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Bacteriological evaluation and antimicrobial sensitivity test of common herbal concoctions in Ogbomoso metropolis

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Herbal concoctions are extracts from plants such as its bark, leaves, stems, roots, seeds and flowers. Plant ingredients were obtained from Oja-igbo market in Ogbomoso, Oyo State and were prepared in the form of concoctions (soup or drink made usually from ingredients after boiling) or infusions (soaking the plant material and allowing it to stand for varying lengths of time) and assessed for their microbial load and type of microorganisms present and also for the degree of resistance and sensitivity to selected antibiotics .The following bacteria were isolated from the herbal concoctions: *Micrococcus sendentarius, Corynebacterium renale, Clostridium tertium, and Clostridium butyricum.* Antimicrobial susceptibility screening of the isolated bacteria indicated multiple resistance to most commonly used antibiotics such as Ofloxacin (75%), Erythromycin (100%), Ciprofloxacin (100%), Clindamycin (100%), Gentamycin (75%), Cephalexin (100%), Cotrimoxazole (100%), Cloxacillin (100%), Ceftriaxone (100%) and Augmentin (100%). Only Ofloxacin and Gentamycin had (25%) sensitivity rate. The microbial quality of herbal concoctions should be of great importance to the community in order to reduce harm to the consumers and spread of resistance strains.

Key words: Antimicrobial, herb concoctions, susceptibility, resistance, Ogbomoso.

INTRODUCTION

Plants produce a diverse range of bioactive molecules, making them rich source of different types of medicines. Most of the drugs today are obtained from natural sources or semi synthetic derivatives of natural products used in the traditional systems of medicine (Sukanya et al., 2009). Medicinal plants are finding their way into pharmaceuticals, cosmetics, and neutraceuticals. In pharmaceutical field medicinal plants are mostly used for the wide range of substances present in plants which have been used to treat chronic as well as infectious diseases (Okigbo et al., 2009).

Plants have been utilized as a source of medicine for thousands of years and continue to play an important role globally in primary health care, mostly in developing countries (Balunas and Kinghorn, 2005). The use of medicinal plants is increasing because people believe they are safe for human consumption.

Traditional medicines are used not only for primary health care of the poor in developing countries, but also in countries where conventional medicine is predominant in the national health care system (Lanfranco, 1999). Herbal medicines serve the health needs of about 80%

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of the world's population, especially for millions of people in the vast rural areas of developing countries; more than 65% of the global population uses medicinal plants for their primary health care needs (WHO, 2001).

Antibiotic resistance has become a global concern in recent years. This problem is of great significance especially in developing countries because infectious diseases are one of the major causes of mortality in these countries. Due to a rapid increase in the rate of infections, antibiotic resistance in microorganisms and due to side effects of synthetic antibiotics, medicinal plants are gaining popularity over these drugs (Babu and Subashree, 2009).

Medicinal plants have been found useful in the cure of a number of diseases including bacterial diseases. Medicinal plants are a rich source of antimicrobial agents (Mahesh and Satish, 2008). Although medicinal plants produce slow recovery, the therapeutic use of medicinal plant is becoming popular because of their lesser side effects and low resistance in microorganisms (Seyyednejad and Motamedi, 2010).

A herb is a plant or any part of a plant valued for its' medicinal, aromatic, or savory qualities (Bodeker et al., 2005; Bisset, 1994). Herbs can be viewed as any biosynthetic laboratory ingredients, producing a number of chemical compounds. Herbal remedies or medicines consist of portions of plants or unpurified plant extracts containing several constituents, which often work together synergistically. According to the World Health Organization (WHO), ''Herbal Preparations'' contain plant parts or plant material in the crude or processed state as active ingredients and may contain excipients (foreign substances) (WHO, 1998; Busse, 1999). Herbal preparations are comminuted or powdered plant material, extracts, tinctures, fatty or essential oils, expressed juices, processed resins or gums and so forth prepared from different plant parts such as roots, bark, stems, leaves, and fruits whose production involves a fractional, purification, or concentration process (Evans, 1989; Evans, 1996).

There is also an increase in infectious diseases worldwide caused by both drug resistance; and lack of sufficient affordable medicine for people living in poor communities. Infectious diseases represent an important cause of morbidity and mortality among the general population, particularly in developing countries.

Microbial infections have posed a health problem throughout the world, and plants are a possible source of antimicrobial agents (Burapadaja and Bunchoo, 1995; Adenisa et al., 2000). Medicinal plants contain active principles which can be used as an alternative cheap and effective herbal drug against common bacterial infections. During the past decade, medicinal plants have been extensively used for medical treatments. They have also been traditionally utilized in the history including some of their biological activities which have been scientifically characterized. Product quality is obviously one of the major criteria that could affect not only the efficacy of the herb but also the safety of patients or consumers.

Ogbomoso, a city in Oyo state, South-western part of Nigeria are endowed with wide varieties of indigenous medicinal plants. These plants are used by the local herbalists for treatment of a number of diseases, both bacterial and non-bacterial type. Medicinal plant materials normally carry a large number of microbes originating from the soil and handlers. Microorganisms of various kinds are normally adhered to leaves, stem, flowers, seeds, and roots. Additional contaminants may also be introduced during harvesting, handling, and production of various herbal remedies since no conscious efforts are made to decontaminate the herbs other than washing them (Sofowora et al., 1982). Therefore, bacteriological contents in herbal concoctions should be evaluated. Microbial contaminations are frequently involved in herbal products since all products come from plants. In this study, the bacteriological and antimicrobial sensitivity assessment of bacterial of herbal concoctions prepared locally for the treatment of malaria, fistula, convulsions, and skin rashes was investigated.

MATERIALS AND METHODS

Sample collection

All the different plant ingredients for the preparation of the herbal concoction for various treatments were obtained from different sellers in Oja-igbo market in Ogbomoso, Oyo state, Nigeria, as shown in Table 1. Based on the treatment, the samples were collected differently in separate sterilized containers. Some of the herbal ingredients were prepared in the form of concoctions (soup or drink made usually from ingredients after boiling) or infusions (soaking the plant material and allowing it to stand for varying lengths of time).

Microbiological analysis

Materials used

The materials used for this experiment included: the various herbal mixtures, autoclave, Petri-dishes, cotton wool, nutrient agar, Potato dextrose agar, inoculating loop, distill water, slant bottles, beakers, measuring cylinders, jars, electrical cooker, measuring scale, aluminum foil, spatula, spirit lamps, test tubes.

Sterilization

All the test tubes, beakers and glass wares were placed in the autoclave and then subjected to a temperature of 121°C for 15 min. After sterilization, they were allowed to cool and kept for use.

Preparation of culture media

Nutrient agar

This is a microbiological growth medium commonly used for the routine cultivation of non-fastidious bacteria. The medium was prepared based on manufacturer's directives. Depending on the

Table 1. The botanical and common names, parts used and family of the plants used in the preparation of the herbal concoctions and the family which they belong.

Usage of herbal concoction/ botanical names	Common/local names (Y-Yoruba; H- Hausa; I-Igbo)	Parts used	Family
Malaria (Iba)			
Enantiachlorantha	Awopa (Y), yellow wood	Bark	Annonacease
Citrus aurantifolia	Osanwewe (Y), lime	Juice	Rutaceae
Cymbopoqoncitratus	Ewe tea (Y), Lemon grass	Leaf	Poacease
Maqniferaindica	Ewe mangoro (Y), Mango	Leaf	Anacardiaceae
Azadirachta indica	Dogonyaro (H), Neem tree, Aforo-oyingbo (Y),	Leaf	Meliaceae
Pile (Jedijedi)			
Sabiceacalycina	Ogan (Y)	Bark	Rubiaceae
Lanneawelwitschii	Orira (Y)	Bark	Anacardiaceae
Aristolochiaalbida	Akoigun (Y)	Leaf	Aristolochiaceae
Lophiralanceolata	Panhan pupa/funfun (Y)	Bark	Ochnaceae
Syzygiumaromaticum	Konofuru (Y), clove	Fruit	Myrtaceae
Tetrapleuratetraptera	Aidan (Y)	Fruit	Mimosaceae
Convulsion (Giri)			
Ocimum gratissimum	Efirin (Y), Nchianwu (I)	Leaf	Lamiaceae
Black alum	Omiroro	ND	ND
Skin rashes (Narun)			
Lophiraalata	Uda, pahan (Y),	Roots	ND
Ceibapentandra	Poripola(Y)	Stem	ND
Pergulariadaemia	Eseatufa, Kole-agbe (Y)	ND	ND

number of plates to be prepared, certain grams of the powder was dissolved in specific litre of distilled water in a beaker or jar, mixed vigorously and heated on an electrical cooker (hot plate) till the powder was completely dissolved. Thus was followed by sterilization at 121°C for 15 minin the autoclave.

Culturing of organisms

Serial dilution

The concentration of the original solution and the desired concentration will determine how much the dilution needs to be and how many dilutions are required and the total volume of solution needed. Using a sterile pipette, 1 ml of each of the prepared herbal concoctions were transferred to separate sterile test tube containing 9 ml of sterile distilled water and shaken to obtain 10^{-1} dilution of sample. 1 ml of 10^{-1} dilution of sample was taken into sterile test tube containing 9 ml sterile distilled water to obtain further dilution of 10^{-2}. The procedure was continued until the desired diluents were derived.

Pour plate method

Media pouring is defined as the process by which sterile media are being poured into sterile Petri dishes and this was done in the inoculating room in the laboratory. Before pouring of the media, the air conditional was put on and the working bench/ slabs were sterilized to avoid contamination.

Isolation and preservation of isolates

After inoculation and incubation of isolates to ensure growth of associated bacteria, colonies which develop on the plates were counted and recorded as colony forming unit per gram of the (Cfu g^{-1}) sample. From the mixed cultures, distinct colonies were picked and streaked unto a freshly prepared media under aseptic conditions to obtain pure cultures. All the pure cultures for bacterial isolates were kept on nutrient agar slants and stored in the refrigerator as working and stock cultures.

Characterization of isolates

Identification and taxonomic studies were carried out on the purified isolates on the basis of their cultural, morphological, biochemical, and physiological characteristics.

RESULTS

The results of the bacterial isolates present in the four samples of the herbal concoctions indicated that all the bacterial isolates obtained were Gram positive and they all appeared in rod form except for the bacteria-*Micrococcus sedentarius* which was isolated from Agboiba that is coccoid in shape. The biochemical analysis which included test for catalase, oxidase, indole, motility, glucose, xylose, sucrose and so on indicated diversity among the different isolated bacteria. Table 2 shows the specific bacterium isolated from the various samples of herbal concoction. The bacteria *Clostridium* was isolated from two herbal concoctions but the species varied; *Clostridium tertium* was isolated from the concoct-tion used in the treatment of fistula (Agbojedi) while *C. butyricum* was isolated from the concoction used in the treatment of convulsion (Agbogiri). Other bacteria isolated

Table 2. Biochemical Characteristics of the Bacterial Isolates from the Four Herbal Concoctions.

Sample	Catalase test	Oxidase Test	Indole test	Motility test	Methyl red test	Vogesproskauer	Urease activity	Citrate utilization	Starch hydrolysis	Gelatin hydrolysis	Casein hydrolysis	NO_3 reduction	Spore test	Coagulase test	Glucose	Xylose	Lactose	Sucrose	Maltose	Mannitol	Raffinose	Arabinose	Sorbitol	Sorbose	Salicin	Fructose	Probable organisms
Agbo Iba	+	–	–	–	–	+	–	–	–	+	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	***Micrococcus sendentarius***
Agbo Narun	+	–	–	–	–	+	+	–	–	+	–	–	–	–	+	–		–	+	–	–	–	–	–	–	+	***Corynebacteriumrenale***
Agbo Jedi	+	+	–	+	–	–	–	–	–	+	–	+	+	–	+	–	+	+	–	–	–	–	–	–	+	–	***Clostridium tertium***
Agbo Giri	+	+	–	+	–	–	–	–	–	–	–	+	+	–	+		+	+	–	–	–	–	–	–	+	–	***Clostridium butyricum***

+ = positive; - = negative.

Table 3. Antimicrobial Test for the Bacterial Isolates in Millilitre (mm).

Samples	Organism	OF	E	CIP	CD	GN	CX	CO	AP	FX	AU
Agboiba	*Micrococcus sedentarius*	R	R	R	R	R	R	R	R	R	R
Agbonarun	*Corynebacterium renale*	R	R	R	R	R	R	R	R	R	R
Agbojedi	*Clostridium tertium*	R	R	R	R	R	R	R	R	R	R
Agbogiri	*Clostridium butyricum*	6.0	R	R	R	7.0	R	R	R	R	R

OF, Ofloxacin; E, Erythromycin; CIP, Ciprofloxacin; CD, Clindamycin; GN, Gentamycin; CX, Cephalexin; CO, Cotrimoxazole; AP, Cloxacillin; FX, Ceftriaxone; AU, Augmentin; R, Resistance.

from concoctions used in the treatment of malaria and skin rashes are *Micrococcus sedentarius* and *Corynebacterium renale* respectively.

Table 3 shows the result of the antimicrobial test of the isolated bacteria. Virtually all the micro-organisms were resistant to the antibiotics except for *C. butyricum* which was susceptible to Ofloxacin (OF, 5 µg) and Gentamycin(GN, 10µg) within the range of 6.0-7.0 mm. Other antibiotics which the bacteria were resistant to are; Erythro-mycin (E, 10 µg), Ciprofloxacin (CIP, 5 µg), Clin-damycin (CD, 10 µg), Cephalexin (CX, 30 µg), Cotrimoxazole (CO, 50 µg), Cloxacillin, (AP, 30 µg), Ceftriaxone (FX, 30 µg) and Augmentin (AU, 30 µg).

DISCUSSION

Results obtained from this research shows that the common herbal concoction contained different bacterial isolates. The samples were all water based. Majority of the herbal ingredients are always exposed not following good manufacturing practices and this accounts for the high rate of bacterial contaminants that renders the concoct-tions virtually fearful especially when consumed excessively with improper prescription.

C. tertium is a spore forming anaerobic Bacillus found in the gut of many animal species including humans (Miller et al., 2001). This bacterium is a non-toxin producing, aerotolerant, non-histotoxic and non-lipolytic specie. *C. tertium* has traditionally

been considered non-pathogenic (Ray et al., 2003), but it is found associated with meningitis, septic arthritis and pneumonia (Ferrell and Tell, 2001). *Clostridium butyricum* is a strictly anaerobic endospore forming, butyric acid producing Bacillus (Seki et al., 2003). *C. butyricum* is a soil inhabitant and uncommonly reported as a human pathogen (Meng et al., 1997). *Corynebacterium renale* has not been reported as human pathogen but a pathogenic veterinary bacterium that causes cystitis and pyelonephritis in cattle. The bacterium is sensitive to the majority of antibiotics, such as Penicillin, Cephalosporin, Tetracycline and Chloramphenicol. *M. sedentarius* is also a soil inhabitant. This organism cannot by itself initiate infection but is an opportunistic pathogen particularly in hosts with immune-compromised immune system (Smith et al., 1999).

Conclusion

This present study has shown that there are varieties of microorganism present in our various herbal concoctions which could have resulted from contaminated soils, plants and its products, preparation processes, quality of water, containers and processing equipment. However, these microorganisms exhibit multi-resistance to many antibiotics. Since herbal concoctions are mainly prepared for human consumption, there is a very high chance of passing the antibiotics resistant microorganisms into the human ecosystem. This poses a great danger to human health. Since herbal concoctions are prepared using varieties of medicinal plants which contain active constituents that are cheap and effective against common bacterial infections. Therefore, it is suggested that proper hygienic conditions should be maintained in all preparation processes starting from plant collection, processing, packaging and storage. There is need for mass education to enlighten the public on excessive consumption of herbal products and other drugs since many microorganisms isolated from this study are resistant to most of the common antibiotics. Also, herbal practitioners should be encouraged to send their products regularly to laboratories for quality assessment to ensure consistency and quality before marketing.

REFERENCES

Adenisa SK, Idowu O, Ogundaini AO, Oladimeji H, Olugbade TA, Onawunmi GO, Pais M (2000). Antimicrobial constituents of the leaves of *Acalyphawilkesiana* and *Acalyphahispida*. Phytother. Res.14: 371-374.

Babu PD, Subhasree RS (2009). Antimicrobial activities of *Lawsonia inermis* - a review. Acad. J. Plant Sci. 2(4): 231-232.

Balunas MJ, Kinghorn DA (2005). Drug discovery from medicinal plants. Life Sci. 78:431–441.

Bisset NG (1994). Herbal Drugs and Phytopharmaceuticals. CRC Press, Boca Raton, FL.

Bodeker C, Bodeker G, Ong CK, Grundy CK, Burford G, Shein K (2005). WHO Global Atlas of Traditional, Complementary and Alternative Medicine. World Health Organization, Geneva.

Burapadaja S, Bunchoo A (1995). Antimicrobial activity of tannins from *Terminalia citrina*. Planta Medica 61: 365-366.

Busse W (1999). The Processing of Botanicals. In Botanical Medicine: Efficacy, Quality Assurance and Regulation. Madison N.Y, Mary Ann Liebert Inc. 143 – 145.

Evans WC (1989). Trease and Evans Pharmacognosy, 13th edition, ELBS Imprint, Great Britain

Evans WC (1996). Trease and Evans Pharmacognosy, 14th edition, ELBS Imprint, Great Britain

Ferrell ST, Tell L (2001). *Clostridium tertium* Infection in a Rainbow Lorikeet with Enteritis. J. Avian Med. Surg. 15(3):204-208.

Lanfranco G (1999). Invited review article on traditional medicine. Elect. J. Biotechnol. 2: 1-3.

Mahesh B, Satish S (2008). Antimicrobial activity of some important medicinal plant against plant and human pathogens. World J Agric Sci. 4 (S): 839-843.

Meng X, Karasawa T, Zou K, Kuang X, Wang X, Lu C, Wang C, Yamakawa K, Nakamura S (1997). Characterization of a neurotoxigenic Clostridium butyricum strain isolated from the food implicated in an outbreak of food-borne type E botulism. J. Clin. Microbiol. 35:2160–2162.

Miller DL, Brazer S, Murdoch D, Barth RL, Corey GR (2001). Significance of *Clostridium tertium* Bacteremia in Neutropenic and Non-neutropenic Patients: Review of 32 Cases. Brief Reports CID 2001: 32.

Okigbo RN, Anuagasi CL, Amadi JE (2009). Advances in selected medicinal and aromatic plants indigenous to Africa. J. Med. Plant Res. 3(2): 86-95.

Ray P, Das A, Singh K, Bhansali A, Yadav TD (2003). *Costridium tertium* in Necrotizing Fasciitis and Gangrene. Emerg. Infect. Dis. 9(10):1347-8.

Seki H, Shiohara M, Matsumura T, Miyagawa N, Tanaka M, Komiyama A, Kurata S (2003). Prevention of antibiotic-associated diarrhea in children by *Clostridium butyricum* MIYAIRI. Pediatr. Int. 45:86–90

Seyyednejad SM, Motamedi H (2010). A review on native medicinal plants in Khuzestan, Iran with antibacterial properties. Int. J. Pharmacol. 6(50): 551-560.

Smith K, Neafie R, Yeager J, Skelton H (1999). *Micrococcus folliculitis* in HIV-1 disease. Br. J. Dermatol. 141 (3): 558–61.

Sofowora A (1982). Medicinal plants and traditional medicine in Africa. Chichester: Wiley, p. 256.

Sukanya SL, Sudisha J, Hariprasad P, Niranjana SR,Prakash HS, Fathima SK (2009). Antimicrobial activity of leaf extracts of Indian medicinal plants against clinical and phytopathogenic bacteria. Afr. J. Biol. 8(23): 6677-6682.

World Health Organization (1998). Quality control methods for medicinal plants: Determination of microorganisms. Geneva: WHO: 64-73. (Technical report series no. 18).

World Health Organization (2001). General guidelines for methodologies on research and evaluation of traditional medicine. WHO, Geneva, Switzerland. p. 1.