plant modified aPTT ratio and this could be attributed to their hemostatic effect. Further investigations are required to confirm the results of the present study, and this could open a new research area in the field of hemostasis in Mali.

Key words: Hemostasis, medicinal plants, prothrombin, anticoagulants, Mali.

Abbreviations: aPTT, Activated prothrombin time; PBS, phosphate buffered sulfate; PPP, poor plasma; PT, prothrombin time; TLC, thin layer chromatography.

INTRODUCTION

Bleeding remains a leading cause of mortality and morbidity around the world and the leading sites being the gastrointestinal, gynecologic and intracranial bleeding (Holmstrom et al., 2016; Yang et al., 2017; Ganchimeg et al., 2014; Say et al., 2014). Whilst most bleeding events are appropriately managed with well-equipped healthcare facilities in the developed world. In many developing countries patients succumb to bleeding due to poor access to appropriate hemostatic interventions and agents (O’Mahony and Black, 2005; Konar and Chakraborty, 2013). In these settings, many patients consult and are reliant on complementary and alternative medicines for the management of their bleeding diathesis (Sanogo, 2011; Wang et al., 2012).

The tools of trade for many complementary and alternative medicine practitioners include herbal plants,
owing to their medicinal effects for the treatment of bleeding patients. Herbal medicines are widely used in many parts of the world including China, Africa and South America for their hemostatic activity and antiplatelet activity (Saidu et al., 2000; Kendler, 1987). The clinical conditions in which herbal medicines are used are diverse including post trauma hemorrhage, postpartum hemorrhage, epistaxis and menorrhagia (Farnsworth et al., 1986).

The active pharmaceutical ingredient in the herbal medicines used for the treatment of bleeding remain poorly characterized and in many instances unknown. In these settings, the question often arises whether herbal medicines used for treatment of bleeding do or do not have hemostatic activity. It is therefore logical to measure the hemostatic activity of herbal medicines as the first step in understanding their value in hemostatic management. Herbal medicines in Mali are yet to be characterized for their potential hemostatic effects.

The Malian pediatric mortality associated with bleeding is high at 464 deaths per 100 000 new born babies (Samake et al., 2007; Aa et al., 2011). Due to poor access to health facilities, a number of herbal medicines are used in the pre hospital management of patients both for the prevention and treatment of bleeding diatheses (Sanogo, 2011; Dai et al., 2002). Of the many herbal medicines used in Mali, only a handful is used specifically for the treatment of bleeding. These include roots, trunks, barks and leaves from diverse herbal medicine preparations. Whilst the use of some of these agents is widely accepted by the complementary and alternative medicine practitioners, but there are paucity of data showing that they indeed have hemostatic activities.

Therefore, the aim of this study was to investigate the hemostatic properties of herbas plants traditionally used to treat bleeding events in Mali.

MATERIALS AND METHODS

Plants selection

Plants were selected after an ethnobotanical survey conducted in the district of Dioila, Koulikoro, Mali. The survey included 50 traditional medicine practitioners recognized and accredited by the Malian Department of Traditional Medicine. The practitioners were asked about their options for treating a variety of bleeding diathesis including postpartum hemorrhage, epistaxis and stroke associated acute bleed. Of the 100 herbal plants used by different practitioners, the top 10 with the highest frequency of use by practitioners were selected for assessment of hemostatic activity and biochemical characterization in this study. The 10 herbal plants used were the Pteleopsis suberosa leaves and stem bark, Erythrina senegalensis trunk and roots barks, Baissea multiflora, Anona senegalensis and Entada africana stem bark, Guiera senegalensis, Cassia sieberiana and Detarium microcarpum leaves, Gossypium barbadense seeds and the roots of Carica papaya. Twelve extracts were prepared from the 10 herbal plants.

Plants extracts preparation and chemical characterization.

Water, dichloromethane and methanolextracts were prepared using a 10% weight/volume proportion of powdered mater in 24 h maceration. The chemical composition of dried and powdered plant material was characterized using chemical group's specific detection reagents and thin layer chromatography (TLC). Briefly, the presence of alkaloids was shown by precipitation of salts and reaction with Mayer’s reagent (potassium tetra-iodomercurate solution). The presence of gallic and catechin tannins was demonstrated using ferric perchloride. Cyanidin reaction shows the presence of free flavonoids (flavones and dihydroflavonols). Leucoanthocyanans were detected by the cyanidin reaction carried out without the addition of magnesium shavings. The saponosides, which occur widely in plants, were characterised by their foaming power in aqueous solution. The presence of sterols and triterpenes were demonstrated using concentrated sulphuric acid. Several reducing compounds could be detected, as oses and holosides and muclageswere detected by adding sulphuric acid and saturated solution of thymol in ethanol to an aqueous decoction of the samples.

Blood collection and platelet poor plasma (PPP) preparation:

Five milliliters of whole blood from healthy volunteers was drawn in evacuated container tubes (Vacutainer®, Becton-Dickinson) containing 0.129 M trisodium citrate (1vol/9vol blood). The blood sample was incubated at the room temperature with extract solution at the final concentration of 0.25 g/L. The platelet poor plasma was prepared by centrifugation at 4000 g for 15 min at 20°C. Under the same incubation condition, phosphate buffered saline (PBS) was used as a control.

Measurement of hemostatic properties of the extracts

An aliquot of each of the extracts suspended in water, ethanol and dichloromethane was prepared. Activated Prothrombin Time (aPTT) and Protrombin Time (PT) were measured immediately (time 0) and following 30 min incubation by aSTA satellite® coagulation instrument (Diagnostic Stago, New jersey, USA) using the Stagoreagents. The aPTT results were expressed as a ratio
Table 1: Moisture, total ash (using sulphuric acid) and insoluble ash (using hydrochloric acid) of the 12 extracts.

<table>
<thead>
<tr>
<th>Plants</th>
<th>H₂O (%)</th>
<th>Totales Ash H₂SO₄ (%)</th>
<th>Insolubles Ash HCl (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Annona senegalensis (Barks of the trunk)</td>
<td>5.26</td>
<td>7.8</td>
<td>1.7</td>
</tr>
<tr>
<td>Baisseamultiflora (Stems barks)</td>
<td>5.69</td>
<td>9.8</td>
<td>1.7</td>
</tr>
<tr>
<td>Caricapapaya (Root)</td>
<td>5.71</td>
<td>15</td>
<td>3</td>
</tr>
<tr>
<td>Cassia sieberiana (Leaves)</td>
<td>5.48</td>
<td>3.2</td>
<td>0.5</td>
</tr>
<tr>
<td>Detariummicrocarpum (Leaves)</td>
<td>5.42</td>
<td>4.1</td>
<td>0.3</td>
</tr>
<tr>
<td>Entadaafricana (Barks of the trunk)</td>
<td>3.30</td>
<td>12.6</td>
<td>0.5</td>
</tr>
<tr>
<td>Erythrina senegalensis (Barks of the trunk)</td>
<td>3.83</td>
<td>9.5</td>
<td>1.4</td>
</tr>
<tr>
<td>Erythrina senegalensis (Roots)</td>
<td>4.25</td>
<td>5.1</td>
<td>0.6</td>
</tr>
<tr>
<td>Gossipumbarbadense (Seed)</td>
<td>4.45</td>
<td>5.1</td>
<td>0.3</td>
</tr>
<tr>
<td>Guierasenegalensis (Leaves)</td>
<td>4.78</td>
<td>3.9</td>
<td>0.6</td>
</tr>
<tr>
<td>Pteleopsis suberosa (Barks of the trunk)</td>
<td>4.52</td>
<td>5.5</td>
<td>1.1</td>
</tr>
<tr>
<td>Pteleopsis suberosa (Leaves)</td>
<td>7.18</td>
<td>4.9</td>
<td>3</td>
</tr>
</tbody>
</table>

H₂O- water, H₂SO₄- sulphuric acid, HCl- hydrochloric acid.

Table 2: Proportion of plants components soluble in water and 70% ethanol.

<table>
<thead>
<tr>
<th>Plants</th>
<th>Extractable substances in H₂O (%)</th>
<th>Extractable substances in EtOH 70° (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Annona senegalensis (Barks of the trunk)</td>
<td>11</td>
<td>8</td>
</tr>
<tr>
<td>Baisseamultiflora (Stems barks)</td>
<td>23</td>
<td>20</td>
</tr>
<tr>
<td>Caricapapaya (Root)</td>
<td>22</td>
<td>2</td>
</tr>
<tr>
<td>Cassia sieberiana (Leaves)</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Detariummicrocarpum (Leaves)</td>
<td>11</td>
<td>4</td>
</tr>
<tr>
<td>Entadaafricana (Barks of the trunk)</td>
<td>15</td>
<td>5</td>
</tr>
<tr>
<td>Erythrina senegalensis (Barks of the trunk)</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>Erythrina senegalensis (Roots)</td>
<td>13</td>
<td>5</td>
</tr>
<tr>
<td>Gossipum barbadense (Seed)</td>
<td>43</td>
<td>10</td>
</tr>
<tr>
<td>Guierasenegalensis (Leaves)</td>
<td>12</td>
<td>16</td>
</tr>
<tr>
<td>Pteleopsis suberosa (Barks of the trunk)</td>
<td>27</td>
<td>25</td>
</tr>
<tr>
<td>Pteleopsis suberosa (Leaves)</td>
<td>12</td>
<td>15</td>
</tr>
</tbody>
</table>

H₂O- water, EtOH- ethanol.

RESULTS

Plants extract characterization

The results of the chemicals composition of all 12 plants extracts are shown in Tables 1 and 2. Table 1 shows the moisture and ash composition of the 12 extracts. The water contents in all extracts was less than 10%. All 12 extracts showed a low level of hydrochloric acid insoluble ash content, the highest being 3% for both P. suberosa (leaves) and C. papaya (roots) extracts. The proportion of extracts soluble in water and 70% ethanol are shown in Table 2. The extract with the highest water solubility was G. barbadense with 43% solubility in water. Components extractable by ethanol were very variable ranging from 2% for C. papaya (roots) to 25% for P. suberosa (bark of the trunk).

Statistical analysis

Each experimental point was performed in duplicate. The results from at least 3 experiments were expressed as mean ± SEM and range between minimum and maximum values. Comparison between variables was performed using Mann-Whitney test for continuous variables with non-Gaussian distribution and Student t test for Gaussian distribution. Chi square was used to test the relationships between the categorical variables. A P value ≤ 0.05 was considered statistically significant. All statistical analyses were performed using the SPSS 17.0. (IBM, USA).
### Table 3: Phytochemical composition of the 12 herbal extracts.

<table>
<thead>
<tr>
<th>Chemicals groups</th>
<th>CS</th>
<th>PM_et</th>
<th>ES_t</th>
<th>EA</th>
<th>PM_f</th>
<th>GB</th>
<th>ES_r</th>
<th>AS</th>
<th>CP</th>
<th>GS</th>
<th>BM</th>
<th>DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carotenoids</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>-</td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Coumarins</td>
<td>-</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Leucoanthocyanes</td>
<td>+</td>
<td>+++</td>
<td>-</td>
<td>+++</td>
<td>+++</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Oses/Holosides</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Saponosides</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sterol/triterpenes</td>
<td>-</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Tannins</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
</tbody>
</table>

CS: Cassia sieberiana (leaves), PM_et: Pteleopsis suberosa (bark of the trunk), ES_t: Erythrina senegalensis (bark of the trunk), EA: Entada africana (bark of the trunk), PM_f: Pteleopsis suberosa (leaves), GB: Gossypium barbadense (seed), ES_r: Erythrina senegalensis (root bark), AS: Annona senegalensis (bark of the trunk), PC: Carica papaya (root), GS: Guiera senegalensis (leaves), BM: Baissea multiflora (bark of the trunk), DM: Detarium microcarpum (leaves).

(-): Absence; (+): Presence; (++): Abundant; (+++): Very abundant; (++++): Very-very abundant.

**Figure 1**: Methanolic and aqueous extracts chromatograms revealed with FeCl₃(a) and with the Godin reagent, 3 deposits by extracts (b). CS: Cassia sieberiana (leaves), PM_et: Pteleopsis suberosa (bark of the trunk), ES_t: Erythrina senegalensis (bark of the trunk), EA: Entada africana (bark of the trunk), PM_f: Pteleopsis suberosa (leaves), GB: Gossypium barbadense (seed), ES_r: Erythrina senegalensis (root bark), AS: Annona senegalensis (bark of the trunk), PC: Carica papaya (root), GS: Guiera senegalensis (leaves), BM: Baissea multiflora (bark of the trunk), DM: Detarium microcarpum (leaves).

**Phytochemical components**

Table 3 shows the phytochemical composition of the 12 herbal extracts. All extracts contained tannins, oses and holosides. Only C. sieberiana did not contain sterols and triterpenes and was also the only plant that contained reducing compounds. P. suberosa contained both catechic and gallactanins. P. suberosa (leaves and stem bark), E. africana and E. senegalensis showed high content of coumarins (Table 3).

**Thin layer chromatography (TLC)**

Chromatographic characterization of the extracts using FeCl₃ and Godin reagent is shown in Figure 1. TLC of aqueous and methanolic extracts showed purple, yellow, red stains constituents after exposure to Godin reagent and greyish-black after exposure to FeCl₃. With these reagents, triterpenes usually appear in purple stain, sterols become blue, and flavonoid show yellow or orange stains. All studied plants parts were very rich in catechic tannins, sterols and terpenes derivatives, flavonoids (mostly anthocyanins type) and sugars (oses and holosides) (Figure 1).

**Hemostatic properties of plants extracts incubated with whole blood**

Hemostasis properties were evaluated using aPTT and PT on PPP. The results were expressed as a ratio and then converted in percentage for the PT. aPTT was expressed in terms of sample time over normal control time. The result was considered lengthened if this ratio was greater than or equal to 1.2. The PT was normal if it is greater than or equal to 70%. An elongated value of aPTT was compatible with a high haemorrhagic risk; a short value of aPTT indicates a state of hypocoagulability (Figure 2).

Water extracts from P. suberosa (leaves and stem bark),
**B. multiflora** (stem bark) and **E. africana** (stem bark) showed high haemostatic activity. aPTT measurement directly after incubation showed that eleven extracts had a ratio 1.2. Only extracts from **P. suberosa** bark and trunk induced an aPTT beyond 1.2. After 30 min of incubation, aPTT value from all extract was lower than 1.2. In contrast, it seems that PT was not modified by any of the 12 extracts.

**DISCUSSION**

Mortality and morbidity associated with bleeding disorders are a challenge for many African rural communities who are reliant on alternative and traditional practitioners. In developed countries, most bleeding events are appropriately managed in well-equipped hospitals with easy access to drugs. But in many developing countries such as black African countries, bleeding patients succumb to bleeding due to poor access to appropriate haemostatic interventions and agents (O’Mahony and Black, 2005; Konar and Chakraborty, 2013). Herbal medicines are widely used in many parts of the world including China, Africa and South American owing to their hemostatic activity and antiplatelet activity (Saidu et al., 2000; Apitz-Castro et al., 1994). This could explained the largest herbal medicines used for management of a variety of conditions including post trauma hemorrhage, postpartum hemorrhage, epistaxis and menorrhagia in these countries (Farnsworth et al., 1986).

The present study was conducted to improve bleeding disease treatment in developing countries with limited access to haemostatic drugs. The aim was to identify different extracts from plants frequently used by traditional practitioners to treat hemorrhages. In Mali, 80 to 90% of rural Malian population successfully uses medicinal plants whose hemostatic activity has not yet been characterized to treat bleeding disorders. We retained the 10 plants following an ethnobotanical survey carried out in Mali which included 100 traditional medicine practitioners on the means to treat bleeding events. The 10 plants were **A. senegalensis**, **B. multiflora**, **C. papaya**, **C. sieberiana**, **D. microcarpum**, **E. africana**, **E. senegalensis**, **G. barbadense**, **G. senegalensis**, and **P. suberosa**.

Numerous herbal medicines used in Mali are poorly or not investigated. It is not clear exactly which of these herbal remedies have proven hemostatic activity. The
ethnobotanical survey remained the best means to identify the best plants used to treat every diathesis. This survey included traditional practitioners recognized by the Department of Traditional Medicine in Mali.

The active pharmaceutical ingredient in many herbal medicines used for treatment of bleeding remain poorly characterized and in many instances unknown. This study aimed to measure the hemostatic activity of 12 extracts from these 10 herbal medicines; which remained the first step to understand their value in hemostatic management.

We prepared water, dichloromethane and methanol extracts from the 10 selected plants in the laboratory of Malian Department of Traditional Medicine, known for his strong expertise in herbal plant study. The materials used were as follows: *P. suberosa* leaves and stem bark, *E. senegalensis* trunk and roots barks, *B. multiflora*, *A. senegalensis* and *E. africana* stem bark, the leaves of *G. senegalensis*, *Cassia sieberiana* and *D. microcarpum* the seeds of *G. barbadense* and the roots of *C. papaya*.

Extractions were carried out according to the traditional mode of preparation (decoction or maceration). Dichloromethane and methanol extracts were also prepared in order to test compounds that are soluble in those solvents. The majority of the extracts obtained had more or less a brilliant flake appearance. In general, the color of the extracts was similar to that of the starting drugs. Among the extraction solvents, water gave the best yield with 43% (*G. barbadense*). This confirms the interest of using the traditional form with water which can be considered as least expensive extraction solvent. The water content in the samples was low and less than 10% which assure a better long-term conservation of the material.

The results of the present study showed a low hydrochloric acid insoluble ash content, with the highest value being 3.06% for *P. suberosa* (leaves). This indicates the absence of dust contamination in the sample. The total ash content in the samples was 15% for *C. papaya* (roots), 12.6% for *E. africana*, 9.8% for *B. multiflora* and 9.5% for *E. senegalensis* (bark of the trunk). This indicates a presence of minerals.

Phytochemistry investigations allowed highlighting the presence of tannins, oses and holosides in all samples. The presence of tannins in *A. senegalensis*, *B. multiflora*, *C. papaya*, *E. africana*, *E. senegalensis*, *G. senegalensis* and *P. suberosa* had already been reported in literature (Saidu et al., 2000; Apitz-Castro et al., 1994; Yeo et al., 2011; Obidike and Emeje, 2011; Zerihun and Workineh, 2013; Lamien et al., 2005; Soulama et al., 2013; Soulama et al., 2014).

Sterols and triterpenes were present in all samples except *C. sieberiana*. These results are similar to those reported by some researchers who showed the presence of sterols and triterpenes in the leaves of *P. suberosa* (De Leo et al., 2006) and *E. africana* (Obidike and Emeje, 2011). Leucoanthocyanins were present in all samples except *E. senegalensis* (bark of the trunk) and *G. barbadense*. Saponosides were also present in all our samples except *P. mylitolia* (leaves), *E. senegalensis* (root) and *B. multiflora* (bark of the trunk). This result is in line with those reported in literature (Saidu et al., 2000; Obidike and Emeje, 2011; Zerihun and Workineh, 2013). Flavonoids were present only in *C. sieberiana*, *A. senegalensis* and *B. multiflora*. Flavonoids were shown to enhance capillary toxicity (Bossokpi, 2003), which could promote reflex vasoconstriction to stop bleeding.

Reducing substances were rarely found in this study's extracts. Absolutely, carotenoids and coumarins were found only in *P. suberosa* (leaves and bark of the trunk), *E. senegalensis* (bark of the trunk and root) and *E. africana*. However, some researchers have demonstrated the presence of coumarins in leaves of *A. senegalensis* (Yeo et al., 2011) and in fruits of *B. multiflora* (Soulama et al., 2013). The rarity of these substances known for their anticoagulant properties especially coumarin derivatives could support the hypothesis that these plants do actually have procoagulant properties. Muclilages were found in *A. senegalensis*, *C. papaya* and *D. microcarpum*.

TCL confirmed some of our tube reaction results by the presence of certain fluoroscences such as violet fluorescence for triterpene and saponosides; yellow or orange for the flavonoids after exposure to Godin reagent, and a brown-black coloration for the tannins after exposure to FeCl₃.

Polyphenolic substances, such as anthocyanosides, flavonoids and tannins in aqueous extracts, possess the properties of decreasing the permeability of blood capillaries, increasing the resistance of venous tone and stabilizing collagen (Bossokpi, 2003). These properties, which favor the strengthening of vessels and capillaries, prevent hemorrhages. This confirms the richness of our plants in these substances and the antihemorrhagic properties they contain. The hemostasis properties of these plants which are traditionally used to treat bleed, could be attributed to tannins that precipitate proteins. Tannins are polyphenolic compounds that stop bleeding and fight infections (Diallo, 2005).

The evaluation of the impact of aqueous extracts on haemostasis tests was done after incubation with whole blood at different times. We have tested different concentrations of extracts with whole blood. Finally, we have retained the ¼ dilution (final concentration of 0.25 g/L). All other high concentrations and other types of extracts have led to massive haemolysis of red blood cells making impossible haemostasis tests realizable. aPTT is a global test that explores the intrinsic pathway of coagulation. It was expressed as a ratio of an explored time (sick or extracted as it was our case) on a normal time (control). It was considered lengthened if this ratio was greater than 1.2. A modification of aPTT could reflect an effect on the coagulation proteins involved in the intrinsic pathway, such as contact factors XII, XI, IX, VIII, X and II. The lengthening of the aPTT reflects a state of hypocoagulability in favor of hemorrhagic risk. On the other
hand, a shortening of the aPTT translates the opposite effect of what is sought in case of haemorrhage. PT explores the extrinsic pathway including factors VII, X, II of coagulation. Similar to the case of aPTT, any modification of the PT could reflect an effect on the proteins involved in this pathway.

Some plants extracts caused a decrease in coagulation time in whole blood. A better activity was obtained respectively with the extracts of P. suberosa (bark of the trunk), B. multiflora (bark of the trunk), P. suberosa (leaves) and E. africana (bark of the trunk).

In this study, it was found that P. suberosa(bark of the trunk) caused a modification of aPTT, this was immediately after contact with the whole blood. This could reflect a negative effect on the coagulation induction phase. On the other hand, there is a tendency to normalization after 30 min which could translate a haemostatic effect of this plant. This effect was observed both in the bark of the trunk and the leaves. This could be attributed to a delay effect. No noticeable change in PT in the extracts immediately after contact with whole blood was observed. On the other hand, after 30 min, an increase of PT in P. suberosa (bark of the trunk), E. senegalensis (bark of the root) and C. papaya was observed, but there was a decrease in D. microcarpum. This decrease could be explained by the massive presence of saponosides that cause hemolysis of the red blood cells. Moreover, the possibility that it can have an effect after a rather long time with the substances cannot be excluded. At the same time, several other extrinsic actors are involved in hemostasis.

The inadequacy of the technical platform does not allow us to exclude a haemostatic effect of the extracts studied on the other stages of haemostasis, such as primary haemostasis, thrombin generation, cell activation or inhibition pathways and fibrinoinformation. The presence of flavonoid in the leaves of C. sieberiana, bark of A. senegalensis, trunk of B. multiflora, known for their effect on vascular tone, reinforces this hypothesis. Therefore, the rarity of the reducing substances, allow us to believe that these extracts could have procoagulant properties.

The haemostatic activity of the studied plants has rarely been studied, and A. senegalensis has shown inhibitory activity of fibrinogen coagulation on venom (Emmanuel et al., 2014).

Conclusion

Medicinal plants traditionally used to treat bleeding event in Mali contain substances known for their pro-coagulant activity; others compounds having anticoagulant activity were also found in smaller amount. Some extract from herbal plant modified aPTT and this could be attributed to their haemostatic effect. More investigations are needed in order to confirm these results and as a result, could open a new research area in the field of hemostasis in Mali and in other developing countries.

REFERENCES


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