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ANTIBACTERIAL SCREENING OF AQUEOUS EXTRACTS OF SOME MEDICINAL PLANT AND THEIR FRACTIONS USED AS ANTIDIARRHEAL AGENTS IN KINSHASA-DEMOCRATIC REPUBLIC OF CONGO

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ABSTRACT

Thirteen aqueous extracts (decoctions) used as antidiarrheal in Kinshasa, Democratic Republic of Congo and their forty two fractions were submitted to an evaluation for their potential antibacterial properties against isolated pathogenes bacteria implicated in diarrhea. Results indicated that five extracts including those from *Carica papaya* root, *Hensia pulchella* leaf, *Morinda morindoides* leaf, *Psidium guayava* leaf, *Tithonia diversifolia* leaf as well as their respective ethylacetate fractions rich in flavonoids inhibited the growth of all tested bacteria with minimum inhibitory and bactericidal concentrations ranging from 15.62 to 62.50 µg/ml (good activity). Aqueous extracts from other selected medicinal plants exhibited a combined level of activity as good (MIC and MBC < 100 µg/ml), moderate (125 < MIC and MBC < 250 µg/ml and weak (250 < MIC and MBC < 500 µg/ml) or were inactive (MIC and MBC > 500 µg/ml) against some selected bacteria according to the case. These results can partly justify and support the tradition use of these medicinal plants for the preparation of remedies used for the treatment of diarrhea of bacterial origin.

KEYWORDS: Medicinal plants, aqueous extracts, fractions, diarrhea, antibacterial activity.

1. INTRODUCTION

For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. Medicinal plants have been used as herbal medicines for their healing properties since ancient times proven to have antibacterial activity (Chauhan et al., 2015). They contain different bioactive compounds belonging to different phytochemical groups such as alkaloids, flavonoids, terpenes, steroids, tannins, anthraquinones, coumarins and so one playing un important role in the manifestation of this biological activity and others. The use of plant compounds for pharmaceutical purposes has gradually increased in more countries in the world. According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. Therefore, such plants should be investigated to better understand their properties, safety and efficiency.

Plants based antimicrobial effects represent the vast untapped source. The use of plant extracts for medicinal plants treatment has become popular when people realized that the effective life span of known antibiotics is limited and over prescription and misuse of traditional antibiotics are causing microbial resistance (Alan et al., 2009). Traditionally, used medicinal plants produce a variety of compounds of known therapeutic properties (Chopra et al., 1992; Harborne and Baxter, 1995).

The non-aviability and high cost of new generation antibiotics with limited effective span have resulted in increase of morbidity and mortality (Williams, 2000). Therefore, there is a need to look for substances from other sources with proven antibacterial activity. Consequently, thus has led to search for more antibacterial agents among materials of plant origin with the aim of discovering potentially useful active compounds that can serve as templates for synthesis of new antibacterial agents (Pretorius et al., 2003; Moreillon et l., 2005). The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. Many plants have been used because of their antimicrobial traits, which are due to compounds synthetized in the secondary metabolism of the plant.

Leaves, root and stem bark of various medicinal plants are currently used in traditional medicine for the treatment of diarrhea, gastro-intestinal disorders and other various ailments (Richard et al., 2013).

In recent years, antimicrobial properties of different plant part extracts from various medicinal various medicinal plants are being increasingly reported *in vitro* and *in vivo* tests in the world to prove their efficiency (Inamul, 1984; Almagboul et al., 1985; Jansen et al., 1987; Kubo et al., 1993; Shapoval et al., 1994; Ikran and Izzo et al., 1995; Elfof, 1998; Chopra et al., 1992; Harborne and Baxter, 1995; Saxena, 1997; Nimri et al., 1999; Williams, 2000; Prectorius et al., 2003; Moreillon et al., 2005; Alam et al., 2009; Seukep et al., 2012; Wendakoon et al., 2012; Shinde and Mulay, 2015,; Traoré et al., 2015). The present study was undertaken to evaluate the antibacterial activity of some medicinal plant extracts and their fractions currently used for the treatment of diarrhea in traditional medicine in Kinshasa, Democratic Republic of Congo.

2. MATERIALS AND METHODS

2.1. Plant materials

Leaves, stem and root of the selected medicinal plants were collected in Kinshasa in December 2010. The plants were identified by Mr. Nlandu Lukebiako B. of the Institut National d'Etudes et de Recherches en Agronomie (INERA), Department of Biology, Faculty of Sciences, University of Kinshasa. A voucher specimen of each plant was deposited in the herbarium of this institute. Leaves, stem and root stem and root barks were dried at room temperature and reduced to powder keepted in brun bottles.

2.2. Preparation of extracts

20 g of each powdered medicinal plant part were mixed with 200 ml distilled water and boiled for 15 minutes on a hoteplate. After cooling and filtration, each filtrate was evapored *in vacuo* yielding corresponding dried extract.

2.3. Phytochemical screening

Chemical tests were carried out on the aqueous dried extracts of selected medicinal plants using standard procedures described in the literature (Harborne, 1998). Saponins were identified by using the frothing test. Alkaloids were detected using Draggendorf's and Mayer's reagents resulting in the formation of an orange or yellow-white precipitate respectively as positive test. The presence of flavonoids was determined using aluminium

chloride 5% in methanolic solution and Shinoda's reagent (HCl + magnesium turnins) after heating for 10 min giving a yellow and purple color respectively as positive test. The test for anthraquinones was performed by adding Bortranger's reagent (NH_4OH 10% or NaOH 10%) producing a red to red-purple color as positive test. Steroids and terpenes were screened by adding Lieberman-Buchardat's reagent (acetic anhydride/conc. H_2SO_4) followed by heating for 10 min until to the appearance of purplish-blue color or other colors as positive test. Tannins were identified with gelatin and Stiasny`reagent (Formol + HCl).

Moreover, the presence of alkaloids, flavonoids, steroids, terpenoids and anthraquinones was confirmed by TLC (thin layer chromatography) analysis performed on silicagel $60F_{250}$ plates Merck (thickness layer 0.25 mm) using $CHCl_3$/Methanol/NH_3 25%: 8:2:0.5 and Ethyl acetate/*iso*-Propanol/NH_3 25%: 16:3:1 as mobile phases, and Draggendorf's reagent for alkaloids; *n*-Butanol/Acetic acid/ Water: 4:1:5 (top layer) as mobile phase and Neu's reagent (1% diphenylboric ethanolamine acid in methanol) for flavonoids; Ethyl acetate/Methanol/Water: 8:1:1 as a mobile phase and magnesium acetate 5% in methanol, and NaOH 10% or NH_4OH 10% as reagents for anthraquinones; Chloroform/Methanol: 9:1 and *n*-Hexane/Dichloromethane:1:9 as mobile phases and Lieberman-Buchardat's reagent and Vanilline 1% and H_2SO_4 5% in methanol for steroids and terpenoids. Coumarins were detected under UV (366 nm) based on their blue fluorescence which becomes intense after spraying KOH 10%. Anthocyanins were detected after heating 1m g of each extract dissolved in 5 ml distilled water with 2M HCl for 5 min at 100°C producing a red color after heating for 10 min, which can be extracted into isoamyl alcoohol or by adding 2M NaOH dropwise giving a blue color which changes to green and becomes fader slowly.

2.6. Antibacterial testing

The following microorganisms were used: *Escherichia coli*, *Escherichia parcoli*, *Citrobacter diversifolia*, *Shigella dysenteria*, *Salmonella enteritidis*, *Staphylococcus aureus* and *Shigella flexneri*, These microorganisms were clinical isolates obtained from patients at the Laboratory of Microbiology, Cliniques Universitaires du Mont Amba, University of Kinshasa. Microorganisms were cultured overnight at 37°C in agar base (Difco). Colonies were directly suspended into a small volume 0.9% saline. 5 ml of this suspension was added to 100 ml Muller-Hinton medium (Difco). The minimum inhibitory concentrations (MIC) of plant extracts were determined by the dilution method as previously described by Tona et al. (1999). For this, 5 mg of plant extract were dissolved in 5 ml distilled water to obtain

respective stock solutions of 1 mg/ml. These were separately further diluted in two fold dilutions to have a series of test concentrations from 500 to 0.1 µg/ml. To 1 ml of bacterial suspension, 1 ml of test sample with known concentration was added. Test tubes were well mixed and incubated at 37°C. A limpid tube indicated antibacterial activity while a cloudy tube indicated an inactivity of the tested sample. The lowest concentration of the test sample that inhibited the bacterial growth after incubation was taken as the MIC value. A volume of 10 µl from each test tube was placed on new culture medium in order to determine the minimal bactericidal concentration (MBC) which was defined as the lowest concentration yielding negative subcultures or only one colony. All samples were tested in triplicate. MIC and BC were expressed in µg/ml.

3. RESULTS AND DISCUSSION

Table 1 presents the traditional uses of the selected medicinal plant parts and indicate that all plant parts are used to treat diarrhea and other various ailments. For these selected plant parts, the decoction was the principal mode of preparation of the used antidiarrheal remedies and the most plant part used is the leaf. In general, the plant part is used alone and in some cases, in combination of other parts of other medicinal plants to prepare antidiarrheal remedies aiming the renforcing or enhancing of the activity. This is the case of *Alchornea cordifolia* leaf in combination with *Morinda lucida* leaf, *Psidium guayava* leaf in combination with *Bridelia ferruginea* stem bark or *Mangifera indica* leaf or stem bark (Kerharao and Adam, 1974, Kambu, 1990).

Table 1: Traditional used of selected medicinal plants.

Plant name and family	Part used	Traditional indications of the selected plant parts
1. *Alchornea cordifolia* Mull. Arg. (Euphorbiaceae) NB28122010L1	Leaf	The leaves are used as purgative, cholagogue, anti-icteric and against tachycardia (Kerharo and Adam, 1974). They are also used as an aqueous decoction to treat diarrhea, dysentery, conjonctivis, gastro-intestinal disorders, fever alone or in combination with *Morinda lucida* leaves according to the case, to treat cough, as healing and vomitive agent (Kambu, 1990). The leaves and stem as an infusion or chewed fresh are taken for sedative and antispasmodic properties to treat a variety of respiratory problems including throat, cought and bronchitis, or other ailments such as gastric ulcers, diarrhea, dysentery and worms, genital urinary problems such as venereal diseases and female sterility. They are uses as an enema, tonic and emetic at high dose, as blow purifier, to treat anemia, epilepsy, malaria and fever and tachycardia (Newinger,

		2000;(http://www.prota4u.org/rotav8.asp?p=Alchornea+cordifolia, 2015). The leaves are eaten in West Africa and Congo as an emmenagogue and to facilitate delivery, and in Gabon as an abortifacient. A cold infusion of the dried and crushed leaves acts as a diuretic. Leaf and root decoctions are widely used as mouth wash to treat ulcers of the mouth, toothache and caries, and twigs are chewed for the same purposes. Crushed fresh leaves or powdered dry leaves are applied externally as a cicatrizing to wounds, to relieve pains, e.g. backache and headache, to fracture or improve healing and to treat eye infections and numerous skin affections including venereal diseases, sores, abscesses, yaws and filariasis. A decoction or paste of leafy twigs is applied as a wash to treat fever, malaria, rheumatic pains, enlarged spleen and as a lotion or poultice to sore feet; vapor baths can also be taken. In Ivory Coast and Ghana the leaves are applied as a haemostatic to stop prolonged menstruation and a decoction of roots or leaves is applied in the vagina to stop post-partum haemorrhagia and to treat vaginitis. In Sierra Leone and Congo, young leaves or pounded bark are made into a suppository to treat hemorrhoids. In DR Congo bruised leaves are applied as an enema to treat impotency diarrhea and , dysentery (Kambu, 1990). In West Africa pulped root is widely taken to treat venereal diseases. Dried leaves or roots, alone or with tobacco, are smoked to cure cough. The leaves and root bark are externally applied to treat leprosy and as an antidote to snake venom (http://www.fam.ucl.ac.be/Full-tests/full-test-FARM/Mavar-Manga-2007, 2015).
2. *Cajanus cajan* (L.) Millsp (Fabaceae) NB28122010L2	Leaf	The leaves are used to treat burns, dizziness, vision problems, rheumatism, intestinal parasites, epilepsy, diarrhea, earache, gonorrhea, measles, sckile cell anemia in combination with *Chassalya kolly* leaf, sprains, stomach problems, threadworms, throat problems and toothache (Newinger, 2000). In Trinidad and Tobago, the leaves of *C. cajan* are used in food poisoning, as colic and in constipation. In Chinese folk medicine, pigeon pea leaves are used to staunch blood, as an analgesic and to kill parasites. In some parts of Tamil Nadu, India, the leaf, seeds and young stems are used to cure gingivitis, stomatitis and as a toothbrush. It is also an important folk medicine in eastern Rajasthan as fresh juice/boiled leaves are given orally to nullify the effect of intoxication and as a laxative. Leaf paste is applied in oral ulcers and inflammations. Leaves and seeds are applied as poultice over the breast to induce lactation. *C. cajan* is indicated in the relief of pain in traditional Chinese medicine and as a sedative. In recent years it has also been explored for the treatment of ischemic necrosis of the caput femoris, aphtha, bedsore and wound healing (Lans, 2007; Ganeshan, 2008; Upadhyay et al., 2010).
3. Carica papaya L.	Root bark	The aqueous decoction of the root bark is used as diuretic,

(Caricaceae) NB2812201Rb1		antiblennorragic, antinvenimous, hemostatic and lactogenic agent (Kerharo and Adam, 1974). It contributes to the medication of Anasarc (Adjanhoum et al., 1986; Kambu, 1990), root bark and leaves are used as aqueous decoction against malaria and fever, diarrhea and dysentery (Kambu, 1990). Il is also used to treat blennorragia in enema or as a boisson (Aké Assi et al., 1980) abdominal pains, oedema in enema (Adjanohoum and Aké Assi, 1979). The methanol extract of the root bark is used in Nigeria for the treatment of malaria, hepatitis, jaundice and antifertility (http://www.sciencedirect.com/science/article/pű/s18785351 4002305, 2015). Its aqueous decoction is used as aphrodiac (http://ed.org/pages:58682, 2015, against stomach pains, oedema of testicles, caries, vesical diseases, asthma in children, anthrax, cough, gastric ulcers, tonic, malaria and fever, abdominal pains, headaches, back pains, swollen glands, mental illness and gingivitis (Newinger, 2000). Juice from papaya roots is used in some countries of Asia to ease urinary troubles. A decoction formed by boiling the outer part of the roots of the papaya tree in the cure dyspepsia (Pal et al., 211). Papaya can be used as a diuretic (the roots and leaves), and to treat bilious conditions (the fruit). Parts of the plant are also used to combat dyspepsia and other digestive disorders (papaya contains a proteolytic enzyme which soothes the stomach and aides in digestion) and a liquid potion has been used to reduce enlarged tonsils (Damson, 2015). The roots or their extracts for cancers of the uterus, syphilis, the tropical infection, hemorrhoids, and to remove mineral concretions in the urine (http://www.drugs.com/npc/papaya.htm, 2015). Decoction of pounded papaya roots are used as digestive tonic and to cure dyspepsia. Extracts from papaya roots are used to abort early pregnancy (http://www.medicalcalhealthguide.com/articles/papaya.htm, 2015).
4. Datura arboreal L. (Salicaceae) NB28122010L	Leaf	For the treatment of asthma by means of the leaves and in external uses for rheumatic pains (Newinger, 2000). *Datura arborea* (*Brugmansia*) has also traditionally been used in many South American indigenous cultures in medical preparations and as an entheogen in religious or spiritual ceremonies. Medicinally, its is mostly used externally as part of a poultice, tincture, ointment, or where the leaves are directly applied transdermally to the skin. Traditional external uses have included the treating of aches and pains, dermatitis, orchitis, arthritis, rheumatism, headaches, infections, and as an anti-inflammatory. It is internally used much more rarely due to the inherent dangers of ingestion. Internal uses, in highly diluted preparations, and often as a portion of a larger mix, have included treatments for stomach and muscle ailments, as a decongestant, to induce vomiting,

		to expel worms and parasites, and as a sedative. The decoction of the leaf is used to treat diarrhea and amoebic dysentery (Newinger, 2000). Several South American cultures have used *Brugmansia* as a treatment for unruly children, that they might be admonished directly by their ancestors in the spirit world, and thereby become more compliant. Mixed with maize beer and tobacco leaves, it has been used to drug wives and slaves before they were buried alive with their dead lord (https://en.wikipedia.org/wiki/Brugmansia, 2015). The leaves are used in Chile to ooze the furuncles and as a substitute of *Datura stramomium*. Treatment of asthma by means of cigarettes of its leaves, and in external use for rheumatic pain (http:www.botanicalonline.com/alacloidestrompetasperuangle, 2015).
5.Euphorbia hirta L. (Euphorbiaceae) NB28122018L4	Whole plant	The decoction of the whole plant is employed for the treatment of dysentery, diarrhea in children and adults, colics, as diuretic, antiblennorragic and galactagogue (Kerharo and Adam, 1974, Adjanohoum et al., 1988). It is also used to treat female disorders, respiratory ailments such as cough, coryza, bronchitis and asthma, worms infestations in children, jaundice, gonorrhea, digestive problems and tumors (Kumar et al., 2012). Decoction of dry herbs is used for skin diseases. *E. hirta* is used in the treatment of gastrointestinal disorders (diarrhea, dysentery, intestinal parasitosis, etc.), bronchial and respiratory diseases (asthma, bronchitis, hay fever, etc.), and in conjunctivitis. Hypotensive and tonic properties are also reported in *E. hirta*. The aqueous extract exhibits anxiolytic, analgesic, antipyretic, and anti-inflammatory activities. Extracts of *E. hirta* have been found to show anticancer and antidiabetic activities. The aqueous extract of the herb strongly reduced the release of prostaglandins $I_{2,}E_{2,}$ and, D_2. The aqueous extract also inhibits aflatoxin contamination in rice, wheat, maize, and mustard crops. Methanolic extract of leaves have antifungal and antibacterial activities. The leaves pounded with turmeric and coconut oil are warmed and rubbed on itchy soles. Decoction of fresh herbs is used as gargle for the treatment of thrush (Kumar et al., 2010).
6.Garcina kola Heckel (Clusiaceae) NB28122010Rb2	Root bark	*Garcinia kola* is traditionally used by African medicine men who believe that it has purgative, antiparasitic, and antimicrobial properties (https://en.wikipedia:wiki/Garcinia_kola, 2015). *Garcinia kola* is used in many tropical countries to fight infectious diseases such as Aids and the Ebola virus. It has shown to possess anti-inflammatory, antimicrobial and antiviral properties and as a cold remedy. *Garcinia kola* is often used to treat the symptoms of colds. It is suggested, in particular, for coughs and sneezing, knee Osteoarthritis. Garcinia kola has been successfully used

		to treat patients suffering from knee osteoarthritis. It reduce pain and swelling and improve movement and immunity. *Garcinia* kola is known for its anti-inflammatory and antioxidant properties. It is used to prevent infections and viruses, especially of the immune system (https://bitter.wordpress.com/2013/05/24/benefits, 2015).
7. Harugana madagascariensis (Hypericaceae) NB29122010Sb	Stem bark	The aqueous decoction of the stem bark is used for the treatment of angina, circumussion wounds, cicatrization, stem sap, diarrhea, dysentery, dysmenorrhea, hypertension, jaundice, parasitic skin diseases, scabies, skin ailments of all kinds, as antiseptic and purgative agent (Kerharo and Adam, 1974; Kambu, 1990). It is also used to treat cardiac affections, gastrite, constipation, diarrhea, articular rheumatism, acute enteritis, asthma, icteria, fever, diabetes and as antienteralgic agent (Kerharo and dam, 1974, Adjanohoum et al., 1984,198). Decoction of the bark is used to treat malaria and jaundice (https://www.wikipedia/wiki/Harungana, 2015).
8. Heinsia pulchella (G. Don) K.Schum (Rubiaceae) NB29122010Rb	Root bark	The aqueous decoction of the root bark is used for the treatment of pneumonia, tuberculosis, inflammation of gums, as aphrodisiac and intestinal worms agent, against diarrhea and scabies (Kambu , 1990), dysentery, diarrhea, parasitic infections, genital stimulant, depressant, dropsy, swelling, oedema, goot, paralysis, epilepsy, convulsions and spams (Newinger,2000; http://plants.ptor.org/scable/10.55555/al.ap.uputa.4893, 2015), fever and malaria (Newinger, 2000). 4).
9. Mangifera indica L NB2912010Sb	Stem bark	The stem bark is used as aqueous decoction for the treatment of hemmorroids, mouth diseases as a mouthwath, syphilis (sap of trunk), score gums, score throat, hypertension (leaves + bark), diabetes (+ roots and leaves of Latana camara), dysmenorrhea, fever and malaria (Newinger, 2000). Various parts of M. indica are used as dentifrice, antiseptic, astringent, diaphoretic, stomachic, vermifuge, tonic, laxative, diuretic, to treat diarrhea, dysentery, anaemia, bronchitis, cough, hypertension, insomnia, rheumatism, toothache,leucorrhea, haemorrhage and piles. They are also used to treat abscesses, broken horn, rabid dog, jackal bite, tumours, snabite,stings,Datura poising, heat stroke, miscrriage, anthrax, blisters, wound in the mouth, tympanitis, colic, glossitis, indigestion, bacillosis, bloody dysentery, livers disorders, excessive urination, tetanus and asthma (Oyele, 2005). The bark have astringent properties used as a lotion in Nigerai to reliev tootchache, sore gums, sore throat or as an infusion in malaria, diarrhea and dysentery treatment. It is also employed in cancer, diabetes, asthma, infertility, lupus, prostatis, prostatichyerplasia, gastric disorders, arthralgies, mouth sores and tooth pain (Okwu and Ezenagu, 2008). It possesses anti-snake properties (Dhananiaya et al., 2016). The resinus gum from

		the trunk is appliedon cracks in the skin of the feet and on scabies, and believed helpful in cases of syphilis (http://rain-tree.com/mang.htm, 2018). The bark has also antibiotic properties (https://en.wikipedia.org/wiki/Mangifera_indica, 2018).
10. *Morinda morindoides* (Baker) Milne Readhed (Rubiaceae) NB29122010L	Leaf	The aqueous decoction is employed for the treatment of intestinal worms, diarrhea, dysentery , blennoragia, gastro-intestinal disorders, bacterial infections, cutaneous irruptions, malaria and fever, colic, constipation, hemorrhoids, gale, urogenital infections, wounds, diabetes, rheumatism, as appetitive stimulant, purgative and decongestive pelvine agent, revigorant for rachitic children and tonic, to treat general tredness (Kerharo and Adam, 1974; Adjanohoum et al., 1988; Kambu, 1990).
11. *Nauclea latifolia* Smith. (Rubiaceae) NB29122010L	Leaf	The aqueous decoction is used to regularize intestinal function, used as antienetralic against colis, vermifuge, and diuretic. Root bar and leaves are used as aqueuous decoction to treat malaria and fever (Kerharo and Adam, 1974). Its leaves in combination with *Afromomum melegueta* leaves are used to treat hemorrhoids (Adjanohoum et al., 1986). Leaf is used in application to treat abces (Adjanhoum et al., 1986).
12.*Psidium guajava* L. (Myrtaceae) NB30122010L	leaf	The aqueous decoction of the leaves is used against diarrhea, dysentery, fever and malaria, icteria (Kerharo and Adam, 1974), as cholagogue associated to *Euphorbia trucalli* leaf for the treatment of intercostal nevralgia in combination with *Conyza sumatremis, Cyanthula prostata, Euphorbia permifolia* leaf, diarrhea in association of *Bridelia ferruginea* stem bark or *Mangifera indica* leaf or stem bark, in pate of the leaf mixed with rabbit mink for the treatment of epilepsy (Adjanouhoum et al., 1986). It is used against abdominal pains, as arbortifiant and cholagogue. Il is also employed to treat jaundice, stomach pains, insanity, tooche, cough, whooping cough, gastric ulcers, vomiting, convulsions fever, measles (Newinger, 2000). Traditionally the leaves where either brewed to treat intestinal tract issues or ground into a poultice and applied to the skin to treat open wounds and rashes. In Northeast India, it is used as a folk medicine against intestinal helminthic parasites. Later, an experimental study showed that the leaf extract of *P. guajava* possesses significant anthelminthic effects in experimental rodent host-tapeworm model. Some experiments have found that certain extracts from the leaves and bark can act as an anti-inflammatory, prevent bacterial growth, and in some cases inhibit the spread of cancer. Extracts of the leaves or stem bark are reported to have antiplasmodial, antidiabetic and antimicrobial, antifungal and antiviral activities (Kambu. 1990; https://en.wikipedia.org/wiki/Psidium_guajava, 2015).
13.*Tithonia diversifolia* (Hesml.) A. Gray (Asteraceae)	Leaf	Leaves are used to treat abces, microbial infections smoke bites, malaria and fever, diabetes hematomas, muscular cramps, diabetes mellitus, amoebic dysentery, sore throat,

NB30122010L		liver pains, abdominal pains during pregnancy, diarrhea, enema, bleeding during pregnancy, stomach pains, indigestion, throat pains, bronchial diseases, pneumonia, rheumatism, snakebites, wound healing and as diuretic agent (Newinger, 2000). An infusion of leaves is used as an anti-malarial (Oyewole et al., 2008), as a medicine for constipation, stomach pains, liver pains, indigestion and sore throats, as an antiviral (Cos et al., 2002; Chiang et al., 2004), antidiabetic, antidiarrheal, antimicrobial (Tona et al., 1999), antispasmodic (Goffin et al., 2002), vasorelaxant and cancer-chemopreventive (Agboola et al., 2006).
14.*Vitex madiensis* Oliv. (Verbenaceae) NB30122010Rb	Root bark	The aqueous decoction is used to treat abdominal pains (Kerharo and Adam, 1974) diarrhea, dysentery, women sterility and to facilitate delivery (Kambu, 1990).

Table 2: Phytochemical screening.

Plant names	Alkaloids	Flavonoids	Anthraquinones	Coumarins	Terpenes and steroids	Tanins
A. cordifolia (L)	++	++	-	+	++	++
C. cayan (L)	-	+	-	+	++	+
C. papaya (Rb)	++	-	-	-	++	++
D. arborea (L)	++	+	-	-	++	++
E.hirta (Wp)	-	++	-	-	++	++
G. kola (Sb)	-	-	-	-	++	++
H. madagascariensis (Sb)	-	-	-	+	++	++
H. puchella (L)	-	+	-	+	++	++
M. indica (Sb)	-	+	-	+	++	++
M. morindoides (L)	-	++	++	-	++	++
N. latifolia (L)	++	++	-	+	++	++
P. guajava (L)	-	++	-	+	++	++
T.diversifolia(L)	-	+	-	+	++	++
V. madiensis (L)	-	+	-	+	++	++

(L): leaf, (Rb): root bark, (Sb): stem bark, (Wp): whole plant

All aqueous extracts (decoctions) and their respective fractions were submitted to an evaluation of their potential antibacterial properties against a series of bacteria implicated in diarrhea. For good appreciation of the level of the activity of each tested sample, the following criteria were adopted: MIC or MBC < 10 µg/ml: strong activity, 10 < MIC or MBC ≤ 100 µg/ml: good activity, 100 < MIC or MBC ≤ 250 µg/ml: moderate activity, 250 < MIC or MBC ≤ 500 µg/ml weak activity, MIC or MBC > 500 µg/ml: inactive.

The antibacterial activity of the selected plant extracts and their respectives fractions are presented in Tables 3 and 4 showing minimum inhibitory concentrations (MIC) and

minimum bactericidal concentrations (MBC) respectively. In general manner, none of the selected medicinal plant has shown strong antibacterial activity in our experimental conditions against all selected microorgamisms. But, the reported results seem to be interesting since it was found that these plants contain some bioactive components capable of inhibition the growth bacteria.

According to the results in Table 3 and considering the level of antibacterial activity of extracts depending to the susceptibility of each tested microorganigm, the active extracts could be classified into three groups as followed: the first group includes five aqueous extracts from *Carica papaya* root bark, *H. pulchella* leaf, *Morinda morindoides* leaf, *Psidium guayava* leaf and *Titonia diverisofolia* leaf exhibited good antibacterial activity against all selected bacteria with MIC values ranging from 31.25 to 62.50 µg/ml.

Fractions from respective aqueous extracts of these five medicinal plants also exerted antibacterial activity at different extents. It was observed that the ethylacetate fraction F2 rich in flavonoids from *C. papaya*, *M. morindoides*, *H. pulchella*, *P. guajava* and *T. diversifolia* inhibited the growth of all selected bacteria with MIC < 100 µg/ml and their activity was considered as good (Vanden Berghe and Vlietinck, 1991). Their respective fractions chloroform F1, *n*-butanol F3 and residual aqueous F4 soluble fractions rich in terpenes and steroids, saponins and phenolic compounds respectively exhibited a combined intensity of antibacterial activity as good, moderate and weak or were inactive according to the case against a determined number of tested bacteria (Table 3).

The second groups includes extracts from *A. cordifolia* leaf, *C. cajanus* leaf, *E. hirta* whole plant, *M. indica* stem bark an *V. madiensis* leaf which exhibited good activity against five tested bacteria with MIC < 100 µg/ml. They showed moderate activity against other bacteria such as *E. coli*, *C. diversus*, *K. pneumonia*, *S. dysentery*, *S. aureus* and *S. fexneri* according to the case (Table 3). The ethylacetate fractions F2 rich in flavonoids *A. cordifolia* and *E. hirta* exhibited good antibacterial activity against all selected bacteria (MIC < 100 µg/ml) while the same fraction from *C. cajan* leaf, *M. indica* and *V. madiensis* displayed good activity against only five bacteria and show moderate or weak activity against the remaining bacteria according to the case. Some of them were devoid of antibacterial activity against some tested bacteria (Table 3). Their respective fractions chloroform F1, *n*-butanol F3 and residual aqueous phase F4 rich in steroids and terpenes, saponins and phenolic compounds respectively were found to display good antibacterial against one or three tested bacteria and

showed in general moderate activity against a high number of selected microorganisms (Table 3).

Table 3: Antibacterial activity of selected plant extracts and their respective fractions (MIC, µg/ml).

Plant names	Samples Code	Ec	Ep	Cd	Sd	Sa	Se	Sf
cordifolia	Decoction	125	62..5	62.50	125	62.50	62.50	62.50
	F1	125	125	125	62.50	31.25	125	125
	F2	62.50	31.25	62.50	31.25	31.25	15.62	15.62
	F3	125	62.50	125	250	62.50	62.50	125
	F4	62.50	125	62.50	250	125	31.25	31.25
C. cajan	Decoction	62.50	62.50	62.50	125	125	31.25	62.50
	F1	125	125	62.50	250	500	62.50	31.25
	F2	62.50	62.50	31.25	250	250	62.50	31.25
	F3	125	125	250	500	500	62.50	62.50
	F4	62.50	125	125	250	250	31.25	31.25
C. papaya	Decoction	31.25	31.25	31.25	62.50	31.25	31.25	31.25
	F1	62.50	62.50	62.50	125	125	62.50	62.50
	F2	31.25	31.25	15.62	62.50	125	15.62	15.62
	F3	125	125	62.50	125	125	31.25	31.25
	F4	62.50	62.50	125	250	250	125	62.50
D. arborea	Decoction	125	62.50	62.50	250	250	62.50	62.50
	F1	250	250	62.50	250	500	125	125
	F2	62.50	62.50	31.25	125	250	31.25	31.25
	F3	250	250	125	250	500	25	62.25
	F4	125	62.50	62.50	500	500	125	125
E. hirta	Decoction	62.50	62.50	31.25	125	62.50	31.25	125
	F1	125	62.50	250	250	250	62.50	125
	F2	62.50	31.25	31.25	62.50	62.50	31.25	31.25
	F3	125	125	250	250	250	125	125
	F4	125	62.50	62.50	250	250	125	62.50
G. kola	Decoction	125	62.50	125	125	125	62.50	125
	F1	125	125	250	250	250	125	125
	F2	62.50	62..5	125	62.50	250	62.50	31.25
	F3	250	125	125	500	500	125	125
	F4	125	62.50	125	250	500	62.50	62.50
H. madagascariensis	Decoction	500	250	62.50	250	500	62.50	250
	F1	250	125	125	125	250	62.50	125
	F2	62.50	62.50	125	125	125	31.25	62.50
	F3	125	125	62.50	250	250	62.50	62.50
	F4	125	62.50	62.50	125	125	62.50	62.50

H. pulchella	Decoction	62.50	31.25	31.25	62.50	15.62	62.50	15.62
	F1	125	62.50	125	250	250	62.50	62.50
	F2	31.25	15.62	15.62	31.25	15.62	31.25	15.62
	F3	250	125	62.50	125	62.50	62.50	31.25
	F4	62.50	62.50	31.25	125	125	31.25	15.62
M. indica	Decoction	62.50	31.25	125	62.50	125	125	62.50
	F1	125	250	250	250	250	62.50	62.50
	F2	62.50	125	62.50	125	62.50	62.50	125
	F3	250	125	250	125	125	62.50	62.50
	F4	250	125	125	500	500	125	250
M. morindoides	Decoction	31.25	31.25	15.25	62.50	125	31.25	31.25
	F1	125	125	125	250	250	62.50	62.50
	F2	31.25	31.25	62.50	62.50	62.50	31.25	15.62
	F3	125	125	62.50	250	250	125	62.50
	F4	250	125	62.50	125	250	250	62.50
P. guajava	Decoction	31.25	31.25	15.62	62.50	31.25	31.25	62.50
	F1	62.50	125	62.50	250	250	62.50	125
	F2	31.25	15.62	15.62	62.50	31.25	15.62	15.62
	F3	125	62.50	62.50	250	250	62.50	62.50
	F4	62.50	125	62.50	500	500	62.50	62.50
ersifolia	Decoction	31.25	31.25	15.62	31.25	31.25	62.50	62.50
	F10	62.50	62.50	62.50	125	125	62.50	31.25
	F2	31.25	15.62	15.62	62.50	31.25	62.50	31.25
	F3	125	125	125	250	250	125	62.50
	F4	62.50	62.50	62.50	250	250	62.50	31.25
V. madiensis	Decoction	62.50	62.50	125	125	62.50	62.50	31.25
	F1	125	62.5	62.5	>500	>500	31.25	62.5
	F2	62.50	31.25	31.25	250	500	62.50	31.25
	F3	250	125	125	>500	250	125	62.5
	F4	62.5	62.5	31.25	500	500	62.50	62.50

EC: *Escherichia coli*, EP*; Escherichia paracoli*, CD: *Citrobacter diversifolia*, SD: *Shigella dysenteria*, SA: *Staphylococcus aureus*, SE*: Salmonella enteritidis*, SF: *Shigela flexneri*, F1: chloroform, F2: ethylacetate, F3:. *n*-butanol, F4: residual aqueous soluble fractions from the partition of the aqueous extract (decoction).

Table 4: Bactericidal activity of selected medicinal plant extracts and their respective fractions (MBC, µg/ml).

Plant names	Samples Code	EC	EP	CD	SD	SA	SE	Sf
A. cordifolia	Decoction	250	125	62.5	250	125	62.50	62.50
	F1	125	250	125	125	62.5	125	125
	F2	125	62.50	62.50	62.50	62.50	31.25	31.25
	F3	250	125	125	500	125	62.50	125
	F4	62.50	250	125	500	250	62.50	62.50
C. cajan	Decoction	62.50	125	62.50	250	250	62.50	62.50
	F1	250	250	125	500	>500	62.50	62.50
	F2	125	62.50	62.50	500	500	125	62.50
	F3	250	250	125	>500	>500	125	62.50
	F4	125	62.50	250	500	500	62.50	62.50
C. papaya	Decoction	62.50	62.50	62.50	62.50	62.50	62.50	31.25
	F1	125	125	62.50	250	250	125	62.50
	F2	31.25	62.50	31.25	125	250	31.25	31.25
	F3	250	125	62.50	500	500	125	62.50
	F4	62.50	62.50	250	250	125	31.25	62.50
D. arborea	Decoction	250	125	125	500	500	62.50	125
	F1	500	250	62.50	500	>500	125	250
	F2	125	125	62.50	500	500	62.50	62.50
	F3	500	250	250	500	>500	125	125
	F4	250	125	125	>500	>500	125	250
E. hirta	Decoction	125	62.50	62.50	250	125	62.50	125
	F1	250	250	125	500	500	62.5	125
	F2	62.50	62.50	31.25	125	125	31.25	62.50
	F3	125	250	500	500	250	125	125
	F4	250	62.50	125	500	500	125	62.50
G. kola	Decoction	250	125	125	250	250	62.50	125
	F1	125	250	500	500	500	250	125
	F2	62.50	125	250	500	500	125	62.50
	F3	250	250	125	>500	>500	250	125
	F4	250	125	250	500	>500	62.50	62.50
H. madagascariensis	Decoction	500	250	125	>500	500	62.50	500
	F1	500	125	125	500	500	62.50	125
	F2	62.50	125	125	250	250	62.50	62.50
	F3	125	250	62.50	500	500	125	125
	F4	250	125	125	>500	>500	125	250
H. pulchella	Decoction	62.50	62.50	31.25	125	31.25	125	31.25
	F1	250	125	125	500	500	62.50	62.50

	F2	31.25	31.25	15.62	250	125	62.50	62.50
	F3	250	250	62.50	125	125	62.50	62.50
	F4	125	62.50	62.50	250	250	62.50	31.25
M. indica	Decoction	125	62.50	125	125	250	125	125
	F1	250	250	500	250	500	125	62.50
	F2	125	125	62.50	250	62.50	125	125
	F3	500	125	250	250	125	125	62.50
	F4	500	250	125	>500	>500	125	250
M. morindoides	Decoction	62.25	31.25	31.25	125	250	31.25	62.25
	F1	250	125	250	500	125	62.5	62.50
	F2	62.50	31.25	62.50	125	125	31.25	31.25
	F3	250	250	62.50	250	500	250	125
	F4	250	250	500	250	250	500	62.50
P. guajava	Decoction	62.50	31.25	31.25	125	62.50	31.25	62.50
	F1	62.50	250	250	500	500	125	250
	F2	62.50	31.25	31.25	62.50	31.25	31.25	15.62
	F3	250	62.50	125	500	500	62.50	125
	F4	125	250	62.50	>500	>500	62.50	62.50
rsifolia	Decoction	62.50	62.50	31.25	62.50	125	62.50	62.50
	F1	62.50	125	62.50	250	250	62.50	62.50
	F2	62.50	31.25	31.25	31.25	62.50	62.50	62.50
	F3	250	250	250	500	500	125	62.50
	F4	125	125	125	500	500	62.50	62.5
V. madiensis	Decoction	62.50	250	250	250	125	62.50	62.50
	F1	250	62.50	125	>500	>500	62.50	62.50
	F2	62.50	62.50	31.25	500	>500	62.50	62.50
	F3	250	125	250	>500	>500	250	125
	F4	62.50	125	62.50	>500	>500	62.5	62.50
Ampicilline		0.05	0.025	0.025	1.50	0.025	0.075	0.025
Tetracycline		1.50	0.50	0.75	2.50	0.50	1.50	1.50

See Table 1

The third group includes plant extracts showing moderate or weak antibacterial activity with MIC and MBC ranging from 125 to 500 µg/ml against four and six bacteria. It concerns extracts from *D. arborea* leaf (4), *H. madagacariensis* (6), stem bark and *G. kola* stem bark (6). Some extracts in this group also showed good activity against two or three bacteria according to the case (Table 3). The ethylacetate soluble fractions F2 of *D. arborea* and *G. kola* exhibited good activity against 5 bacteria while that of *H. madagascariensis* showed the same effect against 4 microorganisms (MIC < 100 µg/ml). Fractions F1, F3 and F4 of these

extracts displayed in general moderate activity against a high number of tested microorganisms, and in some cases, good activity was observed against a limited number of tested bacteria (Table 3).

Concerning their bactericidal activity, it was observed that aqueous extracts from *C. papaya* root bark, *H. pulchella* leaf, *M. morindoides* leaf, *P. guajava* leaf and *T. diversifolia* leaf exhibited good bactericidal activity against all selected bacteria (MBC < 100 µg/ml) (Vanden Berghe and Vlietinck, 1991). Aqueous extracts from *A. cordifolia* and *V. madiensis*, and *C. cajanus*, exhibited the same level of activity against 3 and 4 tested bacteria respectively while the remaining plant extracts displayed good bactericidal activity against one or two selected bacteria according to the case and displayed in general moderate bactericidal activity on a large number of tested bacteria (MBC = 125 or 250 µg/ml) (Table 4).

Among fractions, it was observed that the ethylacetate fraction F2 of *P. guayava* and *T. diversifolia* showed good bactericidal activity against all selected bacteria with MBC < 100 µg/ml) (Vanden Berghe and Vlietinck, 1991) while the same fraction F2 from *A. cordifolia*, *C. papaya* and H. *pulchella*, *E. hirta* and *M. morindoides* displayed the same magnitude of activity against six and five bacteria respectively. The bactericidal activity of their fractions F1, F3 and F4 were considered as good or weak against a limited number of tested bacteria, and moderate against a large number of selected bacteria according to the case. In some cases, fractions were devoid of bactericidal activity (MBC > 500 µg/ml). This observation is also valid for the some fractions of other selected medicinal plant extracts in the present study (Table 4).

A literature search has indicated the responsible phytochemical responsible for the antibacterial activity of various medicinal plants. They include flavonoids and phenolic acids (Molsen et al., 1977; Krishana Rao and Ganapaty., 1983; le Grand, 1989, alkaloids (El-Tayeb et al., 1974; Al-Shamman and Mitcher, 1979; Deeni and Hussain, 1981; Cimanga et al., 1998), steroids and terpenes (le Grand, 1989). The presence of these phytochemical groups in the selected medicinal plant extracts as demonstrated by results from the phytochemical screening in the present study, has largely contribute to the observed biological activity, in which they can act alone or in synergic manner according to the case.

These results clearly demonstrate that the selected medicinal plant extracts possess antibacterial at different extents with can in part, justify and support their use for the treatment of diarrhea of bacterial origin in traditional medicine.

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