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IN VITRO AMOEBICIDAL ACTIVITY OF AQUEOUS EXTRACTS AND THEIR FRACTIONS FROM SOME MEDICINAL PLANTS USED IN TRADITIONAL MEDICINE AS ANTIDIARRHEAL AGENTS IN KINSHASA-DEMOCRATIC REPUBLIC OF CONGO AGAINST *ENTAMOEBA HISTOLYTICA*

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ABSTRACT

40 aqueous extracts and 126 fractions obtained from the partition of these aqueous extracts were submitted to an assessment in vitro for their potential amoebicidal activity against *Entamoeba histolytica*. Results revealed the presence of five aqueous extracts and their respective soluble fractions exhibiting pronounced amoebicidal activity with minimal amoebicidal concentrations (MAC) and inhibitory concentration 50 (IC_{50}) < 10 µg/ml. They included aqueous extracts from *Carica papaya* ripe seeds, *Morinda* morindoides leaves, *Paropsia brazzeana* root, *Psidium guajava* stem bark and *Mangifera indica* stem bark. Seventheen aqueous extracts from *Alcornea latifolia* root bark, *Carica papaya* unripe seeds, *Bridelia feruginea*, *Dialium engleriaum* stem bark, and *Dracena refl*exa, *Sida rhombifolia* and *Tithonia diversifolia* leaves, *Euphorbia hirta* whole, *Heinsia pulchella* root bark, *Harungana madagacasriensis*, *Hymenocardia acida* stem bark, *Jatropha curcas*, *Maprounea africana*, *Morinda lucida*, *Psidium guyava* leaves, *Quassia africana* and *Rauwolfia obscura* root bak and their respective soluble fractions exhibited good amoebicidal activity with MAC and IC_{50} < 100 µg/ml. A moderate amoebicidal activity was also found in six aqueous extracts form *Alcornea cordifolia* and *Datura arborea* leaves, *Cassia siamea*, *Ceiba pentandra* and *Garcinia kola* stem bark, and *Nauclea latifolia* root bark while their respective fractions exhibited the same level of activity with MAC and IC_{50} < 100 µg/ml. One aqueous extract from *Pentaclethra macrophylla* and its fractions showed weak amoebicidal activity with MAC and IC_{50} values of 250 and 162 µg/ml. Eleven aqueous extracts including *Cajanus cajan* leaves and root bark, *Cissius areloides*, *Myrianthus arborea*, *Nauclea latifola Justisia insularis*, *Ongokea gore*, *Phytolacca dodecandra*, *Pteridium aquilinum*, *Vitex maddiensis* leaves and *Costus afer* fruit juice stem bark, were devoid with amoebicidal activity at the highest tested concentration of 500 µg/ml. These results demonstrate that these medicinal plants possess amoebicidal activity and can be used for the treatment of dysentery (amibiasis) and at some extents diarrhoea in traditional medicine.

KEYWORDS: Medicinal plants, aqueous extract, diarrhoea, dysentery.

1. INTRODUCTION

Diarrhoea can be defined as an alteration in the normal bowel movement, characterized by a situation in which a person daily stools exceeds 300 g and contains 60 to 95% water. During diarrhoea, the normal bowel movement becomes changed, which results in an increase of water content, volume and frequency of stool (Rhaman et al., 2015). It causes severe dehydration that can lead to death (Wansi et al., 2014). The disease accounts for more than 5-8 million deaths worldwide each year, especially in developing countries (WHO, 2006). The most common reasons for causing diarrhoea is gastrointestinal disorders and infections related to the presence of microorganisms such as *Shigella flexneri*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella thyphi*, *Vibrio cholerae*, and *Candida albicans*. *Entamoeba hytolytica* causes amoebiasis which can provocate diarrhoea and virus, and enterotoxins produced and secreted by these same bacteria (Yakubu et al., 2015; Tenório et al., 2016).

Amoebiasis is a major cause of morbidity and mortality in tropical areas and causes severe diarrhoea. It is a major problem in some countries such as China, South America, West Africa and Asia and many other developing countries (WHO, 1985). The occurrence of amoebiasis is known to be more related to the sanitation and socio-economic conditions and poor life (Stanley, 1996). To combat these diseases, WHO has initiated a diarrhoea disease control program to study traditional medicine practices and other related aspects, together with the evaluation of health education and preventive approaches (Syner and Merson, 1986). Thus, population rely to traditional medicine using many preparations based medicinal plant and find some reliefs since medicinal plant are kwon to be a rich source of bioactive constituents against various human ailments. Many medicinal plants claimed to have amoecidal activity by traditional healers are now scientifically investigated for this activity *in vitro* and *in vivo* tests proved in different scientific studies (Tona et al., 2000, Anturlikar aet al., 1993; Sohni et al., 1995; Goshal et al., 1996; Sharma and Sharma, 2001). The concern of the present study was to assess *in vitro* amoebicidal activity of aqueous extracts some medicinal plants used as antidiarrhoeal agents in Kinshasa- Democratic Republic of Congo and their respective fractions against *Entamoeba histolytica*.

2. MATERIALS AND METHODS

2.1. Plant materials

All plant materials were collected in Kinshasa and plants were identified at National Institute of Studies and Research in Agronomy (NISRA), Department of Biology, Faculty of Sciences, University of Kinshasa. The voucher specimen of each plant was deposited in the herbarium of this institute. All plant materials were dried at room temperature and reduced to powder by using an electronic blender.

2.2. Preparation of aqueous extracts

Aqueous extracts were prepared by boiling 20 g of each plant material in 200 ml distilled water. After cooling, each mixture was filtered on a paper filter Watman N° 1. Each filtrate was evaporated *in vacuo* to give corresponding dried extract. The fractionation of all aqueous extracts was carried out by using the following schema:

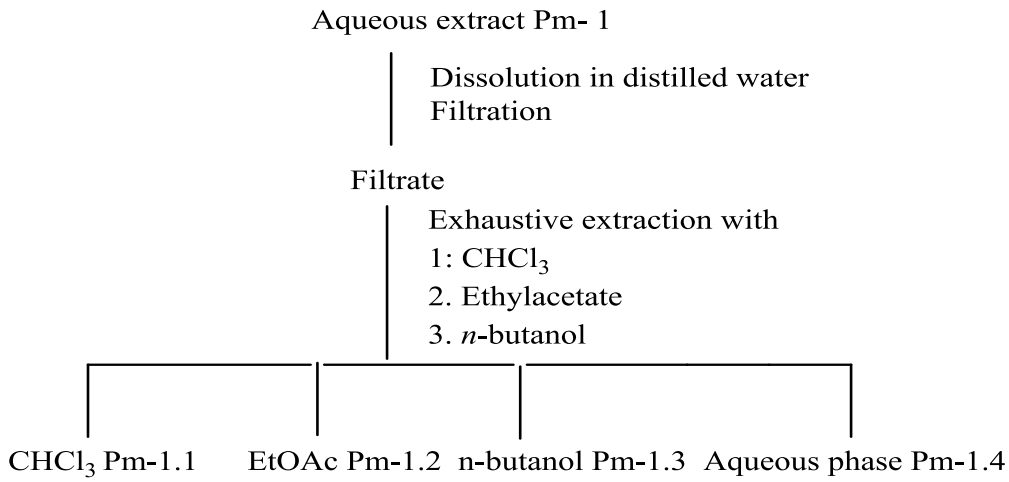

Figure 1: Fractionation of aqueous extracts with solvents of different polarities. 10 g of each dried extract were separately dissolved in 200 ml distilled water and filtered as described above Each filtrate was extracted with solvents of different polarities chloroform, ethylacetate, *n*-butanol (Fig. 1). The residual aqueous phase and all fractions was treated as described above yielding corresponding dried extract.

2.3. Qualitative phytochemical screening

Each aqueous extract was submitted to qualitative phytochemical screening carried out by TLC technic using precoated silica gel plates (thickness layer 0.25, Merck, Germany) using different reagents and mobile phase described in the literature (Harorne, 1998, Trease and Evans, 1996) to identifiy major phytochemical groups such as alkaloids, anthocyanins anthraquinones, coumarines, falvonoids, saponins, steroids, terpenes and tannins.

2.4. *In vitro* amoebicidal testing

Entamoeba histolytic used in the present study is a laboratory isolated strain from patients with acute dysentery diagnosed in the Tropical Medicine Institute, Faculty of Medicine, University of Kinshasa. The evaluation of activity was performed using the methods previously described by Tona et al., (2000) and Cimanga et al.(2010).

Briefly, the parasite was grown and cultured in sterile tubes containing 9 ml of diphasic medium (medium N of Pasteur Institute) called Dobbell and Laidlaw medium. The mixture was stirred and incubated for one week at 37°C. The daily examination and counting of amoebae through a optic microscope with the aid of Neubauer's cells were performed in order to monitor the parasitic growth and to detect possible contamination.

Uncontaminated tubes containing an average number of $2.5.x10^6$ amoebae/ml culture medium were selected as test tubes. 10 mg of each test sample was dissolved in 10 ml hydroethanol solution (eau-ethanol :9:1) to have corresponding stock solutions of 1 mg/ml. These last solutions were diluted in two fold dilutions to give a series of test solutions ranging from 500 to 0.1 µg/ml. Next, 1 ml of the test solution with a known test concentration was added to a separated 1 ml of test tubes containing parasites. On the other hand, two tubes were used as controls, one containing parasites in hydroethanol solvent (9:1) without test sample as negative control and another containing test tubes with parasites and Metronidazole or Dehydroemetine (10 to 0.1 µg/ml) as positive controls.

All tubes were plugged with sterile cotton, vigorously stirred and incubated at 37°C for one week. The daily counting of dead and living amoebae was done described above. The test was considered as positive when the vegetative or kystic forms of amoebae was not microscopically observed. The minimum amoebicidal concentration (MAC) was immediately determined and inhibitory concentrations 50 (IC_{50}) were derived by using linear-courbes doses-responses (n=3).

3. RESULTS AND DISCUSSION

3.1. Traditional used of some selected medicinal plants

In a previous study, traditional uses used of some selected medicinal plants in the present study were already reported by Cimanga et al. (2018). This list is now completed with other medicinal plants.

Table 1: Traditionnal used of selected medicinal plants.

Plant names and family	Used parts	Traditional uses
Bridelia ferruginea Benth. (Euphrbiaceae)	leaves	Treatment of oedemes, diarrhoea, decoction as a drink and for batht. A decoction of leaves is used to treat oedema, children irritations, diarrhoea, epilepsy, psychique troubles in association with *Sterculia stigera*, *Diospyros mespiliformis* and *Bombax costatum*, gastralgia, anemia, dysentery and rhumatisms.
Cassia siamea Lam. (Caelpiniaceae)	Stem bark	Aqueous decoction of the stem bark is used for the treatment of diarrhoea, amoebiasis, blennorragia. Stem bark are mixed with those of *Abrus pectorius* and *Musanga cepropiodes* is used to treat hypertension per os.
Ceiba pentandra (L.) Gaertn (Bombaceae)	Stem bark	Batch mouth to treat teeth pains, gingivitis, stomatite, used as vomitive, to treat infantile rachitism, diarrhoea wounds, asthma, hernia, blennorragia, heart and abdominal pains, stomacal wounds.
Costus afer Ker Grawl. (Zingiberaceae)	Stem bark juice	To treat eye diseases, otitis, fever, hypertension, denral caries, mouth infections, rumatism, diarrhoea, wounds and rheumatism.
Dialum englerianum Henriq. (Ceaslpinaceae)	Stem bark	Diarrhoea.
Draceana reflexa var. Nittens Welw ex Back. (Agavaceae)	Leaves	Treatment of heamorroids, dirrhoea, infantile cachexy, dysentery, smallpox, diarrhoea, hickenpox, ovulation troubles, urinary infections, uses as antiseptic.
Jatropha curcas L. (Euphorbiaceae)	Leaves	To treat articular pains, intestinal worms, fever, otitis, leprosy, constipation, enteralgia, to make easier deliverance, used as abortive, diuretic, rubefiant, drastic purgative, used as an hepato-renal regulato rin anury, blennorragia, diarrhoea and icteris.
Justicia insularis Mull.Arg (Acantaceae)	Leaves	Used in cataplam to treat anal fistules and to treat diarrhoea.
Maprounea africana Mull. Arg (Euphorbiaceae)	Leaves	To treat cough, mixed with leaves of *Crossopterix febrifuga* to treat blennoragia, teeth pains, epilepsy, aqueuse decoction to treat diarrhoea, abdominal pains, constipation.
Morinda lucida Benth (Rubiaceae)	Leaves	Used as febrifuge, cicatrisant of wounds, against fever, malaria, diabetes, oedema, antienteralgic, leprosy mixed with *Vitex cuneata* and diarrhoea.
Myrianthus arboreus P. Beauv. (Moraceae)	Leaves	To treat kidney and heart pains, and diarrhoea.
Ongokea gore (Hua) Pierre	Leaves	Not reported

Olacaceae		
Pentaclethra macrophylla Benth. (Mimosaceae)	Stem bark	Aqueous decoction used as aphrodisia**c,** galactogene, stimulant antalgic Aqueous decoction used to treat gastric, heart pains, bennrragia, diarrhoea 0dysentery, adominal pains, dysmenohrrea and cephalgia.
Phytolocca dodecandra L'Herit (Phytolaccaea)	Leaves	Aqueous extract used to treat abdominal pains, stomach bloated, woonds, enflures, scabies, to sooche intercostal nevralgy dysentery, diarrhoea, dermatosis, epilespsy.
Pteridium aquilunum (L.) Khun var. caudatum (Pteridophytes)	Leaves	Diarrhoea.
Quassia africana Baill. (Simarubaceae)	Root bark	Aqueous decoction in wine to treat blennorragia, sexual asthenia and weakness sexual, mixed to *Citru*s juice or *Afromomum stipulatum* used to treat intestinal worms aqueous decoction used to treat diarrhoea, dysentery, fever, gastric haemorroids, pains teeth, head pains, angine, hypertension, woods, bronchopneumonia, hernia emphysemes, rheumatism, abdominal pains, used as cholague, anthelmintic, antalgic.
Rauwolfia obscura K. Schum (Apocynaceae)	Root bark	Aqueous decoction is used to treat fever, diarrhoea, diabete, gargarism, dental caries.
Tetracera poggei Gilg. (Dilleniaceae)	Leaves	Not reported
Vocanga africana Staph. (Apocynaceae)	Root bark	Wine decoction is used for the treatment of blennorragia, hypertension, tuberculosis, breathing difficults mixed with *Ocimum gratissinum* leaves, hernia pains, rheumatism and diarrhoea. dysentery, Aqueous decoction is used to treat hernia pains and to avoid aftemaches of prematurated and precipitated childbirth, to treat epilepsy and diarrhoea.

Kerharo and Adam, (1974); Adjanohoun et al., (1988, 1989); Kambu, (1990).

3.2. Qualitative phytochemical screening

Results from the qualitative phytochemical screening are presented in Table 1. They revealed that alkaloids were presents in aqueous extract from *Carica papaya ripe and unripe seeds*, *Vocanga Africana* root bark. Anthraquinones were only found in *Cassia siamea* stem bark, *Morinda morindoides* and *M. lucida* leaves, and *Rauwolfia obscura* stem bark. Coumarins were revealed to be present in *A. cordifolia* leaves, *B. ferruginea* stem bark, *C. cajan* leaves, *H. acida* stem bark, *J. curcas* leaves, *P. macrophylla* stem bark, *P. dodecanda* root bark, *T. poggei* leaves and *T. diversifolia* leaves.

Table 1: Results of Phytochemical screening.

A/B	*A. cordifolia*	*B. ferruginea*	*C. cajan*	*C.papaya*	*C.siamea*	*C. pentandra*
Alkaloids	-	-	-	++	-	-
Anthraquinones	-	-	-	-	++	-
Anthocyanins	-	-	-	-	-	-
Aminated compounds	++	+++	+++	+++	+++	+++
Cardiotonic glycosides	-	-	-	-	-	-
Coumarins	++	+	++	-	-	-
Flavonoids	++	++	-	-	+	-
Proanthocyanidins	+	++	+	-	++	++
Reducing sugars	++	++	++	++	++	++
Saponins	++	++	-	+	++	+
Steroids	++	++	++	++	++	++
Terpenes	++	++	++	++	++	++
Tannins cathechics	+	++	+	-	++	++
Tannins gallics	+	++	+	-	++	++

A/B	*C.areloides*	*C.. afer*	*D.arborea*	*D.englerianum*	*E. hirta*	*G. kola*
Alkaloids	-	-	+++	-	-	-
Anthraquinones	-	-	-	-	-	-
Anthocyanins	-	-	-	-	-	-
Aminated compounds	+++	+++	++	-	++	++
Cardiotonic glycosides	-	-	-	-	-	-
Coumarins	-	-	-	-	-	-
Flavonoids	-	++	-	-	+++	+
Proanthocyanidins	-	++	++	++	++	++
Reducing sugars	-	-	++	++	+++	++
Saponins	-	-	++	++	++	++
Steroids	++	+	++	++	++	++
Terpenes	++	+	++	++	++	++
Tannins cathechics	-	++	++	++	++	++
Tannins gallics	-	++	++	++	++	++

A/B	*H. madagascariensis*	*J. curcas*	*J. insularis*	*M. indica*	*M. africana*
Alkaloids	+	-	-	-	-
Anthraquinones	-	-	-	-	-
Anthocyanins	-	-	-	-	-
Aminated compounds	++	-	++	+++	+++
Cardiotonic glycosides	-	-	-	-	-
Coumarins	-	++	-	-	-
Flavonoids	+	++	-	++	++
Proanthocyanidins	++	++	-	+++	++
Reducing sugars	+++	+++	++	+++	++
Saponins	+	+++	-	++	++
Steroids	++	+++	++	+++	++
Terpenes	++	+++	++	+++	++
Tannins cathechics	++	++	-	+++	++
Tannins gallics	++	++	-	+++	++

A/B	*N. latifolia*	*O. gore*	*P. brazzeana*	*P. macrophylla*	*P. dodecandra*	*P. guajava*
Alkaloids	++	-	-	-	-	-
Anthraquinones	-	-	-	-	-	-
Anthocyanins	-	-	-	-	-	-
Aminated compounds	++	++	+++	+++	+++	+++
Cardiotonic glycosides	-	-	-	-	-	-
Coumarins	-	-	-	++	++	-
Flavonoids	++	++	-	++	-	+++
Proanthocyanidins	++	+	+++	+++	++	-
Reducing sugars	++	++	+++	+++	++	++
Steroids	+++	++	+++	+++	+++	++
Saponins	+	-	++	++	++	++
Terpenes	+++	++	+++	++	+++	++
Tannins cathechics	++	+	+++	++	++	-
Tannins gallics	++	+	+++	++	++	-

A/B	*P. aquilinum*	*Q. africana*	*R. obscura*	*S. rhombifolia*	*T. poggei*	*T. diversifolia*
Alkaloids	-	++	++	++	++	-
Anthraquinones	-	-	+	-	-	-
Anthocyanins	-	-	-	-	-	-
Aminated compounds	++	++	++	++	-	+++
Cardiotonic glycosides	-	-	-	-	-	-
Coumarins	-	-	-	-	++	++
Flavonoids	-	-	-	+	++	++
Proanthocyanidins	+	++	++	++	++	++
Reducing sugars	+++	+++	+++	+++	++	++
Steroids	+++	+++	+++	+++	-	++
Terpenes	+++	+++	+++	+++	-	++
Tannins cathechics	+	++	++	++	++	++
Tannins gallics	+	++	++	++	++	++

A/B	*V. madiensis*	*V. africana*	*D. reflexa*	*H. acida*	*M. arboreus*
Alkaloids	-	+++	-	++	-
Anthraquinones	-	-	-	-	-
Anthocyanins	-	-	-	-	-
Aminated compounds	+++	+++	++	+++	++
Cardiotonc glycosides	-	-	-	-	-
Coumarins	-	-	-	+	-
Flavonoids	-	++	+	-	-
Proanthocyanidins	+	++	++	++	-
Reducing sugars	++	++	++	++	++
Saponins	-	+	+	++	-
Steroids	++	++	++	+++	++
Terpenes	++	++	++	+++	++
Tannins cathechics	+	++	++	++	-
Tannins gallics	+	++	++	++	-

D. arborea leaves, *H. madagascariens* stem bark, *Nauclea latifolia* leaves, *Quassai africana* root bark, *Rauwolfia obscura* root bark, *Sida rhomifolia* leaves, *Tetracera pogei* leaves, and Aminated compounds, flavonoids, tannins (gallics, cathechics and proanthocyanidins), steroids terpenes and reducing sugars were main phytochemicals detected in a wide range of aqueous extracts of more selected medicinal plants. Anthocyanins and cardiotonic glycosides were not detected in all plant extracts in our experimental conditions.

3.3. *In vitro* amoecidal activity of aqueous extracts from selected medicinal plant and their fractions

Minimal amoebicidal (MAC) and inhibitory concentrations (IC_{50}) of plants aqueous extracts and fractions are presented in Table 2. For good interpretation of these results, following criteria were adopted: MAC, IC_{50} < 10 µg/ml: pronounced activity, MAC, IC_{50} < 100 µg/ml good activity, 125 ≤ MAC, IC_{50} < 250 µg/ml: moderate activity, 250 ≤ MAC, IC_{50} < 500 µg/ml weak activity, MAC, IC_{50} ≥ 500 µ/ml: inactive. According to the level of their amoebicidal activity, the plant aqueous extracts can be divided into following groups:

The first group included extracts and fractions exhibiting pronounced amoebicidal activity with MAC and IC_{50} values < 10 µg/ml. It concerned aqueous extract of *Carica papaya* ripe seeds and its fraction F2 showing amoebicidal activity with MAC value of < 7.81 and 8.96 µg/ml and IC_{50} values of 3.20 and 5.62 µg/ml respectively. All samples from *C. papaya* ripe seed exhibited high activity compared to the same samples from *C. papaya* unripe seeds. The amoebicidal principles of seed extracts of this medicinal plant are known to be attributed to alkaloids named carpasemine and carpaine synthetized in high amount in ripe seeds than in unripe seeds (Etkin and Roos, 1982; Grandvaux, 1986). Aqueous extract of *Morinda morindoides* exhibited amoebicidal activity with MAC and IC_{50} values of < 7.81 and 3.15 µg/ml respectively and its fractions F2 rich in flavonoids displayed this activity with MAC and IC_{50} values of 9.25 and 6.52 µg/ml respectively. The amoebicidal activity of aqueous extract and fractions from *Morinda morindoides* leaves are due to the presence of isolated flavonoids such apigenin, quercetin, kaempferol, luteolin and their derivative glycosides, 7,4-quercetin dimethylether and chrysoeriol (Cimanga et al., 1995), and iridoids such as

Table 1: Minimal amoebicidal (MAC) and inhibitory (IC_{50}) concentrations of selected medicinal plant aqueous extracts and their fractions.

Extracts and fractions	UP	Preparation	MAC, µg/ml	IC_{50}, µg/ml
Alcornea cordifolia Rb	L	Decoction	125.00±0.02	75.2±0.01
		F1	62.50±0.03	24.50±0.03
		F2	31.25±0.12	21.14±0.09
		F3	125.00±0.11	80.30±0.05
		F4	62.50±0.06	35.20±0.01
	R	Decoction	62.50±0.03	29.57±0.08
		F1	125.00±0.11	72.03±0.06
		F2	31.25±0.02	18.65±0.04
		F3	125.00±0.06	80.24±0.08
		F4	31.25±0.13	22.15±0.11
Bridelia ferruginea	L	Macerate	62.50±0.05	27.80±0.03
		F1	125.00±0.13	62.30±0.09
		F2	62.50±0.02	45.30±0.01
		F3	125.00±0.11	75.14±0.07

		F4	31.25±0.07	23.24±0.02
*Cajanus caja*n	L	Decoction	>500	-
	R	Decoction	>500	-
Carica papaya	RS	Decoction	< 7.81	3.20±0.02
		F1	62.50±0.15	32.41±0.12
		F2	7.81±0.04	5.62±0.01
		F3	62.50±0.04	40.01±0.07
		F4	31.25±0.13	20.78±0.10
	US	Macerate	62.50±0.04	25.40±0.06
		F1	62.50±0.02	46.08±0.05
		F2	31.25±0.01	17.56±0.04
		F3	62.50±0.11	29.36±0.09
		F4	31.25±0.01	20.00±0.03
Cassia siamea	Sb	Decoction	125.00±0.12	80.60±0.05
		F1	125.00±0.02	75.60±0.05
		F2	62.50±0.13	37.80±0.08
		F3	125.00±0.02	82.30±0.05
		F4	62.50±0.05	40.35±0.08
Ceiba pentandra	Sb	Decoction	125.00±0.03	90.30±0.06
		F1	250.00±0.05	142.30±0.02
		F2	62.50±0.05	52.30±0.02
		F3	125.00±0.12	95.30±0.10
		F4	125.00±0.05	81.03±0.03
Cisius areloides	L	Decoction	>500	-
Costus afer		Jsb	>500	-
Datura aroborea	L	Decoction	125.00±0.06	67.50±0.03
		F1	62.50±0.02	42.30±0.05
		F2	31.25±0.04	23.50±0.07
		F3	125.00±0.12	75.20±0.09
		F4	62.50±0.01	36.50±0.03
Dialum englerianum	Sb	Decoction	62.50±0.06	28.25±0.02
		F1	125.00±0.09	85.30±0.07
		F2	31.25±0.01	25.74±0.03
		F3	125.00±0.06	75.20±0.04
		F4	31.25±0.02	29.60±0.05
Draceana reflexa	L	Decoction	62.50±0.11	24.30±0.09
		F1	62.50±0.01	53.50±0.04
		F2	31.25±0.06	27.20±0.04
		F3	125.00±0.10	70.50±0.08
		F4	31.25±0.02	25.60±0.04
Euphorbia hirta	Wp	Decoction	31.25±0.06	19.41±0.02
		F1	62.50±0.08	40.15±0.05
		F2	31.25±0.03	24.20±0.01
		F3	62.50±0.07	37.50±0.04
		F4	31.25±0.09	27.30±0.07
Garcinia kola	Sb	Decoction	125.00±0.12	72.03±0.10
		F1	125.00±0.01	80.50±0.03
		F2	62.50±0.08	48.20±0.02
		F3	250.00±0.11	163.51±0.10
		F4	62.50±0.05	42.60±0.03
Harugana madagascarensis	Sb	Decoction	62.50±0.01	40.16±0.04
		F1	125.00±0.02	82.30±0.04
		F2	62.50±0.05	39.20±0.03
		F3	125.00±0.13	73.14±0.10
		F4	62.50±0.03	43.26±0.05
Heinsia pulchella	Rb	Decoction	15.62±0.02	6.53±0.05
		F1	62.50±0.08	37.12±0.05

		F2	31.25±0.03	23.41±0.01
		F3	62.50±0.06	40.02±0.02
		F4	31.25±0.07	26.35±0.05
Hymenocardia acida	Sb	Decoction	31.25±0.04	22.36±0.01
		F1	62.50±0.06	41.20±0.04
		F2	15.62±0.01	9.12±0.02
		F3	62.50±0.05	32.50±0.03
		F4	31.25±0.06	18.75±0.02
Jatropha curcas	L	Decoction	31.25±0.12	20.51±0.10
		F1	62.50±0.10	35.20±0.08
		F2	31.25±0.02	19.50±0.04
		F3	125.00±0.03	65.25±0.06
		F4	31.25±0.05	22.30±0.02
Justicia insularis	L	Decoction	>500	-
Mangifera indica	Sb	Decoction	< 7.81	4.25±0.02
		F1	62.50±0.10	38.15±0.07
		F2	7..81±0.02	5.35±0.01
		F3	62.50±0.01	32.06±0.03
		F4	15.62±0.02	10.11±0.04
Maprounea africana	L	Decoction	62.50±0.05	36.25±0.02
		F1	125.00±0.10	52.36±0.07
		F2	62.50±0.03	38.25±0.05
		F3	125.00±0.02	42.31±0.04
		F4	62.50±0.01	40.15±0.03
Morinda morindoides		L	< 7.81	3.15±0.01
		F1	62.50±0.06	30.25±0.04
		F2	9.25±0.02	6.52±0.03
		F3	62.50±0.06	41.06±0.05
		F4	31.25±0.03	19.75±0.05
Morinda lucida	L	Decoction	62.50±0.03	25.41±0.07
		F1	62.50±0.08	40.57±0.02
		F2	31.25±0.05	23.60±0.04
		F3	62.50±0.02	36.24±0.03
		F4	31.25±0.06	26.78±0.02
Myrianthus arboreus.	L	Decoction	>500	-
Nauclea latifolia	L	Decoction	>500	-
	Rb	Decoction	125.00±0.12	84.30±0.10
		F1	250.00±0.11	172.39±0.09
		F2	62.50±0.05	42.12±0.03
		F3	250.00±0.02	152.6±0.04
		F4	62.50±0.06	37.92±0.08
Ongokea gore	Sb	Decoction	>500	-
Paropsia brazzeana	Rb	Decoction	< 7.81	4.56±0.03
		F1	62.50±0.02	32.01±0.05
		F2	15.62±0.03	6.52±0.01
		F3	125.00±0.09	65.32±0.03
		F4	31.25±0.05	19.54±0.02
Pentaclethra macrophyla	Sb	Decoction	250.00±0.10	162.50±0.13
		F1	250.00±0.09	185.00±0.07
		F2	125.00±0.03	115.05±0.06
		F3	250.00±0.05	175.00±0.06
		F4	125.00±0.03	96.25±0.06
Phytolacca dodecandra	L	Decoction	>500	-
Psidium guajava	L	Decoction	62.50±0.02	25.65±0.05
		F1	62.50±0.06	32.04±0.03
		F2	31.25±0.04	17.25±0.02
		F3	125.00±0.02	78.25±0.05
		F4	31.25±0.07	15.68±0.05

	Sb	Decoction	< 7.81	4.15±0.02
		F1	62.50±0.07	35.12±0.04
		F2	8.65±0.02	5.66±0.03
		F3	62.25±0.09	41.08±0.05
		F4	15.75±0.03	9.07±0.05
Pteridium aquilinum	Sb	Decoction	>500	-
Quassia africana	Rb	Decoction	31.25±0.02	20.06±0.06
		F1	62.50±0.01	35.21±0.05
		F2	31.25±0.03	16.52±0.07
		F3	125.00±0.08	70.05±0.06
Rauwolfia obscura	Rb	Decoction	31.25±0.05	19.25±0.03
		F1	62.25±0.08	35.21±0.04
		F2	31.25±0.03	17.33±0.05
		F3	125.00±0.10	67.25±0.03
		F4	31.25±0.01	19.56±0.04
Sida rhombifolia	L	Decoction	62.50±0.03	32.04±0.05
		F1	125.00±0.01	62.54±0.05
		F2	31.25±0.03	22.14±0.02
		F3	125.00±0.12	75.06±0.09
		F4	31.25±0.05	28.65±0.02
Tetracera poggei	L	Decoction	>500	-
Tithonia diversifolia	L	Decoction	62.50±0.06	26.50±0.01
		F1	62.50±0.03	35.74±0.06
		F2	32.25±0.01	24.89±0.03
		F3	125.00±0.09	69.25±0.06
		F4	31.25±0.02	26.78±0.05
Vitex madiensis	L	Decoction	>500	-
Vocanga africana	Rb	Decoction	62.50±0.03	21.03±0.01
		F1	125.00±0.11	79.05±0.09
		F2	62.50±0.08	35.07±0.06
		F3	250.00±0.01	165.35±0.03
		F4	62.5±0.05	38.50±0.07

Gaertneroside, acetymgaertneroside, gaertneric acid, acteylgaertneroside methoxygaertneroside and epoxygaertneroside (Cimanga et al., 2006a). Flavonoid aglycones were more active compared to their respective glycosides (Cimanga et al., 2006,b). Iridoids were reported to exhibit high amoebicidal activity ($1.3 < IC_{50} < 7.5$ µg/ml) compared to flavonoids ($64 < IC_{50} < 121$ µg/ml) (Cimanga el al., 2006 a,b) suggesting that iridoids are responsible of the amoebical activity of aqueous extract of *M. morindoides* leaves. Aqueous extract of *Psidium guajava* stem bark exhibited amoebicidal activity with MAC and IC_{50} values of < 7.81 and 4.15 µg/ml respectively and its fraction F2 showed this activity with MAC and IC_{50} values of 8.65 and 5.66 µg/ml respectively. Its amoebicidal activity was due to the presence of its flavonoid content for the same reasons evoked before. Aqueous extract of *Mangifera indica* stem bark and *Paropsia brazzeana* root bark displayed amoebicidal activity with MAC and IC_{50} values of < 7.81 and 4.25, and < 7.8 ad 4.56±0.03 µg/ml respectively with their fractions F2 riche in flavonoids exerting this activity with MAC and IC_{50} values of 7.81±0.02 and 5.35±0.01 and 15.62±0.03 and 6.52±0.01 µg/ml respectively.

The second group included aqueous extracts and their fractions exhibiting amoebicidal activity with MAC and IC_{50} < 100 µg/mg. Aqueous extract and fractions from *Euphorbia hirta* for which it amoebicidal activity was attributed to the presence of a crystalline substance named substance E showing similar properties to those of choline (Krishna-Rao and Ganapaty, 1983). In addition, this medicinal plant also contains flavonoids such as quercetin, rutin and quercetrin (Bakana, 1983) which can be considered also as responsible for its amoebicidal activity since their amoebicidal was previously reported (Cimanga et al., 2006a). The amoebicidal activity of aqueous extract and fractions from *Quassia africa* root bark was attributed to the presence of quassin reported firstly to exhibit pronounced amoebicidal activity (Keene et al., 1986), but this compound was found after to display weak activity (Phillipson et al., 1995).

Compounds such as atropine and scopolamine isolated from *Datura arborea* leaves were reported to be inactive against *E. histolytica* (Keene et al., 1987), thus the activity of this medicinal plant are probably due the presence of other alkaloids or other constituents not yet identified. Other samples exhibiting good amoebicidal activity in our biological screening with MAC and IC_{50} <

100 µg/ml included aqueous extracts and fractions from *Carica papaya* unripe seeds, *Dracena reflexa* leaves, *Heinsia pulchella* root bak, *Hymenocardia acida* stem bark, *Jathropha curcas* leaves, *Morinda lucida* and *Tithonia diversifolia* leaves, and *Psidium guayava* leaves for which its amoebicidal activity may be due to the presence of its flavonoid content such as quercetin and its derivative glycosides (Lutterdot, 1989, Luzoya et al., 1994), *Rauwolfia obsura* and *Vocanga africana* root bark for which their amoebicidal activities may be due to their alkaloid contents since some alkaloids from medicinal plants belonging to the Apocynaceae family such as *Alstonia angustifolia* roots were previously reported to exhibit pronounced amoebicidal activity *in vitro* (Wright et al., 1992). The amoebicidal activity of aqueous extract of *Sida rhombifolia* leaves is due to the presence of the alkaloid cryptolepine.

For these plants extracts, theirs different soluble fractions displayed amoebicidal activity with MAC and IC_{50} values < 100 µg/ml and showed good amoebicidal activity. In general, the most active fraction for each plant extract is the fraction F2 rich in flavonoids followed by fraction F4 rich in other polyphenolic compounds than flavonoids. Thus the observed amoebicidal activity observed may be due their flavonoids and other polyphenolics content. Fractions 1 and F3 rich in steroids and terpenes, and saponins respectively also showed good amoebicidal activity and largely contribute to the manifestation of the evaluated activity since some isolated compounds belonging to these phytochemical groups were previously reported to exhibit *in vitro* amoebicidal activity (Sharma and Bhutani, 1987; Alani et al., 2003).

The third group included plant extracts exhibiting the evaluated activity with MAC = 125 or 250 µg/ml and their effect was considered as moderate. In concerned aqueous extracts from aqueous extract from *Alchornea cordifolia* leaves, *Casssia siamea* stem bark, *Ceiba pentandra* stem bark, *Datura arborea* leaves, *Garcinia kola* stem bark, *Nauclea latifolia* root bark and *Pentaclethra macrophylla*. Interesting, by calculating their IC_{50} values, it was observed that these extracts inhibited the growth of *E. histolytica* with IC_{50} values < 100 µg/ml. This last observation is also valid for more of these soluble fractions with factions F2 as the most active than others.

All soluble fractions of these aqueous extracts from medicinal plants cited above displayed good antiamoebic activity with Mac and IC_{50} < 100 µg/ml (Table 1) with the fraction F2 as the most active sample. Based on previous studies of reported isolated antiamoebic constituents in medicinal plants, it was observed that the antiamoebic activity of selected medicinal plants in the present study is due to present of alkaloids, flavonoids, steroids, terpenes and tannins identified in these extracts and fractions and previously reported to displayed amoebicidal activity with different magnitudes (Ahmed et al, 1966; Cedeno et al., 1987; Sharma and Bhutani, 1987; Wright et al., 1994; Marshall et al, 1994; Calzada et al, 1999; Alani et al., 2003, Cimanga et al., 2006b).

The last group included nine aqueous extracts devoid with amoebicidal activity at the highest tested concentration of 500 µg/ml. They included aqueous extract from *Cajanus cajan* leaves and roots, *Costus afer* juice stem bark, *Cissius areloides*, *Justicia insularis*, *Nauclea latifolia*, *Phytolacca dodecandra*, *Pteridium aquilinum*, *Vitex madiensis* leaves and. Our results are in good agreement with Tona et al., (2000) concerning the amoebicidal activity of aqueous extracts of these selected medicinal plants, but in their work any fraction from these aqueous extracts were not tested and the IC_{50} values of both samples were not reported.

4. CONCLUSION

This biological investigation on the amoebicidal activity of 40 aqueous extracts and 120 fractions had lead to the discovery five aqueous extracts and their fractions with exhibiting pronounced amoebicidal activity against *E. histolytica* with MAC and IC_{50} values < 10 µg/ml. It concerned aqueous extract from *C. papaya* ripe seeds, *M. indica* stem bark, *M. morindoides* leaves, *P. brazzeana* root bark and *P. guajava* stem bark. For these aqueous extracts, active principles for this activity are well known as described above. At this group, it could be add twenty aqueous extracts who had shown good amoebicidal activity with MAC and IC_{50} < 100 µg/ml. All fractions of these aqueous extracts exhibited also good amoebicidal activity with the fraction F2 rich in flavonoids as the most active. Thus, it could speculated that flavonoids have contributed actively in the manifestation of this evaluated activity and some time they react in a synergistic manner with other constituents present in the extracts. Seven aqueous extracts were found to exhibit amoebicidal activity with MAC values of 125 µg/ml, and IC_{50} values < 100 µg/ml. but their respective soluble fractions exhibit this activity with MAC and IC_{50} < 100 µg/ml suggesting that a pre-purification of crude extracts can enhance a biological activity as observed for all aqueous extract in the present study. They included aqueous extract from and *D. arborea* leaves, *C. siamea*, *C. pentandra* and *G. kola* stem bark, and *N. latifolia* root bark. Further extensive phytochemical studies are need for the isolation and structure elucidation of active constituents in aqueous extracts with good amoebicidal activity. In general the amoebicidal activity displayed par these selected medicinal plant can partly support and justify their current use for the treatment of diarrhoea and dysentery in traditional medicine in Kinshasa-Democratic Republic of Congo and other African countries.

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