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## Contribution to the ethnobotanical, phytochemical and pharmacological studies of traditionally used medicinal plants in the treatment of dysentery and diarrhoea in Lomela area, Democratic Republic of Congo (DRC)

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## **1. Introduction**

Diarrhoea and dysentery epidemics are mostly common where living conditions are crowded and hygiene is poor. Dysentery is a microbial infection of gastrointestinal tract. Symptoms include fever, vomiting, abdominal pain and diarrhoea which often contains blood and pus. The onset of the disease usually occurs within 2–3 days after infection and lasts for up to several weeks. Dehydration occurs rapidly, especially in children and can cause death if treatment is not given.

Parasitic diseases such as dysentery and diarrhoea are the main causes of high mortality rate in developing countries. In these countries, over five million children under the age of five die annually from severe diarrhoeal diseases (<u>WHO, 1996</u>). The majority of rural people has limited access to formal and adequate health services, thus people seek help from traditional healers who provide alternative health care services. Indigenous plant remedies are widely used in the treatment of a variety of disorders. However, only few of them have been controlled clinically, or studied chemically and biologically to identify their active constituents.

In DRC, numerous plants are traditionally used as medicinal tools with antibacterial and antiparasitic properties in the treatment of diarrhoea and dysentery (<u>Penge</u>; <u>Penge</u>; <u>Penge</u> and <u>Kambu</u>). Since ethnobotanical field research is always the first step in any phytomedicinal research and development work (<u>Mitscher</u> and <u>Rojas</u>), inquiries were studied out in Lomela villages.

This study is the first one performed in this region and to the best of our knowledge, most of the plants hereby investigated have not been previously reported to possess antidiarrhoeal activity. Therefore, the present study gives specific and useful information about; (i) which plants and/or plant parts are used in the treatment of diarrhoea and dysentery, (ii) how the plant material is provided and processed for remedies, and (iii) how these remedies are sued for treatments by traditional healers and traditional people of the Lomela region.

In addition, a preliminary phytochemical screening has been carried out in order to have an idea of the different classes of organic compounds present in the different extracts of each plant. Finally, we have screened crude extracts derived form these plants for their antibacterial and antiamoebic activities, so as to generate some preliminary data on the biological activity of the plant extracts.

## 2. Materials and methods

## 2.1. Study area

Inquiries have taken place in the area of Lomela, sub-district of Sankuru, district of Oriental Kasaï (Congo). This region was chosen due to severe epidemics of dysentery that occurred in 1986 and 1994 which were treated essentially with endemic medicinal plants in complete absence of modern medicine.

## 2.2. Methods used in the interviews with informants

Inquiries were executed from 2 March 1996 to 16 April 1996 among the traditional healers and people of Lomela area, mainly in the villages where epidemics of diarrhoea and dysentery occurred. These places are:

• Lomela centre, in Batetela community;

• Mukumari, in Batetela community, approximately 70 km from Lomela centre, in the South of Lomela area;

- Kutusongo, in Bahamba I community, 50 km from Lomela centre, in the South of Lomela area;
- Wony'Angondo, in Bahamba I community, 40 km from Lomela centre, in the South Lomela area;
- Betembo, in Bakela community, 25 km from Lomela centre, in the West of Lomela area;

• Osangamanga, in Bahamba II community, 30 km from Lomela centre, in the North of Lomela area.

Interviews based on pre-prepared series of questions were administered to the informants. Such questions included among others:

- Researcher's name,
- Informant: name, tribe, village, community, zone, sub-region, age, sex and religion,

• Plant: vernacular name, uses in popular medicine, plant part, plant combinations, additional ingredients, preparation, application and dosage, rites, side effects, interdicts, no medicinal uses.

These interviews were administered by one of the authors (A. Longanga Otshudi) in local languages, with the help of the political authority of each village. In total, 104 interviews were conducted among healers and native people of Lomela villages, of which 33 interviews were carried out in Lomela centre, 30 in Mukumari, ten in Kutusongo, 21 in Wony'Angondo, six in Osangamanga and four in Betembo.

The plants were collected from all the different parts of the investigated Lomela villages, and their botanical identification was carried out with the help of the INERA (Institut National d'Etudes et de Recherches Agronomiques) herbarium at Kinshasa University and the specialists of the 'Herbarium of National Botanical Garden of Meise, Belgium'. Confirmation was obtained by comparing the collected species with the already identified herbarium specimens. A voucher specimen of each species was deposited in the Department of Pharmaceutical Sciences, Laboratory for Pharmacognosy and Phytochemistry, Vrije Universiteit Brussel.

### **2.3. Preparation of extracts**

Each air-dried plant material (10 g) was ground into a fine powder and exhaustively percolated after maceration for 24 h, with solvents having an increasing polarity (diethylether, 70% methanol and  $H_2O$ ). The resulting extracts were evaporated to dryness in vacuum at 40 °C, yielding three crude extracts: F1, F2 and F3, respectively. These extracts were subjected to phytochemical analysis and biological activities.

### 2.4. Antibacterial and antiamoebic testings

The antimicrobial testing was performed by the two-fold serial broth microdilution method (<u>Mitscher et al., 1972</u> and <u>Vanden</u>) whereas the antiamoebic assay was carried out using the microdilution technique in Diamond TPS-1-monophasic medium (<u>Wright Colin et al., 1988</u>).

### 2.5. Test organisms

Test microorganisms used in the preliminary antibacterial and antiamoebic testings included both enteropathogens and protozoan. As enteropathogens, we have used:

## 3. Results and discussion

The rural area of Lomela is located in the district of Oriental Kasai, Sub-district of Sankuru in the DRC. It is the widest area of Sankuru with a surface area of 26 346 km<sup>2</sup> and population estimated to be ca. 45.874 in 1958. This area is located approximately at 22–25° longitude East, 1°30′–3° longitude South at an altitude of 500 m. The area lies entirely in the region of shady equatorial forests, where it rains all year round with less rainfall observed in March, June and August. There is around 1729 mm annual rainfall. The temperatures are tropical, with 30.9°C at the maximum and 19.5°C as a minimum. The area has a variety of different ethnic groups. It is inhabited by the Batetela (in majority), Bakela, Bahamba and Djonga. The main vernacular language spoken by the majority of the population is Otetela followed by Okela and Djonga. The majority of the population is nominally christian, protestant or kimbanguist. The population lives mainly from the income of agriculture (rice, maize, coffee...), hunting and fishing.

An ethnobotanical study of antidiarrhoeal plants used by traditional healers in six communities of Lomela area (DRC) has been carried out. During the course of the study, a total of 104 informants were interviewed

for their knowledge on the uses of plants for the treatment of diarrhoea. The average age of the interviewed informants showed that they were predominantly older members of Lomela communities. The group of interviewed persons had a larger number of males, 83, as compared to 21 females. Educationally, more than half of the informants, 59, had no formal education. The rest have had either primary or secondary education.

Six medicinal plants widely used in this area for the treatment of diarrhoea and dysentery were collected by one of the authors (A. Longanga Otshudi). These plants are listed in <u>Table 1</u>. The result of the preliminary phytochemical screening of these plants is reported in <u>Table 2</u>. The frequency of usage of collected plants and the proportion of detected organic compounds are summarized in <u>Table 3</u>. In <u>Table 4</u> and <u>Table 5</u>, we report the results of biological testings.

# Table 1. Antidiarrhoeal investigated plants, their preparation and traditional uses in the rural area of Lomela is located in the district of Oriental Kasai, Sub-district of Sankuru in the DRC

Non PRELUDE	<i>Roureopsis obliquifoliolata.</i> (Gilg) Schellenb	Connaraceae	lungangula	diarrhoea and dysentery, roots are grounded into fine bits and added to cold water. The mixture is filtered by using a fine clean cloth, then, a small quantity (half a cup) is taken orally twice a day for ten days
Non PRELUDE	<i>Epinetrum villosum</i> . (Excell) Troupin	Menispermaceae	dipapoma	diarrhoea and dysentery, root decoction of the plant VO crushed leaves are used as a wound dressing for fast healing
Non PRELUDE	<i>Croton mubango.</i> Müll	Eurphobiaceae	unganga	diarrhoea and dysentery the maceration of root is drunk (1 cup) three times per day for 7–10 days abdominal pains and fever, stem bark decoction, VO laxative, young fruits added to palm wine are taken, VO.
12800	Vernonia amygdalina Del.	Asteraceae	mpasi nyioso	diarrhoea and dysentery, the leaf maceration, VO gastroenteritis, malaria, hepatitis and worms., roots decoction, VO fever and malaria, some traditional healers use <i>Quassia</i> <i>africana</i> in combination with <i>Vernonia</i> <i>amygdalina</i> , RNS.
Non PRELUDE	Quassia africana. Baill	Simaroubaceae	dokotele	diarrhoea and dysentery, the root bark decoction, VO fever and malaria, some traditional healers use <i>Quassia</i> <i>africana</i> in combination with <i>Vernonia</i> <i>amygdalina</i> , RNS.
03170	Cissus rubiginosa Planch.	Vitaceae	surne	diarrhoea and dysentery, root or stem decoction, VO., 1 cup, 2 X / 1 day for 7 days diarrhoea and dysentery, leaf infusion VO., 1 cup, 2 X / 1 day for 7 days

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The ethnobotanical, phytochemical, antibacterial and antiparasitological information of the collected plants in relation to gastrointestinal disorders is as follows.

## 3.1. Roureopsis obliquifoliolata. (Gilg) Schellenb (Connaracea)

A climbing shrub with brown-pubescent branchlets present in rain forest. Native to South of Nigeria, also in French Cameroon, Spanish Guinea, Congo (DRC) and Angola (<u>Troupin, 1952</u>). In Lomela area (DRC), roots are grounded into fine bits and added to cold water. The mixture is filtered by using a fine clean cloth, then, a small quantity (half a cup) is taken orally twice a day for ten days for the treatment of diarrhoea and dysentery. This plant is also used for the treatment of toothache and elephantiasis (<u>Troupin, 1952</u>). The phytochemical screening showed flavonoids, sterols and/or triterpenes, saponins, reducing sugars and tannins.

The antibacterial screening showed activity against all the tested enteropathogens. The methanolic and aqueous extracts showed the highter potency, with a MIC value of  $31.25 \ \mu g/ml$  for *Vibrio cholerae*. These extracts also acted weakly against *E. histolytica* with a MIC value of  $500 \ \mu g/ml$  after 72 and 144 h incubation. Biological activity of this plant could be attributed to tannins. Most frequently tannin containing plants are used to treat diarrhoea and dysentery (<u>Heinrich et al., 1992</u>).

## 3.2. Epinetrum villosum. (Excell) Troupin (Menispermaceae)

A twining lianca, ca. 2 m high, found in secondary forests in the costal area of Congo (DRC) and Angola (<u>Parvez, 1994</u>). In Lomela area, root decoction of the plant is taken orally for the treatment of diarrhoea and dysentery. The crushed leaves are used as a wound dressing for fast healing. The plant is known to have abortive activity (<u>Troupin, 1951</u>).

Preliminary phytochemical analysis showed alkaloids, sterols and/or triterpenes, saponins and reducing sugars. The isolation of alkaloids in *E. villosum* has been previously described (<u>Parvez, 1994</u>).

In vitro antibacterial screening demonstrated activity in methanolic and water extracts against *Campylobacter coli* and *Campylobacter jejuni* with a MIC value of 62.5  $\mu$ g/ml. They also acted against *E. histolytica* at 125  $\mu$ g/ml. The antibiotic and antiparasitic activities of this plant are thought to be due mainly to alkaloids.

## 3.3. Croton mubango. Müll (Eurphobiaceae)

An elegant tree, 12–20 feet. Native to Congo (DRC), Angola and Guinea (<u>Leondard, 1962</u>). In DRC, the maceration of root is drunk (1 cup) three times per day for 7–10 days for the treatment of diarrhoea and dysentery. The stem bark decoction is taken orally for abdominal pains and fever. In contrast, young fruits added to palm wine are taken orally as a laxative. Preliminary phytochemical screening showed the presence of flavonoids, sterols and/or triterpenes, saponins and reducing sugars.

In vitro antibacterial screening showed no activity against tested enteropathogens. Furthermore, the three tested extracts showed no activity against *E. histolytica*.

## 3.4. Cissus rubiginosa. (Welw.ex Bak) Planch (Vitaceae)

A climbing or trailing herb, present in savannah and secondary forests. The plant is widespread in tropical Africa (<u>Dewit, 1960</u>).

In Lomela area, root or stem decoction is drunk for the treatment of diarrhoea and dysentery (1 cup, 2×1 day for 7 days). Leaf infusion is used for the same purpose.

Preliminary phytochemical analysis showed the presence of flavonoids, sterols and/or triterpenes, reducing sugars and tannins.

Antibacterial screening showed activity in methanolic and aqueous extracts against several tested enteropathogens. The highest activity was observed towards *Shigella dysenteriae* and *Shigella boydii* with MIC values of 31.25 µg/m. In contrast the plant extracts showed weak activity against *E. histolytica* (MIC values of 500 µg/ml after 144 h incubation).

Activity against tested enteropathogens could be due to the presence of tannin and flavonoids in the plant.

### 3.5. Vernonia amygdalina. Del (Asteracea)

A shrub or small tree 6–10 feet. high. The plant is common and often planted, from Guinea to Cameroon. Extending to Congo (DRC), Uganda and Tanganyika (<u>Oliver, 1877</u>).

In Lomela area, the leaf maceration is orally used for the treatment of diarrhoea and dysentery whereas the root decoction is drunk for gastroenteritis, malaria, hepatitis and worms. The use of the root decoction for the treatment of gastrointestinal disorders also has been reported from Rwanda (Vlietinck et al., 1995).

Preliminary phytochemical screening showed the presence of flavonoids, sterols and/or triterpenes, saponins, reducing sugars and tannins. The isolation of saponins in *V. amygdalina* has been previously described (Jisaka et al., 1992).

Antibacterial testing of the plant extracts showed no activity against tested enteropathogens. Furthermore, a weak activity was observed against *E. histolytica*. This result validates previous reports on the antibacterial activity of extracts derived from *V. amygdalina* towards *Escherichia coli* (<u>Vlietinck et al., 1995</u>).

### 3.6. Quassia africana. Baill (Simaroubaceae)

A glabrous shrub, 8–12 feet high; present in rain forest. The plant is native to Cameroon, Congo (DRC) and Angola (<u>Gilbert, 1958</u>).

In Lomela area, the root bark decoction is drunk for the treatment of diarrhoea and dysentery. Some traditional healers use *Quassi africana* in combination with *Vernonia amygdalina* for the treatment of fever and malaria. The use of this plant for the treatment of diarrhoea and dysentery in Kinshasa (DRC) has been previously reported (Kambu et al., 1990).

Preliminary phytochemical screening showed the presence of sterols and/or triterpenes, saponins and reducing sugars. The isolation of quassinoids in this plant has been previously described (<u>Cabral et al., 1993</u>).

Antibacterial screening showed no activity against tested enteropathogens whereas the plant extracts demonstrated good activity against *E. histolytica* with MIC value of 125 µg/ml after 72 h incubation. Certainly the quassinoids in the extracts are effective against *E. histolytica*, since these organic compounds are known to have antiamoebic properties (Wright Colin et al., 1988). In contrast, we did not find out in this study the previous antibacterial activity reported on extracts derived from *Quassia africana* against *Escherichia coli* and *Shigella flexneri* (Kambu et al., 1990).

All the plant materials were collected during the rainy season and early in the morning, since most of interviewed traditional people indicated that some plants have their full therapeutic effect only if collected at certain time or during a certain season. Most of the investigated plants were designated several times by different informants and multiple citations were particularly found within the same study area, exception made for *Vernonia amygdalina* and *Quassia africana* which were cited in all the investigated villages. Most of the collected plants were used separately except *Quassia africana* which is used in combination with *Vernonia amygdalina* for treating malaria and fever.

Root is the plant part most frequently used by traditional people of Lomela for preparing traditional medicine as observed in this study. This agrees with a study carried out in Tanzania, where <u>Chhabra et al. (1993)</u> also found that roots were the most prominent plant part used to prepare remedies. The concentration and the nature of the active compounds can vary significantly between different parts of the same plant. We observed that the stem bark of *Croton mubango* is used against diarrhoea while the seeds (fruits) of the same plant cause diarrhoea and are sometimes used for treating constipation. Another observation made is the way by which remedies are administered for treating diarrhoea. In most cases, the oral route is preferred. The usual modes of preparing remedies are decoction, infusion and maceration, with water and/or palm wine as solvents.

The bacteriological quality of these remedies is solely dependent on their mode of preparation (decoction, infusion) or the antimicrobial properties of palm wine, sometimes used as solvent. No rites or incantations are required to give the desired effect from a remedy. The dosage of a remedy depends mainly upon the nature of the drug (plant part) to be administered, the sex of the patient and the age. In general, pregnant women and children get small doses.

The duration of the treatment varies, depending on the type of remedy and the severity of diarrhoea. In general, at least 10–14 days are assumed to be necessary for treatment. Normally, if after 2 weeks of treatment dysentery persists, patients die of anemia and dehydration.

No interdicts are required during the treatment, but since one knows that dysentery is a very contagious disease, traditional healers recommended no contact with the patient. That is why, sometimes, even food was given to the patients by means of sticks. When asked about secondary effects, only a few of the respondents reported vomiting, abdominal pains, loss of appetite and abortion for *Epinetrum villosum*, but we can not overlook the fact that some of these effects (vomiting and abdominal pains) also occur during dysentery episode.

The preliminary phytochemical screening carried out on the six collected plant materials covered mainly alkaloids, saponins, sterols and/or triterpenes, tannins, flavonoids, anthraquinones and reducing sugars. Out of the six screened plants, only one (1/6), *Epinetrum villosum*, gave a positive test for alkaloids which are reputed to have important physiological activities. Three plant materials (3/6) gave a positive test for tannins which are well documented for their astringent action; this provokes their usefulness in preventing diarrhoea and controlling haemorrhage. In addition, tannins have been reported to possess cytotoxic and antineoplastic activities. Four plant materials (4/6) gave a positive test for flavonoids which are known to possess antiviral, antiinflammatory and cytotoxic activities. Five plant materials (5/6) gave a positive test for saponins lies in their easy conversion to medicinally used steroidal hormones. All the six plant materials (6/6) gave a positive reaction for reducing sugars which are known to be useful in providing a means of

storing energy and transport of energy. In addition, many classes of plant constituent (e.g. the nucleic acids and the plant glycosides) contain sugars as essential features of their structures. Sugars also play a number of ecological roles in protection form wounding and infection and in the detoxification of foreign substances. All the six plant materials (6/6) also gave positive reaction for sterols and/or triterpenes. The therapeutic and industrial interest of triterpenes and steroids make them a group of secondary metabolites of outstanding importance.

No anthraquinones were detected in all the investigated plants.

We were also interested in the frequency of usage of a given plant. Since the observations on the frequency could not be quantified, popularity of the plant was used as an indicator. Thus, considering the frequency of usage of a given plant and the proportion of the organic compounds it contains, we observed that the most frequently used medicinal plant does not, in most instances, contain the highest proportion of organic compounds (Table 3).

The results of the antibacterial screening indicated that methanol and aqueous extracts derived from *Roureopsis obliquifoliolata, Cissus rubiginosa* and *Epinetrum villosum* produced in vitro antibacterial activity against one or more tested enteropathogens (<u>Table 4</u>). This could partly be explained by the widespread occurrence of polyphenolic compounds, which are soluble in water and methanol. Of great importance is *Epinetrum villosum* which also showed high activity against *Entamoeba histolytica* (<u>Table 5</u>). Although *Quassia africana* showed no activity against tested enteropathogens, the antiamoebic activity of its extracts was comparable to those derived from *Epinetrum villosum* (<u>Table 5</u>). *Croton mubango* and *Vernonia amygdalina* did not show any prominent antibacterial and antiamoebic activities in this study. We must point out that sometimes, traditional healers prefer a combination of several plants to treat disease. This combination could have a pharmacological activity whereas separate constituents might not. Evidence is hereby presented that some of the plants used for the treatment of diarrhoea and dysentery by the population of Lomela area (DRC) really have some biological activities (antibiotic and antiparasitic activities) which might explain partly the properties claimed in the treatment of gastrointestinal diseases. Some of these interesting plants are now being subjected to in-depth study for chemical constituents. Such substances in the purified state may be useful for the treatment of diarrhoea and dysentery.

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