



Phytochemical Screening of some medicinal plants traditionally used by African Women in Kinshasa city (DR Congo) for their intimate hygiene and Evaluation of the pH of derived recipes

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ABSTRACT

In Africa, about 80% of population relies on traditional medicine for their health care purpose. The aim of the present study was to evaluate both the chemical composition of plants and the pH of derived recipes used by African Women in Kinshasa city (DR Congo) for their intimate hygiene. Phytochemical screening revealed the presence of phenols and triterpenoids in all extracts. Saponins were found in seven plant species (*Bridelia ferruginea*, *Crossopteryx febrifuga*, *Hibiscus mechowii*, *Lycopersicum esculentum*, *Mangifera indica*, *Ocimum basilicum*, *Vitex madiensis*). The use of plants containing saponins by women for their intimate hygiene could serve as source of spermicidal agents. The results of the pH measurements indicated also that the acidity of twenty seven recipes varied between 2,35 (*Citrus limon* juice) and 7,02 (decoction of the leaves of *Chenopodium ambrosioides*). Only 14% of the recipes presented the value of acidity conforms to vaginal physiological acidity (pH 4 to 5) and should preserve the healthier vaginal microflora through retention of *Lactobacillus*. The acidity of the recipes depends on the factors such as plant species (alone or associated) and the mode of preparation.

Keywords: Intimate hygiene, folk medicine, medicinal plants, phytochemical screening, Democratic Republic of the Congo.

INTRODUCTION

The Congo Basin, whose the major part is located in the Democratic Republic of Congo constitutes a large terrestrial biome considered on a global scale (like the Amazon and Borneo - Mekong basins) as a reservoir of medicinal plants and biodiversity. In the DR Congo, forests cover approximately 62% of the national territory. This is the second largest block of tropical forest in the world and harbors approximately half of the continent's rainforests [1]. Medicinal plants are the key product for the Congolese population. Almost all Congolese populations, both urban and rural, depend on medicinal plants for their health care needs because the costs of conventional drugs are often unaffordable. These plants have found to have therapeutic value for solving major health problems [2-12].

In order to preserve the ethno-medical cultural heritage of the Democratic Republic of the Congo, the present study was undertaken with the aim of evaluating both the chemical

composition of plants and the pH of derived recipes used by African Women in Kinshasa city for their intimate hygiene.

The renewed interest that is given to medicinal plants is due to the fact that despite the efforts of chemists to synthesize new molecules, more than 25% of available drugs derived directly or indirectly from plants. These products usually are not always available, or when they are, they are too expensive for the population [13]. So, the first line of intimate hygiene for African women in Kinshasa city is the use of herbal medicines at home.

MATERIALS AND METHODS

Plant material collection and identification

The tested plant materials used in this study were collected in Democratic Republic of the Congo during a field work in March 2013 and were authenticated by Mr B.L. Nlandu of the INERA (Institut National d'Etudes et Recherches Agronomiques).

Vouchers specimens are on deposit at the INERA Herbarium of the Faculty of Science (Université de Kinshasa).

Extraction and chemical screening

The dried and powdered plant material (10 g) was repeatedly extracted by cold percolation with 95% ethanol and water (100 mL x 2) for 48 hours. Chemical screening was done according to a well known protocol as previously reported [5-8, 14, 15].

Detection of phenols (Ferric Chloride Test)

Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

Detection of flavonoids

The ethanol extract (5 ml) was added to a concentrated sulphuric acid (1 ml) and 0.5g of Mg. A pink or red coloration that disappear on standing (3 min) indicates the presence of flavonoids.

Detection of anthocyanosids

The presence of anthocyanosids is revealed by a color change as a function of pH due to titration of the acidic aqueous solution with a solution of NaOH. If the solution turns a red color, the pH is less than 3, if against a blue color; the pH is between 4 and 6.

Detection of tannins

Two methods were used to test for tannins. First, about 1 ml of the ethanol extract was added in 2 ml of water in a test tube. 2 to 3 drops of diluted ferric chloride solution was added and observed for green to blue-green (catechic tannins) or a blue-black (gallic tannins) coloration. Second, 2 ml of the aqueous extract was added to 2 ml of water, a 1 to 2 drops of diluted ferric chloride solution was added . A dark green or blue green coloration indicates the presence of tannins.

Detection of leucoanthocyanins

To 2 ml of aqueous extract was added few drop of Shinoda reagent in a test tube and then boiled. A red or purple coloration in the supernatant indicates the presence of leucoanthocyanins.

Detection of saponins

To 1 ml of aqueous extract was added few volume of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth for 20 min.

Detection of alkaloids

Five ml of the extract was added to 2 ml of HCl. To this acidic medium, 1 ml of Dragendroff's reagent was added. An orange or

red precipitate produced immediately indicates the presence of alkaloids.

Detection of coumarins

Evaporate 5 ml of ethanolic solution, dissolve the residue in 1-2 ml of hot distilled water and divide the volume into two parts. Take half the volume as a witness and to add another volume of 0.5 ml 10% NH₄OH. Put two spots on filter paper and examined under UV light. Intense fluorescence indicates the presence of coumarins.

Detection of free quinones

To 1 ml of organic extract was added few drops of Borntrager reagent (NaOH 10% ou NH₄OH 10%) in a test tube. The solution was and then shaken vigorously. A sharp red or orange coloration indicates the presence of free quinones.

Detection of steroids

One ml of the extracts was dissolved in 10 ml of chloroform and equal volume of concentrated sulphuric acid was added by sides of the test tube. The upper layer turns red and sulphuric acid layer showed yellow with green fluorescence. This indicated the presence of steroids.

Detection of diterpenes (Copper acetate Test)

Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

Detection of triterpenoids

Ten (10) mg of the extract was dissolved in 1 ml of chloroform; 1 ml of acetic anhydride was added following the addition of 2 ml of Conc.H₂SO₄. Formation of reddish violet colour indicates the presence of triterpenoids.

Evaluation of the pH of the recipes

The receipts were prepared according to the method suggested by the traditional healers. The value of the pH of the various conditioned receipts was taken using a pH-meter apparatus. The experiment was taken again twice.

RESULTS AND DISCUSSION

Chemical screening

The results of chemical screening of medicinal plants traditionally use by African Women for their intimate hygiene is presented in Table 1.

Table 1: Chemical screening of 11 plant species used by African Woman in Kinshasa City for their intimate hygiene

Secondary metabolites	Medicinal plant species and used parts										
	Abrus precatorius (Leaves)	Alchornea cordifolia (Leaves)	Bridelia ferruginea (Stem bark)	Citrus lemon (Fruit juice)	Crossopteryx febrifuga (Leaves)	Hibiscus mechowii (Leaves)	Lycopersicum esculentum (Leaves)	Mangifera indica (Stem bark)	Ocimum basilicum (Leaves)	Vitex madiensis (Leaves)	Zingiber officinale (rhizome)
Phenols	+	+	+	+	+	+	+	+	+	+	+

Catechic tannins	-	+	+	-	+	-	+	+	+	+	+
Gallic tannins	+	+	+	+	-	+	-	+	+	-	+
Anthocyanins	-	+	+	+	+	-	-	+	-	+	+
Leucoanthocyanins	+	+	+	-	+	-	-	+	-	+	+
Alkaloids	+	+	+	+	+	+	+	+	+	-	+
Flavonoids	+	+	+	+	+	-	+	+	+	-	-
Free quinones	-	-	+	-	-	-	-	+	-	+	-
Saponines	-	-	+	-	+	+	+	+	+	+	-
Coumarines	-	-	-	-	-	-	-	+	-	-	-
Diterpenoids	+	+	+	+	+	+	+	+	+	+	+
Steroids	+	+	-	+	+	+	+	-	+	-	+
Triterpenoids	+	+	+	+	+	+	+	+	+	+	+

It is deduced from the table 1 that 13 secondary metabolites were detected in the extracts of the plants used by African Women for their intimate hygiene. 100% of analyzed extracts were found to contain the phenols, diterpenoids and triterpenoids. The alkaloids were present in 90% of plant extracts. 72, 7% of extracts tested contain tannins, flavonoids and steroids. 63, 6% of screened extracts contain anthocyanins, leucoanthocyanins and saponins. While, free quinones were detected in 27% of extracts but coumarins were detected in 9% of plant species.

The presence of these secondary metabolites could partially justify the use of these medicinal plant species by African women for their intimate hygiene and could also prevent on the negative consequences of such practice (side effects). Indeed, recent findings showed that the various types of chemical compounds found in the studied plant extracts have a broad range of biological properties. For example, phenolic compounds are reported to have antibacterial activities. The recipes applied by African women in the vagina could stop the growth of vaginal microbial flora. The users would profit from it if the action of these preparations is limited only to the pathogenic microbes. However, the vaginal flora is made up to 95% of the bacteria which contribute to local protection [16].

The astringency of the tannins could be responsible of vaginal muscles toning up and vaginal diameter contraction. While others phenolic compounds such as anthocyanins, flavonoids and leuco-anthocyanins which are present in some used plants, could inhibit or reduce the vaginal tissue ageing process because of their radical scavenging properties [6-9]. According to Boua et al. [17], the flavonoids possess aphrodisiac effects. However the revealed that the flavonoids are absent in the leaves of *Hibiscus mechowii*, a plant species used to stimulate sexual appetite of the user.

Many reports revealed that saponins possess fungicidal, anti-inflammatory and spermicidal effects. Plant containing secondary metabolites having such properties could be useful in the prevention of vaginal infections as well as inflammatory reaction. They could also down regulate the mobility of spermatozoids or destroy them. The presence of saponins in some screened plants could also induce the cicatrization [18] of the microlesions of the wall of a drained vagina, caused by frictions of genitals at the time of the sex act. Saponins being used as detergent for their surface-active properties could also cause the inhibition of *Lactobacillus* biofilm formation because of the lack of bacterial adhesion on the vaginal wall.

The results of phytochemical screening revealed also the presence of coumarins only in the stem bark of *Mangifera indica*. These compounds were reported to stop the haemorrhages after delivery [19] and could calm the uterine bleedings. The presence of alkaloids in some plant extracts could facilitate the childbirth or cause abortions because of the oxytocic effect of certain alkaloids [20]. The presence of secondary metabolites such as flavonoids, quinones, steroids and terpenoids could have negative effect on the vaginal microbiota because of their antimicrobial activities [21].

Acidity of the recipes

Twenty three used organs of some inventoried plants were used to prepare 27 recipes whose acidity is presented in the following table.

Table 2: Recipes derived from some medicinal plants traditionally used by African Women in Kinshasa city (DR Congo) for their intimate hygiene and their pH values

Mode of preparation and administration of recipes	pH values
The young fresh leaves of <i>Abrus precatorius</i> are crushed or triturated between the hands. They are introduced into the vagina (and sometimes into the anus) from the morning to the evening and are removed before ordinary cleaning with water	6,27
The young leaves of <i>Abrus precatorius</i> are dried in the ambient air then crushed. The powder obtained adheres to the moistened finger and is applied to the vaginal epithelium.	5,7
The young fresh leaves of <i>Alchornea cordifolia</i> are crushed or triturated between the hands. They are introduced into the vagina (and sometimes into the anus) from the morning to the evening and are removed before ordinary cleaning with water.	4,03
The stem bark of <i>Bridelia ferruginea</i> are dried then crushed. Crushed is applied to the surface of the wall of the vagina.	4,45
The young fresh leaves of <i>Chenopodium ambrosioides</i> are crushed or triturated between the hands. They are introduced into the vagina (and sometimes into the anus) from the morning to the evening and are removed before ordinary cleaning with water.	6,72
The sap of <i>Cissus aralioides</i> is extracted from its stem and is used to stretch the small lips.	5,4
Leaves of <i>Chenopodium ambrosioides</i> , <i>Mangifera indica</i> , and <i>Ocimum gratissimum</i> are carried to boiling together in water. The decoction is used for the sitzbath.	5,42
The juice of <i>Citrus lemon</i> is extracted from its fruit and soaks the plug to be introduced into the vagina.	2,35
The juice of <i>Citrus lemon</i> is extracted from its fruit and is added in the water which is used for cleaning of the vagina (ratio 3:1, v/v).	2,40
The juice of <i>Citrus lemon</i> is extracted from its fruit and is added in the water which is used for cleaning of the vagina (ratio 1:1, v/v)	2,77
The young fresh leaves of <i>Crossopteryx febrifuga</i> are carried to boiling in water. The decoction is used for the sitzbath.	4,98
The follicules of <i>Dewewrea bilobata</i> and <i>Monodora myristica</i> are crushed with the root bark of <i>Lannea antiscorbutica</i> as well as nodosities of <i>Cyperus articulatus</i> . The mixture is introduced into the vagina.	5,37
The young fresh leaves of <i>Gossypium barbadense</i> are crushed or triturated between the hands. They are introduced into the vagina (and sometimes into the anus) from the morning to the evening and are removed before ordinary cleaning with water.	6,36
The young fresh leaves of <i>Gossypium barbadense</i> are carried to boiling in water. The decoction is used for the sitzbath	6,69
The young fresh leaves of <i>Hibiscus mechowii</i> are crushed or triturated between the hands. They are introduced into the vagina (and sometimes into the anus) from the morning to the evening and are removed before ordinary cleaning with water.	2,79
The fresh leaves of <i>Hibiscus mechowii</i> are spread out over a hotplate a few moments to be tenderized. They are triturated then introduced into the vagina.	6,73

The young fresh leaves of <i>Lycopersicum esculentum</i> are crushed or triturated between the hands. They are introduced into the vagina (and sometimes into the anus) of the morning at the evening and are removed before ordinary cleaning with water.	5,57
The fresh barks of the trunk of <i>Mangifera indica</i> are crushed and subjected to the maceration in water. After filtration, extract is used for the sitzbath.	5,48
The young fresh leaves of <i>Ocimum basilicum</i> are crushed or triturated between the hands. They are introduced into the vagina (and sometimes into the anus) from the morning to the evening and are removed before ordinary cleaning with water.	6,62
The young fresh leaves of <i>Ricinus communis</i> are crushed or triturated between the hands. They are introduced into the vagina (and sometimes into the anus) from the morning to the evening and are removed before ordinary cleaning with water.	6,34
The fresh leaves of <i>Ricinus communis</i> are spread out over a hotplate a few moments to be tenderized. They are then triturated and introduced into the vagina.	6,78
The barks of the fresh root of <i>Sarcocephalus latifolius</i> are grated and introduced into the vagina.	6,20
The young fresh leaves of <i>Tetradenia riparia</i> are crushed or triturated between the hands. They are introduced into the vagina (and sometimes into the anus) from the morning to the evening and are removed before ordinary cleaning with water.	5,71
The fresh root of <i>Vigna unguiculata</i> is crushed and introduced into the vagina.	4,02
The fresh root of <i>Zingiber officinale</i> is crushed and introduced into the vagina.	6,67

From the table 2 it can deduce that 14% of the recipes have a pH which borders the physiological pH and the remainder present values which deviates some, which could make believe that their acidity would influence the vaginal pH. However, it was reported that, the introduction of a naturally acidic products in the vagina would not have an influence on the vaginal pH. The pH is only little influenced even by very alkaline products such as sperm. The recipes derived from some medicinal plants traditionally used by African Women could thus act on the vaginal pH, not by direct chemical action starting from its own pH (acidity), but by indirect action on the local micro-biota. Indeed, *Lactobacillus acidophilus* is a important organism in the vaginal microbiota, and is responsible for maintaining acidic pH, preventing the growth of potential pathogens [16]. The ideal natural recipes for intimate hygiene should preserve the healthier vaginal microflora through retention of *Lactobacillus*.

From the table 2 it can also observe that two recipes only out of the 27 analyzed (7, 4%) are prepared by associating four medicinal plant species, the remainder of the recipes are made up of only one botanical species.

The values of pH for the recipes vary from 2, 35 (juice of *Citrus limon*) to 7, 03 (decoction of the leaves of *Chenopodium ambrosioides*). These data can be categorized in three groups: the recipes whose pH is lower than 4, those whose pH lies between 4 and 5, those whose pH is higher than 5. Figure 1 the gives the intervals of acidity of prepared recipes.

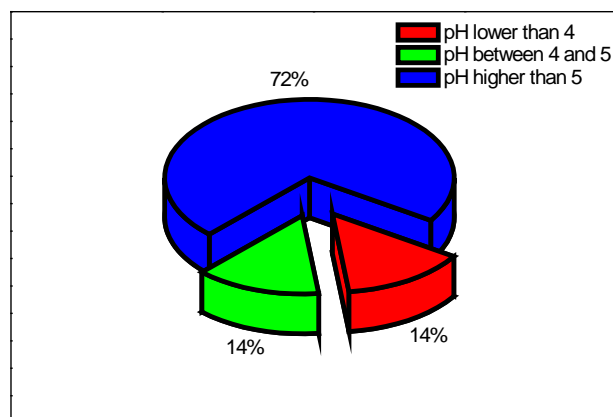


Figure 1: Proportions of the recipes according to intervals of acidity

Figure 1 revealed that 72% of the recipes have a pH higher than 5. Recipes having pH between 4 and 5 represent 14%. This interval of pH is compatible with the vaginal pH. The results indicated that the acidity of the recipes depends on the factors such as plant species (alone or associated) and the mode of preparation.

Conclusion

The present study evaluated the chemical composition and the pH of derived recipes used by African Women in Kinshasa city (DR Congo) for their intimate hygiene. Phytochemical screening revealed the presence of various secondary metabolites of pharmacological relevance. Some recipes presented the pH value conforms to vaginal physiological acidity (pH 4 to 5) and should preserve the healthier vaginal microflora through retention

of *Lactobacillus*. Further studies involving the spermicidal effects of these plants are in progress.

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