Research Journal of Applied Sciences 3 (7): 514-520, 2008
ISSN: 1815-932X
© Medwell Journals, 2008

Chemical Study of the Stems of *Urtica massaica*, a Medicinal Plant Eaten by Mountain Gorillas (*Gorilla beringei beringei)* in Parc National des Volcans, Rwanda

[1]Nahayo Alphonse, [2]M.J. Bigendako, [3]K. Fawcett, [4]H. Nkusi, [5]J.B. Nkurikiyimfura and [6]Yansheng Gu
[1]School of Environmental Studies, China University of Geosciences,
Hubei, 388 Lumo Road, 430074, Wuchang, P.R. China
[2]Director of Pharmacopoeia Center, Institute of Scientific and Technological Research,
IRST, P.O. Box 227, Butare, Rwanda
[3]Director of Karisoke Research Center, P.O. Box 105, Ruhengeri, Rwanda
[4]Pharmacopoeia Center, Institute of Scientific and Technological Research,
IRST, P.O. Box 227, Butare, Rwanda
[5]National University of Rwanda, Faculty of Medicine, P.O. Box 117, Butare, Rwanda
[6]School of Environmental Studies, China University of Geosciences,
Hubei, 388 Lumo Road, 430074, Wuchang, P.R. China

Abstract: This study aimed, at finding out different active components contained in the stems of *Urtica massaica*, a plant eaten by mountain gorillas in Parc National des Volcans (PNV) and used in rwandan traditional medicine to treat diarrhea in humans. Among, the medicinal plants eaten by mountain gorillas (*Gorilla beringei beringei*), the stems of *Urtica massaica* have been chosen to undergo phytochemical tests, those stems are used in humans to treat diarrhea, a disease also found in mountain gorillas. The phytochemical screening showed that the stems of *Urtica massaica* contain tannins in great quantity but terpenes-sterols, saponosides, flavonoids and anthocyanes are in small quantity. The steps of separation on the thin layer chromatography and the column chromatography have been done successively on the hexanic, chloroformic and hydromethanolic extracts of that plant. During the bacteriological test, the extracts and the hydromethanolic fractions of *Urtica massaica* have given satisfactory activities against the enteropathogenes (*Salmonella* B, *Shigella flexneri* and *Escherichia coli)* able to induce bacterial diarrhea.

Key words: *Urtica massaica*, *Gorilla beringei beringei*, parc national des volcans, phytochemical screening, thin layer and column chromatography, bacterial diarrhea

INTRODUCTION

The Park National des Volcans (PNV) extending to the North-West of Rwanda (in the Northern Province) is contiguous with Virunga National Park in Democratic republic of Congo and Mgahinga Gorilla National Park in Uganda. The vegetation of the PNV plays ecological, economic and scientific roles (Plumptre *et al*., 2004). From the ecological point of view, this vegetation takes part not only in the protection against soil erosion but also contributes to the formation of the cloud giving place to precipitations. Moreover, certain plant species of the PNV are eaten by various animals of the park like mountain gorillas (*Gorilla beringei beringei*), buffaloes, elephants, golden monkeys, etc. From an economic and scientific point of view, certain plants of the PNV constitute a primary source of the traditional medicine practiced by the surrounding traditional practitioners. As the mountain gorillas are primarily vegetarians (Robbins *et al*., 2005), their food mode includes stems, roots, sheets, flowers, barks and fruits. According to that last author, the gorillas are selective in the choice of their food. This choice relates in particular to the plants and the parts of the plants highly rich in food value (proteins, carbohydrates, mineral salt). However, the same authors add that the mountain gorillas eat occasionally certain invertebrates like the ants and the caterpillars. According to, the information provided by the guides of the PNV, certain plants consumed by the mountain gorillas are also used in traditional medicine by the practitioners of the surrounding area.

Corresponding Author: Nahayo Alphonse, School of Environmental Studies, China University of Geosciences, Hubei, Wuchang, 388 Lumo Road, 430074, P.R. China

In the PNV, certain plants consumed by the mountain gorillas are supposed to have medicinal properties. According to Bruneton (1999), the medicinal plants contain some active ingredients with therapeutic properties. Sometimes, the mountain gorillas suffer from certain diseases like diarrhea, pneumonia, cough and scale. These diseases can be transmitted to the mountain gorillas by the tourists, the researchers, the guards, the other contagious animals like the monkeys, the cows, the local population, etc. (Homsy, 1999). This study aims at seeking the active substances contained in some plant species (the stems of *Urtica massaica*) consumed by the mountain gorillas in the PNV, but also used in traditional medicine. The active ingredients that they contain would make it possible to acquire the capacity of self-medication. The s pecific objectives are the following: to detect by phytochemical screening and the chromatography, some biologically active substances contained in that plant; to prove the effectiveness of some extracts and certain fractions with respect to some pathogenic microbes, by determining their Inhibiting Minimal Concentration (IMC). The assumption of this study is that the stems of *Urtica massaica*, a plant consumed by the mountain gorillas in the PNV, also used in Rwandan traditional medicine by the practitioners of the surrounding area could contain substances with bacteriostatic activity.

MATERIALS AND METHODS

Choice of the plant (stems of *Urtica massaica*) to be collected for chemical analysis. The choice is related to the medicinal plant, which is both found in the PNV and the fields of traditional practitioners. Moreover, the parts of that plant, are not only consumed by the mountain gorillas in PNV, but also used (nonassociated with the others) in traditional medicine by the surrounding traditional practitioners to treat some diseases both found in humans and the mountain gorillas.

All the collected parts, were washed with tepid water and then dried; after crushing, one hundred grams of the powder of the stems of *Urtica massaica* were filtered to obtain a fine powder having been used for phytochemical screening.

The chemical substances to be detected through phytochemical screening are the alkaloids, the anthocyanes, the flavonoides, the quinones, the saponosides, the terpene-sterols and the tannins. The procedure followed in this study was proposed by Bruneton (1999) and Rwangabo (1993).

To test alkaloids, one proceeds in the following way: to weigh 5 g of dry powder; to place in a percolator and to add 5 mL of hydrochloric acid 10%; to let macerate during 24 h; to filter and collect 3 mL in a test tube; to, respectively add to the filtrate some drops of the reagents of Dragendorff, Mayer and Wagner.

The appearance of a precipitate indicates the presence of alkaloids; the results are marked as follows: -: Absence of precipitate (absence of alkaloids):

(+) : Light opalescence (alkaloids in traces).
\+ : Light precipitate (alkaloids in small quantity).
++ : Precipitate in suspension (presence of easily detectable alkaloids).
+++ : Important precipitate with immediate flocculation (alkaloids in significant amount).

To test the anthocyanes, one proceeds in the following way: to place 2 g of dry powder in a percolator of 50 mL, to add 2 mL of methanol and to let percolate during 24 h; to collect 1 mL in a test tube, to add some ethyl ether drops; to observe the precipitate at the bottom of the test tube. The results are marked as follows: absence of the precipitates (absence of the anthocyanes); (±): small precipitate (anthocyanes in traces) +: Important precipitate (detectable anthocyanes).

Test of flavonoids: to weigh 5 g of dry powder; to place in a percolator and to add 50 mL of distilled water; to let macerate during 24 h; to filter and collect 3 mL in a test tube and to add to the filtrate of methanol, distilled water, hydrochloric acid 10% in the proportions (1: 1: 1); to add a mixture 0.5 g of Magnesium. The solution is colored in red in the presence of flavonol, orange in the presence of flavone and violet in the presence of flavanone. The results are marked as follows: (+): Weak coloring; +: Frank coloring; ++: Intense coloring.

To test quinines: one proceeds in the following way: to moisten during a few moments 2 g of dry powder with a hydrochloric acid 10%; to let macerate during 24 h in 50 mL of the chloroform mixture-ether (75: 25); to filter; 1 mL of the filtrate is treated with 1 mL of soda 10%. A red coloring or violet announces the presence of quinines; The results are noted as follows: (+): Weak positive reaction; +: Strong positive reaction; -: Negative reaction.

The test of the saponosides is done as follows: to place 20 mg of dry powder in a test tube; to add distilled water and to agitate gently; to observe and measure the height of persistent foam after 15-20 min rest. The results are marked as follows: (+): For a height of foam of 0.0-0.5 cm; +: For a height of foam of 0.5-1 cm; +: Followed by arab numeral () indicating the number of cm if the height exceeds 1cm (strongly positive reaction).

The test of tannins is done in the following way: to place 2 g of dry powder in a test tube; to add distilled

water or methanol then some drops of the iron trichloride 5%; to observe the presence of the precipitate; The results are marked as follows: (+): Disorder; +: Weak precipitate; ++: Strong precipitate.

The test of terpenes sterols is done in the following way: We used the method of Liebermann-Burchard as follows: In a percolator, to deposit 1 g of dry powder and to let macerate during 24 h in 20 mL of diethyl ether; to agitate from time to time; After filtration, to evaporate solvent; the residue is then dissolved in some acetic anhydride drops; to add 2-3 drops of concentrated sulphuric acid; In the presence of terpene-sterols, the sulphuric acid addition produces a mauve color, which transfers with the green; the results are noted in the following way: (+): Weak color; +: Frank color; ++: Intense color.

Preparation of the rough extracts: Three solvents of different polarities (by ascending order) were used for the extraction: There are hexane, chloroform and methanol/water.

Hexanic extract: To separately weigh 400 g of the powder of *Urtica massaica*; to macerate in a percolator with hexane (1600 mL) during 24 h; to filter on a pads plug; to evaporate solvent under reduced pressure using the rotary evaporator at the temperature of 40°C; to dry the filtrate of the sample in the drying oven at 40°C; dry hexanic extracts of 1.8 g were obtained; chloroformic extract: the marc of hexanic extract of the sample is macerated in 1400 mL of chloroform during 24 h; to filter macerated on a pads plug; to concentrate the filtrate with rotary evaporator at 40°C; to dry the filtrate of the sample in the drying oven at 40°C; chloroformic dry extracts of 1.6 g were obtained; hydromethanolic extract: The marc of chloroformic extract of the sample is macerated in 1200 mL of methanol/water: 7/3 during 24 h; to filter the macerated sample with a pads plug; to concentrate the filtrate with a rotary evaporator at 40°C; to dry the filtrate of the sample in the drying oven at 40°C; dry hydromethanolic extracts of 1.5 g were obtained.

Thin layer chromatography: Thin layer chromatography was carried out according to the following stages (Chavanne *et al*., 1991): After washing the tank and rinsing, we poured the mobile phase made up of: Heptane/benzene/ethylacetate (EA): 3/3/4 for hexanic extract; ether of petrol/EA: 7/3 for the chloroformic extract; dichloromethane/EA: 6/4 was used for hydromethanolic extract.

Development tank preparation: To pour 10 mL of the system of eluant adapted in the developing tank until a 0.5 cm height from the bottom of the tank. To wait for 10 min to allow saturation in atmosphere of the tank room by the solvent of the mobile phase.

Chromatographic plate preparation: For the stationary phase, to use the induced aluminium foils of silicagel 60 F254 of 12×5 cm. By using the capillary tubes, to deposit approximately 0.5 μL solution in a point located at 1 cm of the lower end of the plate. Chromatogram development: To place the plate in the tank in oblique position and to close again the container. When the face of solvent reaches approximately 0.5 cm of the higher end of the plate, to withdraw the latter and to locate the position of the aqueous solution using a pencil.

Revelation and calculation of the Rf: After having withdrawn the plate from the tank, to dry it with the free air. The spots were observed under UV lamp at 254 nm. Once identified spots, we calculated their Rf (rotating factor) by using the following formula: Rf: d1/d2 with d1: distance covered by the substance on the chromatographic plate (measured starting from the center of the spot); d2: distance covered by solvent (since, the starting line until the face).

Column chromatography: The column chromatography was carried out according to the following stages (Chavanne *et al*., 1991): Preparation of the column according to the method of filling by wet process. To fix the column in driving position using the stand. To prepare in a beaker a homogenized mixture of the adsorbent (stationary phase: silica gel Merk 60) and the least polar of two solvents of different polarities for the development. To make a sufficient fluid pulp being able to run easily (approximately 50 g of the adsorbent per gram of sample). The preparation of the mixture must be done by adding per minor amount at the same time, the adsorbent in solvent. To pour the pulp in the column until having a layer of approximately 2 cm. To open the tap so that the solvent runs slowly. While, the particles of the adsorbent in suspension settle slowly, to shake the walls of the column to support maximum compression. The addition of the pulp homogenized by successive portions continues while, shaking the walls of the column. This one is ready to be used if there is neither crack nor bubbles of air. The surface layer is perfectly horizontal. One must make sure, during elution, that the surface of the adsorbent is always covered with solvent and that it is never in contact with the air.

Deposit of the sample: Once the column is ready, to deposit the sample on the surface of the adsorbent by using a capillary pipette in order to form a uniform layer. To add an eluant till 10 cm height.

Development of the chromatogram: To adjust the flow in bottom of the column at intervals from 5-50 drops per minute. When the volume to be eluated becomes 100 mL, to change eluant by using the mixture of solvents by ascending polarity order. If the zones obtained are coloured, to join together the fractions corresponding to the same zone. If the zones are not coloured, to collect the fractions of constant volume (10 mL).

These various fractions were subjected to thin layer chromatography in order to see whether they corresponded each one to a group of homogeneous product.

Bacteriological tests: The bacteriological test consisted in testing the antibacterial activity of the stems of *Urtica massaica*. We tested the sensitivity of some enterobacteria (*Escherichia coli*, *Salmonella* B, *Shigella flexneri*) to the extracts and the hydromethanolic fractions.

Test of bacteriostasis: The test of bacteriostasis consists in determining the Inhibiting Minimal Concentration (IMC). The method adopted for the test of bacteriostasis is that of dilution in solid medium. This test was done on two samples: the total hydromethanolic extract and 2 hydromethanolic fractions obtained on column. Test of bacteriostasis of the total hydromethanolic extract. The test of bacteriostasis includes the following stages: To have a series of Petri box and to mark them; the box n°1 contains only the culture medium Mueller-Hinton (M-H); the box n°2 contains the culture medium M-H and the bacteria, the other boxes contain the extract, the culture medium M-H and the bacteria; to prepare dilutions of solutions of total hydromethanolic extract by carrying out the increasing concentrations according to the geometric progression of reason two (example: 51.2, 25.6, 12.8, 6.4, 3.2, 1.6, 0.8, 0.4 and 0.2 mg mL^{-1}); To take 1 mL of each dilution and incorporate them in the Petri boxes (a box by dilution) containing 10 mL of medium M-H liquified at 45°C; to let cool; to sow using a platinum handle, some bacteria on the surface of each Petri box; to let incubate during 24 h at 37°C; to make the reading: to observe the presence or the absence of the colonies on the boxes. Test of bacteriostasis of the hydromethanolic fractions: To make this test, we followed the same stages as those used for the test of bacteriostasis of the total hydromethanolic extract (Table 1).

Table 1: Phytochemical screening

	Powder	Hexanic extract	Chloroformic extract	Hydromethanolic extract
Alkaloids	-	-	-	-
Anthocyanes	(+)	-	-	(±)
Flavonoids	(+)	-	-	(+)
Quinones	-	-	-	-
Saponosides	+	-	(+)	(+)
Terpene sterols	(+)	-	(+)	-
Tanins	++	-	-	++

Source: Lab 2008

RESULTS

The powder of the stems of *Urtica massaica* contains tannins in great quantity whereas terpenes-sterols, the saponosides, the flavonoïdes and the anthocyanes are in small quantity. The quinones and the alkaloids are absent.

Thin layer chromatography: Only one product of hexanic extract of Urtica massaica was obtained using an eluant heptane/benzène/EA (3/3/4). The chloroformic extract showed three products in ether of petrol/EA (7/3) whereas hydromethanolic extract with dichloromethane/EA (6/4) gave only 2 products.

Column chromatography: The following photographs show the fractions collected in various test tubes starting from hexanic extracts (left), chloroformic (middle) and hydromethanolic extracts (right) (Fig. 1a-c).

Test of bacteriostasis of the total hydromethanolic extract: In order to determine, the IMC of total hydromethanolic extract, we used three series of increasing concentrations according to a geometric progression of reason two. The results are in the following Table 2.

The results of Table 2 show that the IMC of the total hydromethanolic extract are of 38.4 mg mL^{-1} (found with the second series) for *E. coli* and *S. flexneri*. It is 22.4 mg mL^{-1} (found with the third series) for

Table 2: Values of IMC (mg mL^{-1}) of the total hydromethanolic extract

Stock of bacteria	Series 1	Series 2	Series 3
E. coli	51.2	38.4	44.8
Salmonella B	25.6	38.4	22.4
S. flexneri	51.2	38.4	44.8

Source: Lab 2008

Table 3: Values of IMC (μg mL^{-1}) of the hydromethanolic fractions

Stock of bacteria	Series 1		Series 2	
	Fraction 1	Fraction 2	Fraction 1	Fraction 2
E. coli	51.2	25.6	38.4	38.4
Salmonella B	51.2	25.6	38.4	19.2
S. flexneri	51.2	25.6	38.4	38.4

Source: Lab 2008

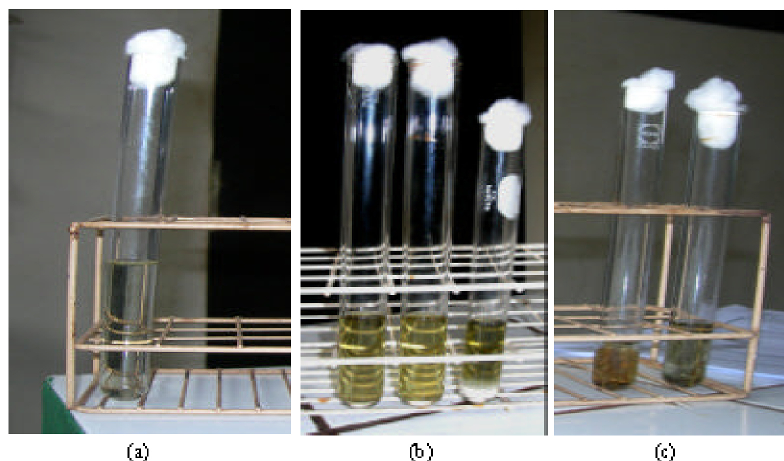

Fig. 1: a) Hexanic extract, b): Chloroformic extract and c) Hydromethanolic extract, Source: Lab 2008

Salmonella B. In short, the total hydromethanolic extract is active against the growth of *E. coli* and *S. flexneri*, more active against that of *Salmonella B*.

The results of the Table 3 show that the IMC of the first hydromethanolic fraction are of 38.4 μg mL^{-1} (found with the second series) for three bacterial strains. Concerning the second fraction, *Salmonella B* is more sensitive (IMC of 19.2 μg mL^{-1}) to the extract than two other bacterial strains, hence the second fraction is more active than the first.

DISCUSSION

The results of Table 1 show that certain groups of products dissolve easily in polar solvents, others in fairly polar or non-polar solvents (Chavanne *et al*., 1991). Then, the tannins, the flavonoïdes and the quinones are soluble in polar solvents (methanol and water) whereas terpenes-sterols are extracted by chloroform (fairly polar solvent). This corresponds to the theory according to which these latter compounds are extracted by solvents allowing the degreasing like chloroform. The presence of tannins in the stems of *Urtica massaica* justifies their use in traditional medicine to fight against diarrhoea because, according to (Hurabielle *et al*., 1981), the tannins have astringent, antibacterial and antidiarrheic properties. The latter were met in the hydromethanolic extracts because, according to (Bruneton, 1999), the extraction of tannins often calls upon hydromethanolic solutions. As the extract and the hydromethanolic fractions of the stems of *Urtica massaica* were active against the bacteria responsible for the diarrhoea, the aforementioned plant would protect the mountain gorillas against bacterial diarrhoea. The hydromethanolic fraction has a great activity in comparison to the corresponding extracts (respective IMC of 19.2 μg mL^{-1} and 22.4 mg mL^{-1}). The fractionation of the rough hydromethanolic extracts thus, led to active fractions justified by the spacing of the elements, which would have an allosteric effect on the starting molecule and this effect would amplify the activity of the molecule deprived of the impurities retained during the fractionation.

The traditional practitioners prepare their extracts with warm water and thus, obtain a decoction in polar matter. The good performances, which they obtain among their patients go in the same direction as those obtained *in vitro* with the polar extracts (hydromethanolic). The antibacteria are characterized by their way of acting in a quite precise site of the bacterial cell. According to the antibacteria, this site can be the bacterial wall, the cytoplasmic membrane or the machinery of synthesis of the proteins (Singleton, 2004). Then, the antibacterial activity of the extract and the hydromethanolic fractions of *Urtica massaica* in the mountain gorillas could appear by one of these mechanisms. Moreover, the same plant could also present an antibacterial activity by acting on the intestinal hypermotricity while, playing on the receivers of the intestinal muscles. By comparing these results with those found by Anastasiah (2000), the methanolic extract of the sheets of *Urtica massaica* was inactive against soil mushrooms of the type *Fusarium oxysporum*. However, according to our results, the hydromethanolic extracts of the stems of *Urtica massaica* as its two fractions were active against some enterobacteria like *Salmonella B*, *Shigella flexneri* and *Escherichia coli* with different concentrations. In addition, we cannot say that the methanolic extract of the sheets of *Urtica massaica* does not have any therapeutic value. Indeed, as the sheets of *Urtica massaica* are also used by the traditional practitioners to treat the diarrhoea,

a disease being able to be caused by the bacteria, these sheets could have an activity on other pathogenic microbes except *Fusarium oxysporum*.

The mountain gorillas seem to tolerate some species of gastrointestinal parasites without expressing signs of diseases. Because of the close genetic relations between humans and gorillas, it is possible that the humans transmit diseases to them once they are in contact (Jonathan *et al*., 2000). Some following examinations proved it: the test of laboratory carried out on droppings of the mountain gorillas suffering from diarrhoea showed that normal droppings contained more eggs of intestinal parasites than liquid droppings. Mucus and blood covering droppings presented a great quantity of eggs of parasites (Hastings *et al*., 1992). The results of this examination showed 9 helminths, namely *Anoplocephala gorillae*, *Impalaia* sp., *Trichostrongylus* sp., *Murshidia devians*, *Oesophagostomum* sp., *Probstmayria gorillae*, *Capillarie hepatica* and 2 nematodes not identified. Moreover, 5 following protozoa were identified: *Entamoeba coli*, *Entamoeba histolytica*, *Entamoeba hartimanii*, *Iodamoeba buetschlii* and *Giardia lamblia*. The same author specifies that the number of eggs contained in droppings depends on the collected sample size. Moreover, droppings of the adult gorillas accustomed to the tourists and the researchers, particularly those of the silver backs showed a great load in intestinal worms than those of the young gorillas. These data thus, suggest that the load in intestinal worms in the mountain gorillas varies according to the age and the contacts with the humans. The same study made in 1996-1997 in Rwanda, by the Mountain Gorilla Veterinary Project and the researchers of University of Tennessee, led to the following results: three intestinal parasites as *Trichuris trichiura*, *Chilomastix* sp. and *Endolimax nana*, were identified in droppings of the accustomed mountain gorillas. These same parasites infect also humans (Jonathan *et al*., 2000). In 2004, the examination of the saddles, realized on 2000 people living around the Virunga National park, showed that 96.4% had an infection of some parasites, especially the intestinal amoebas and worms. Moreover, on 49 samples of droppings collected in 5 families of gorillas living in this park, 92% presented the same parasites as those found in these people. However, the examination of droppings of the gorillas living very far from the villages and, not having known a contact with the humans, presented less parasites in general. Thus, these results show us that taking into account the close genetic relation existing between the humans and the gorillas (Hacia, 2001), there is a similarity of diseases affecting the humans and the gorillas as well as the transmission risk between them. So, the plants having therapeutic properties in humans can also have the same properties in the mountain gorillas.

Thus, the medicinal plants consumed by the mountain gorillas enable them to acquire the capacity of self-medication. In particular, according to the results of these bacteriological tests, the stems of *Urtica massaica* enable them to be protected from the bacterial diarrhoea.

CONCLUSION

The phytochemical screening carried out on the powder of the stems of *Urtica massaica* revealed the presence of four groups of products. The hydromethanolic extracts revealed the tannins in great quantity. The flavonoids are slightly represented. Thin layer chromatography allowed having an idea on the complexity of chemical substances contained in the sample and knowing the solvent systems being able to allow a better separation on the column chromatography.

The total extract and two hydromethanolic fractions of *Urtica massaica* were used for the bacteriological tests (by using certain bacteria responsible for the diarrhoea like *Escherichia coli*, *Salmonella* B and *Shigella flexneri*). The results proved that the total extract has an antibacterial capacity. Indeed, this extract was active against the growth of *E. coli*, *Salmonella* B and *S. flexneri* with the values of the IMC, respectively of 38.4, 22.4 and 38.4 mg mL^{-1}. Thus, the total hydromethanolic extract is active against the growth of *E. coli* and *S. flexneri*, much more active against that of *Salmonella B*. Concerning two fractions, the second is more active than the first. Indeed, *Salmonella* B is more sensitive to the fraction (IMC of 19.2 μg mL^{-1}) than 2 other bacterial strains (IMC of 25.6 μg mL^{-1}). Thus, because of the presence of tannins (having astringent and antibacterial properties) in the stems of *Urtica massaica*, their effectiveness for the treatment of the bacterial diarrhea is proven.

ACKNOLEDGEMENT

I would like to acknowledge the financial support provided by Karisoke Research Center for data collection and analysis and Institute of scientific and technological research (IRST) for lab analysis.

REFERENCES

Anastasiah, N. Ngigi and Paul K. Ndalut, 2000. Evaluation of natural products as possible alternatives to methyl Bromide in soil fumigation. Conference proceedings: 2000 annual International Research Conference on Methyl Bromide Alternatives and Emissions Reduction, Moi University, Eldoret, Kenya, pp: 4-5.

Bruneton, J., 1999. Pharmacognosy. Phytochemistry, Medicinal Plants. 2nd Edn. Agricell Reports, P.O. Box 255, Schrub Oak, N.Y. 10588, USA, pp: 1131.

Chavanne, M., A. Jullien, G.J. Beaudouin and E. Flamand, 1991. Experimental Organic Chemistry. 2nd Edn. Belin, Mont-Royal (Québec), pp: 901.

Hacia, J.G., 2001. Genome of the Apes the Institute for Genetic Medicine, University of Southern California, 2250 Alcazar Street, IGM 240, Los Angeles, CA 90089, USA, 17 (11): 637- 645.

Hastings, B.E., L.M. Gibbons and J.E. Williams, 1992. Parasites of free-ranging mountain gorillas: Survey and epidemiological factors. Proc. Am. Assoc. Zoo. Vet. Am. Assoc. Wildl Vet., pp: 301-302.

Homsy, J., 1999. Ape tourism and human diseases: How close should we get? A critical review and regulations governing park management and tourism for the wild mountain gorilla, *Gorilla gorilla beringei*; report of a consultancy for the International Gorilla Conservation Programme, pp: 86.

Hurabielle, M., M. Malsot and M. Paris, 1981. Contribution to the chemical study of 2 oils of *Artemisia herba-alba Asso* and *Artemisia vulgaris* L. Chimiotaxonomic interest. XXV. Aromatic day, Lourmahn, Rivista ltaliana EPPOS, 6: 296-299.

Jonathan, M., M.B. Sleeman, M.R.C.V.S. Vet, L. Lisa, B.S. Meader, B. Antoine, D.V.M. Mudakikwa, W. James, D.V.M. Foster and Sharon Patton, 2000. Gastrointestinal parasites of mountain gorillas (*Gorilla gorilla beringei*) in the Parc National des Volcans. Rwanda, J. Zoo. Wildlife Med., 31 (3): 322-328.

Plumptre, A.J., A. Kayitare, H. Rainer, M. Gray, I. Munanura, N. Barakabuye, S. Asuma, M. Sivha and A. Namara, 2004. The Socio-Economic Status of People Living near Protected Areas in the Central Albertine Rift, Albertine Rift Technical Reports 4, Wildlife Conservation Soc. New York, USA, pp: 135.

Robbins, M.M., P. Sicotte and K.J. Stewart, 2005. *Mountain gorillas*: 3 decades of research at Karisoke, Cambridge University, pp: 445. ISBN: 0521780047, 9780521780049.

Rwangabo, P.C., 1993. Traditional medicine in Rwanda. Paris, Karthala. CRDI-ACCT, pp: 264. ISBN: 2865374521.

Singleton, P., 2004. Bacteria in Biology, Biotechnology and Medicine. 6th Edn. John Wiley and Sons, pp: 559. ISBN 0470090278, 9780470090275.