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Preliminary Study on Polysaccharides and Certain Secondary Metabolites of Medicinal Plants used in Cote D'Ivoire for Wound Healing

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ABSTRACT

Knowledge of chemical composition of medicinal plants used in wound healing is desirable because many people suffering from wounds are depriving from the benefit of using their traditional medicine. Many medicinal plants are useful in the control of wound healing process in West African countries such as Cote d'Ivoire. This ethnopharmacological study related to some of those plants with the aim to understand and explain their traditional use for treating wounds. Six plant species were selected after ethnobotanical review and investigated with standard phytochemical screening methods. Preliminary chemical composition showed that *Alstonia boonei* (DC) Willd., *Antiaris toxicaria* Lesch., *Ceiba pentandra* Gaertn., *Ficus exasperata* Vahl., *Periploca nigrescens* Afzel and *Tiliacora dinklagei* Engl. contain polysaccharides, mucilages, flavonoids, tannins and alkaloids. These metabolites are known for their wound healing effects. Phytochemicals of these 6 plants justifies their traditional use in West Africa for the treatment of wounds. The presence of polysaccharides highlighted the great interest of studied plants which could be used to develop and formulate improved traditional medicines in the form of ointments for topical application.

Key words: Medicinal plants, polysaccharides, secondary metabolites, Cote d'Ivoire, wound healing

INTRODUCTION

In their daily deed, many people can be confronted with many kinds of injuries which can be classified as opened and closed wounds (Nagori and Solanki, 2011). Wound is defined as a break or disruption in the normal tissues provoking various cellular and molecular changes (Pattanayak *et al.*, 2011; Nithya and Baskar, 2011). Badly treated or untreated, wounds can become chronic and represent huge burden in patients, due to cost and duration of the treatment. Wounds such as buruli ulcer or wound in diabetics are of great public health concern because they are often disabling and difficult to cure by the modern therapy. Diabetes and other disease conditions are aggravating factors increasing susceptibility to wound infection and morbidity (Nagori and Solanki, 2011). For example, 25% of people with diabetes will suffer from a wound problem (Norfarizan-Hanoon *et al.*, 2009) which can compromise their wellbeing, image, independence and capacity to work. This situation has financial and social implications which highlight that a good control of wounds is important not only for sick persons but also communities (Gupta and Jain, 2010).

Since ancient times, a large variety of plants have been used by the populations to accelerate and control wound healing process (Reuter *et al.*, 2009; Schmidt *et al.*, 2009; Khorshid *et al.*, 2010), support formation of blood clots and protect from infectious agents (Dahanukar *et al.*, 2000). According to Nithya and Baskar (2011), plants are more potent healers due to their ability to promote the repair mechanisms in the natural way. In West Africa, in particular in Cote d'Ivoire, ethnobotanical surveys had revealed that a good number of plants were used in wound healing (Kone *et al.*, 2002; Kamanzi, 2002). Experimental assessment of therapeutic value of recorded plants will lead to the development of traditional method of treatment (Haque *et al.*, 2003) or synthesis of complex chemical products (Vaghasiya *et al.*, 2011) for wound healing.

Many west African medicinal plants have been proved to be sources of bioactive compounds including alkaloids, tannins, flavonoids, steroids, glycosides and saponins. In addition, it is believed that the synergistic effect of phytochemicals render crude plant extracts more biologically active than isolated compounds. Such chemicals from medicinal plants are complex polysaccharides which are known for their bioadhesive effects on irritated cells (Deters *et al.*, 2010).

Polysaccharides with complement fixing activity were found in *Entanda africana* Guill. and Perr (Diallo *et al.*, 2001) and *Opilia celtidifolia* Guill. and Perr (Togola *et al.*, 2008), plants used in Mali to treat wounds (Inngjerdingen *et al.*, 2004). Other natural products playing a role in wound healing are terpenes (Patil *et al.*, 2009), essential amino-acids and fatty acids (Hui *et al.*, 2010) and phenolic compounds such as tannins and flavonoids (Shivhare *et al.*, 2010). Phenolics possess beneficial antibacterial and antioxidant (Halliwell, 1995) properties for the reduction of inflammatory process.

The present study investigated the chemical composition, with focus on polysaccharides and some secondary metabolites of 6 medicinal plants used in Cote d'Ivoire for wound healing. These plant species, *Alstonia boonei*, *Tiliacora dinklagei*, *Antiaris toxicaria*, *Ficus exasperata*, *Periploca nigrescens* and *Ceiba pentandra*, were selected after an ethnobotanical review.

MATERIALS AND METHODS

Ethnobotanical review for selection of studied plant species: A review was carried out on the basis of ethnobotanical surveys conducted in Cote d'Ivoire and other areas of West Africa (Dalziel, 1956; Adjanohoun and Ake Assi, 1979, 1989; Burkill, 1985, 1997; Pharmel, 1992; Neuwinger, 1996; Onayade *et al.*, 1996; Tra Bi, 1997; Weiss, 1997; Kone *et al.*, 2004). This approach appeared judicious for selection of plants, since this data is available on medicinal herbs used for treating several diseases including wounds. The authentication of selected plant species was carried out at the herbarium of Centre Suisse de Recherches Scientifiques in Cote d' Ivoire, using the flora of West Africa (Hutchinson and Dalziel, 1954-1972; Lebrun and Stork, 1991, 1992, 1995, 1997; Ake Assi, 2001).

Phytochemical screening

Selection of plant species: Six plants were selected for the phytochemical investigations according to 3 essential criteria: Large scale use, accessibility and availability and lack of information on the presence of polysaccharides. On the basis of these criteria, *Ceiba pentandra*, *Antiaris toxicaria*, *Tiliacora dinklagei*, *Ficus exasperata*, *Alstonia boonei* and *Periploca nigrescens* were selected.

Preparation of plant extracts: The stem bark, leaves and roots of these plants were collected in June 2009 in the forest of Adiopodoumé located at 17 km from Abidjan, the economic capital of Cote d'Ivoire. The samples were identified at the herbarium of Swiss Center for Scientific Research in Cote d'Ivoire (CSRS).

The various plant parts were dried in an air-conditioned room (18°C) during 2 weeks and then grounded in a mortar. The phytochemical screening was carried out on decoction and macerate. For decoction, 200 mL of distilled water were added to 20 g of powder. The mixture was boiled during 15 minutes, then immediately filtered on Whatmann paper. Macerate was obtained from 10 g of powder mixed with 100 mL of distilled water, under mechanical stirring (160 rev/min) during 24 h. Macerates were filtered on Whatmann paper.

Phytochemical screening methods: Polysaccharides were studied by precipitation in ethanol and swelling index while tests for alkaloids, phenolic compounds, flavonoids and tannins were carried out on portions of extracts using standard photochemical procedures (Rizk, 1982; Al-Yahya, 1986; Jana and Shekhawat, 2010).

Test for polysaccharides: The detection of polysaccharides is based on their capacity to inflate in the presence of water. Into a graduated tube, one introduced 1 g of powder and then 50 mL of distilled water. After 30 min, the increase in volume indicates the presence of complex polysaccharides. Then swelling index was determined according to the formula below:

$$\text{Swelling index (SI)} = \frac{(\text{Final volume} - 50 \text{ mL})}{\text{Weight (g)}}$$

The swelling index is expressed in mL g⁻¹. The higher SI is the higher content in polysaccharides.

Test for mucilages: The characterization of mucilages is based on their capacity to precipitate in presence of ethanol. To 1 mL of 10% decoction, 5 mL of ethanol were added and then vigorously shaken during 15 min. The appearance of flocculent precipitate indicates the presence of mucilages.

Test for alkaloids: Six milliliter of each extract were dried in rotary evaporator and dissolved in 6 mL of ethanol 60%. Few drops of Dragendorff's alkaloidal reagent were added to each tube and the presence or absence of any turbidity or precipitates was noted in each test tube.

Test for phenolic compounds: A few drops of 2% FeCl₃ solution were added to 2 mL of each extract. The appearance of deep blue, black or green colour indicates the presence of phenolic compounds.

Stiasny's test for tannins: Five milliliter of each extract were evaporated and dissolved in 15 mL of Stiasny reagent (10 mL of formal 30%, 5 mL of concentrated HCl). The mixtures were boiled over a steam bath at 80°C during 30 min and then allowed to cool. The positive reaction characterized by big brown flakes indicates the presence of non-hydrolysable tannins. The present extract was filtrated and saturated with sodium acetate. When treated with few drops of 10% ferric chloride test solution, a deep green colour indicated hydrolysable tannins.

Cyanidin's test for flavonoids: Filtrate of about 2 mL was evaporated. The residues were then treated with 5 mL of HCl, magnesium shaving and colour were noted. A pink, red, red-orange colour in presence of 0.5 mL of isoamylic alcohol shows positive reaction to flavonoids.

All these tests were carried out in triplicates in order to confirm the results. The intensity of coloration was recorded using a scale: trace (+), abundant (++) and high abundant (+++).

RESULTS

Some medicinal plants used for wound healing: The ethnobotanical review permitted to draw up a non-exhaustive list of 50 medicinal herbs used for the treatment of various kinds of wounds (Table 1). These plants belong to 47 genera and 29 families. The most represented families are

Table 1: Some medicinal plants used in Cote d'Ivoire for wound healing (source: literature)

Plant species	Families	Plant part	Preparation and administration	References
<i>Adansonia digitata</i> L.	Bombacaceae	Stem bark	Decoction for washing, ointment with crushed paste	Kone <i>et al.</i> (2002), Burkill (1985)
<i>Aframomum sceptrum</i> K. Schm	Zingiberaceae	Leaves, stems, seeds	Juice for new wounds	Neuwinger (1996)
<i>Ageratum conyzoides</i> L.	Asteraceae	Leaves	Juice for new wounds	Watt and Breyer-Brandwijk (1962)
<i>Alchornea cordifolia</i> Müll. Arg	Euphorbiaceae	Leaves	Paste for new wounds	Dalziel (1956)
<i>Alstonia boonei</i> (DC) Willd.	Apocynaceae	Sap	Sap for new wounds	Dalziel (1956)
<i>Altermanthera pungens</i> Kunth in H.B. and K.	Amaranthaceae	Whole plant	Mouth wound : decoction for mouthwash	Kone <i>et al.</i> (2007)
<i>Anchomanes difformis</i> Engl	Araceae	Juice	Juice for new wounds	Adjahoun <i>et al.</i> (1991)
<i>Aneilema lanceolatum</i> Benth	Commelinaceae	Sap	Sap for new wounds	Dalziel (1956)
<i>Aneilema pomeridianum</i> A. Chev	Commelinaceae	Whole plants	Decoction for washing old wounds	Dalziel (1956)
<i>Aneilema subnudum</i> A. Chev	Commelinaceae	Leaves	Grounded leaves for wounds, after bath	Kone <i>et al.</i> (2002)
<i>Annichia polycarpa</i> (DC.) Van setten and Maas	Annonaceae	Stems	Decoction for washing old and fresh wounds	Weiss (1997)
<i>Annona senegalensis</i> Pers.	Annonaceae	Leaves, stem bark, roots	Decoction for washing old wounds	Bouquet and Debray (1974)
<i>Antiaris toxicaria</i> Lesch.	Moraceae	Sap	Sap for new wounds	Tra Bi (1997)
<i>Azadirachta indica</i> A. Juss	Meliaceae	Leaves	Decoction for washing old wounds	Davis <i>et al.</i> (1991)
<i>Bidens pilosa</i> L.	Asteraceae	Leaves	Juice for new wounds	Adjahoun and Ake Assi (1983)
<i>Borreria verticillata</i> G. Mey	Rubiaceae	Leaves	Juice for new wounds	Pousset (1989)
<i>Ceiba pentandra</i> Gaertn.	Bombacaceae	Stem bark	Decoction for washing old wounds	Burkill (1985)
<i>Centella asiatica</i> L.	Apiaceae	Whole plant	Grounded plant as ointment	Adjahoun <i>et al.</i> (1993)
<i>Cissus populnea</i> Guill. and Perr	Vitaceae	Roots	Decoction for washing old wounds.	Kone <i>et al.</i> (2002)
<i>Combretum molle</i> R. Br. Ex Don	Combretaceae	Leaves	Decoction for washing wounds	Kone <i>et al.</i> (2002)
<i>Combretum racemosum</i> P. Beauv	Combretaceae	Roots	Decoction for washing old wounds	Kone <i>et al.</i> (2004)
<i>Combretum smeathmanii</i> G. Don	Combretaceae	Leaves, roots	Grounded plant as ointment	Bouquet and Debray (1974)
<i>Costus spectabilis</i> Fenzl.	Zingiberaceae	Whole plant	Decoction for washing toe wounds	Kone <i>et al.</i> (2002)
<i>Craterispermum caudatum</i> Hutch.	Rubiaceae	Leaves	Juice for new wounds	Pharmel (1992)
<i>Chromolaena odorata</i> L.	Asteraceae	Leaves	Juice for infected wounds	Kone (2003)
<i>Eclipta prostrata</i> L.	Asteraceae	Leaves	Juice for new wounds	Bouquet and Debray (1974)
<i>Entada africana</i> Guill. and Perr.	Mimosaceae	Roots	Mouth wound: mix powder with porridge and drink	Kone <i>et al.</i> (2007)
<i>Erythrococca anomala</i> Prain.	Euphorbiaceae	Leaves	Juice for new wounds	Tra Bi (1997)
<i>Euphorbia hirta</i> L.	Euphorbiaceae	Leaves	Juice for wounds	Santhanam and Nagarajan (1990)
<i>Ficus exasperata</i> Vahl	Moraceae	Stem bark	Decoction for washing old wounds	Weiss (1997)

Table 1: Continued

Plant species	Families	Plant part	Preparation and administration	References
<i>Gardenia tenifolia</i> Schum.	Rubiaceae	Unripe Fruits	Powder of calcinate applied	Kone <i>et al.</i> (2002)
<i>Jatropha curcas</i> L.	Euphorbiaceae	Sap	Sap for new wounds	Watt and Breyer-Brandwijk (1962)
<i>Kalanchoe spathulata</i> DC.	Crassulaceae	Leaves	Juice for wounds	Yadav and Yadav (1985)
<i>Khaya senegalensis</i> A. Juss	Meliaceae	Stem bark, Fruits	Decoction for washing old wounds	Neuwinger (1996)
<i>Mansonia altissima</i> A. Chev	Sterculiaceae	Stem bark	Decoction for washing old wounds	Bouquet and Debray (1974)
<i>Mareya micrantha</i> Müll. Arg	Euphorbiaceae	Leaves	Juice for old wounds	Weiss (1997)
<i>Motandra guineensis</i> A. DC	Apocynaceae	Leaves	Mouth wound : decoction for mouthwash	Tra Bi (1997)
<i>Newbouldia laevis</i> Seem.	Bignoniaceae	Stem bark	Decoction for internal wounds	Tra Bi (1997)
<i>Periploca nigrescens</i> Afzel.	Periplocaceae	Sap	Sap for new wounds	Neuwinger (1996)
<i>Paullinia pinnata</i> L.	Sapindaceae	Leaves	Juice for new wounds	Tra Bi (1997)
<i>Piliostigma thonningii</i> Schum.	Caesalpiniaceae	Young leaves	Decoction for washing old wounds	Kone <i>et al.</i> (2002)
<i>Premna lucens</i> A. Chev	Verbenaceae	Roots	Grounded plant as ointment	Kone <i>et al.</i> (2004)
<i>Psidium guajava</i> L.	Myrtaceae	Leaves	Grounded plant as ointment	Ngala (1995)
<i>Securidaca longepedunculata</i> Fres.	Polygalaceae	Roots	Decoction for washing wounds	Kone <i>et al.</i> (2004)
<i>Solenostemon monostachyus</i> A. Chev.	Lamiaceae	Leaves	Mouth wound : decoction for mouthwash	Bouquet and Debray (1974)
<i>Spondias monbin</i> L.	Anacardiaceae	Leaves, stem bark, resines	Decoction for washing old wounds	Adjanohoun and Ake Assi (1989)
<i>Tiliacora dinklagei</i> Engl.	Menispermaceae	Stem bark	Decoction for washing wounds	Weiss (1997)
<i>Trichilia monadelpha</i> (Thonn.) De	Meliaceae	Stems	Decoction for washing old wounds	Dalziel (1956)
<i>Ximeria americana</i> L.	Olcaceae	Roots	Mouth wound: Decoction for mouthwash	Kone <i>et al.</i> (2002)
<i>Xylopiia aethiopica</i> A. Rich	Annonaceae	Fruits	Macerate for old wounds	Dalziel (1956)

Euphorbiaceae and Asteraceae, with 5 and 4 species, respectively. The genera represented by a large number of species were *Aneilema* and *Combretum*, with 3 species.

The modes of preparation mostly used are sap of trees, juices obtained by squeezing fresh leaves, decoction, paste and powder. The remedies are administered by topical route consisting in washing wounds before application of ointment. The sap and juice of leaves are used in the case of new wounds while decoction or macerate are indicated for old wounds.

Phytochemical analysis: The 6 plant species contain various amounts of polysaccharides (Fig. 1). According to the values of SI, the richest plants are *P. nigrescens*, *A. boonei* and *A. toxicaria*. Also, all the studied species contain mucilages in abundance, except *C. pentandra* which contains only trace amount. *A. toxicaria* and *P. nigrescens* are the richest species in mucilages (Table 2).

Phytochemical screening revealed the presence of secondary metabolites such as alkaloids, tannins and flavonoids (Table 2). Catechic tannins are high abundant in *C. pentandra* and *A. toxicaria* and present in trace amount or absent in the other plants. None of the 6 plants contains gallic tannins.

The flavonoids were found only in *F. exasperata* and *C. pentandra*. The alkaloids were detected in trace amount in *C. pentandra*.

Table 2: Certain phytochemicals of studied plants

Plant species	Extracts	Studied metabolites					
		Phenols	Catechic tannins	Gallic tannins	Alkaloids	Flavonoids	Mucilages
<i>Alstonia boonei</i>	Mac	+	-	-	-	-	
	Deco	+	-	-	-	-	++
<i>Antiaris toxicaria</i>	Mac	++	++	-	-	-	
	Deco	+	++	-	-	-	+++
<i>Ceiba pentandra</i>	Mac	+++	+++	-	+	+++	
	Deco	+++	+++	-	+	+++	+
<i>Ficus exasperata</i>	Mac	++	+	-	-	++	
	Deco	++	-	-	-	+	++
<i>Periploca nigrescens</i>	Mac	++	+	-	-	-	
	Deco	++	-	-	-	-	+++
<i>Tiliacora dinklagei</i>	Mac	+	-	-	-	-	
	Deco	+	+	-	-	-	++

-: Absence; +: Presence (trace); ++: Abundant; +++: High abundant; Mac: Macerate; Deco: Decoction

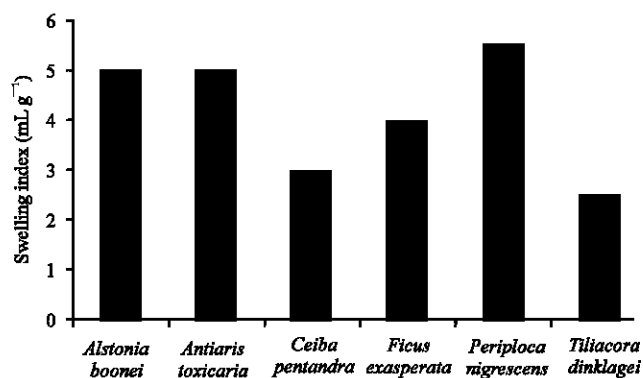


Fig. 1: Polysaccharides content of studied plant species

DISCUSSION

The ethnobotanical review for selection of studied medicinal plants revealed that *Aneilema* and *Combretum* species were more often indicated for treating wounds in Sub-Saharan Africa. Plants of these genera are used in Cote d'Ivoire (Kone *et al.*, 2002, 2004) and Kenya (Kiringe, 2006). Several *Combretum* species also are used in South Africa for wound healing (Onayade *et al.*, 1996).

The mode of administration of remedies seems to be function to the kinds or stages of wounds. The sap of trees or juices of fresh leaves are more often used in the case of new wounds while decoction or macerate are applied for old wounds. This behavior is probably related to the occupations of people who are rural. Topical application of sap or juice on new wound probably aimed at stopping hemorrhage and protecting wound from infectious agents when injuries occur. Certain plants such as *Chromolaena odorata* are known for their hemostatic properties (Gupta and Jain, 2010). In addition, the use of decoction for old wounds intended to exploit beneficial effects of compounds such as tannins and polysaccharides that can accelerate wound healing process. Tannins and polysaccharides are extracted in high amount in warm water.

Interesting, the extracts obtained from the 6 studied plants contain these compounds together with flavonoids and alkaloids. Therefore, all these species are promising for the treatment of

wounds. Their chemical composition supports their use in traditional medicine for repairing disruption of tissue. These compounds are known for their role in mechanical cure and disinfection of wounds (Onayade *et al.*, 1996; Gupta and Jain, 2010).

Decoction of *C. pentandra* is used to wash opened wounds (Burkill, 1985). Stem bark of this plant contains tannins, flavonoids and low amount of alkaloids, polysaccharides and mucilages. The presence of flavonoids, tannins and alkaloids was also reported by Sule *et al.* (2009) for a sample of Nigeria. The action of this plant in healing external wounds is due in part to flavonoids and tannins. These latter phytochemicals are known for their significant role in wound healing process (Shivhare *et al.*, 2010). These compounds have vulnerary, astringent and draining properties (Afaq *et al.*, 2005); but also antibacterial and immunostimulant activities (Pousset, 1992). Flavonoids show antimicrobial activity (Hernandez *et al.*, 2000).

Sap of *Antiaris toxicaria* is indicated for treatment of cuts, wounds and skin diseases, eczemas and leprosy in west tropical Africa (Burkill, 1997). In Cote d'Ivoire, sap is used (Tra Bi, 1997) while stem extract is reported to stimulate proliferation of cells (Jiang *et al.*, 2009). The phytochemical study showed that stem bark of *A. toxicaria* contains tannins and complex polysaccharides in abundance. Complex polysaccharides are known for their healing effects (Ross and Brain, 1977), antibacterial (Sofowora, 1996) and antioxidant activity (Liu *et al.*, 2005). The antioxidants accelerate the healing process by destroying free radicals which are implicated in inflammation (Gupta and Jain, 2010).

Leaves and stem bark of *F. exasperata* are used as decoction for treatment of old wounds (Weiss, 1997). The leave aqueous extract showed antibacterial activity against *Staphylococcus aureus* (Macfoy and Cline, 1990), bacteria implicated in wound infections. The phytochemical studies carried out on this plant revealed low amount of tannins but high quantity of polysaccharides, mucilages and flavonoids. The presence of tannins, flavonoids and alkaloids was reported by Umerie *et al.* (2004) and Mensah *et al.* (2008).

The juice squeezed from *A. boonei* leaves is used for disinfection of external wounds while the latex is recommended in pediatrics for the treatment of skin eruptions (Kerharo, 1967). According to Dalziel (1956), the sap is applied for treatment of new wounds. This plant contains complex polysaccharides in abundance, in particular mucilages but low quantity of alkaloids, tannins and flavonoids. This showed that wound healing properties of *A. boonei* might be mainly attributed to polysaccharides. This plant exhibited antioxidant activity (Akinmoladun *et al.*, 2007) which strengthens its role in reduction of inflammation.

The sap and various parts of *P. nigrescens* are cited for wounds (Neuwinger, 1996). The phytochemical analysis revealed presence of catechic tannins and abundant polysaccharides and mucilages. Awobajo *et al.* (2009) reported flavonoid content but not tannins. *P. nigrescens* showed anti-inflammatory and analgesic effects (Owoyele *et al.*, 2009).

Decoction of *T. dinklagei* is used in West Africa to wash old wounds (Dalziel, 1956). Only catechic tannins and polysaccharides were characterized in this plant. The presence of alkaloids is reported in literature (Tackie *et al.*, 1975).

This ethnopharmacological study permitted drawing up a nonexhaustive list of 50 medicinal herbs traditionally used in West Africa to treat wounds. The preliminary phytochemical investigations carried out on 6 of these plants revealed at least trace amount of polysaccharides, mucilages, tannins, flavonoids and alkaloids. These results clearly demonstrated that studied plants can play a role in the development of treatment for wound healing.

Study is ongoing on these plants in order to elucidate the structure of polysaccharides, evaluate their complement fixing activity *in vitro* and *in vivo*.

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