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Research Paper

Antibacterial activity, phytochemical properties and mineral content of "Aju Mbaise" decoction administered to nursing mothers

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This study was conducted to evaluate the phytochemical properties, mineral composition and antibacterial activity of "Aju Mbaise" plant decoction. The objectives are as follows; To extracts and concentrate the active principles in the plants using hot water, To investigate the antibacterial activity of the extracts of the plants ("Aju Mbaise") against some clinical pathogens, To determine the phytochemical constituent of the plants, To determine the mineral content of the extract/decoction. The antibacterial activity was determined using the discs diffusion method with *Salmonella spp, Staphylococcus aureus* and *Escherichia coli* as test isolates. The mineral content was determined using atomic absorption spectrophotometer while the phytochemical analysis was conducted using standard analytical methods. The result of the phytochemical screening showed that "Aju Mbaise" contained appreciable amount of metabolites: alkaloids (6.343%), tannins (3.240%), flavonoids (0.366%), cyanogenic glycoside (0.068%), and saponin (7.600%). While mineral element analysis showed that the decoction contained high level of potassium (130.30 mg/ml),calcium(12.43 mg/ml), magnesium (10.40 mg/m) and sodium(4.60mg/ml) and low levels of iron (2.15 mg/ml), zinc (2.23 mg/ml), phosphorus (0.80 mg/ml), copper (0.07 mg/ml), manganese(0.05 mg/ml) and chromium (0.06 mg/ml). The antibacterial screening revealed that the decoction inhibited the growth of gram negative bacterial with the maximum inhibition observed against E. coli (9.0 mm), followed by *Salmomellas pp* (8.5 mm) and the minimum inhibition observed against E. Coli (7.5mm) and Salmonella spp (6.5 mm) while the gram positive bacterium (*Staphylococcus aureus*) was resistant to the decoction. These results suggest that, the decoction of "Aju mbaise" plants contains active principles which are responsible for their antibacterial activities and this provides the basis for their folkloric use as cure for various human ailments.

Keywords: Antibacterial activity, Phytochemical, Decoction, Nursing mothers.

INTRODUCTION

From time immemorial, plants have been utilized as therapeutic agent in both organized and unorganized forms (Girach et al., 2003). Plants contain accumulated natural products, biologically active materials and ingredients which have various effects. Some of these active ingredients accumulate in certain parts of the plants. It is only those portions of these plants that contain active ingredients that are used for therapeutic purposes. The part that contains the active ingredient is taken in the form of extract, infusion and decoction (Bukar et al., 2010).

According to the World Health Organization (WHO) (2002),

80% of the world population lives in the developing countries and 80% of them use traditional plant extracts (medicine) to meet most of their needs in primary health care. This means that about 3.2 million people around the world use plant extracts as drugs. It is also estimated that 25 to 50% of the 2500 flowering plants have been used at one time or another for therapeutic purposes. The use of plants and their natural product in Nigeria as either extracts or infusion is a widespread practice in the treatment and management of diseases (Bukar et al*.,* 2010).

According to the World Health Organization (2002), plant that contains subsistence in one or more of its organs that can be used for therapeutic purposes is called medicinal plants. Such a plant will have its parts including leaves, roots, rhizomes, stems, barks, flower, fruits, grains, or seeds, employed in the control or treatment of a disease condition and therefore contains chemical components that are medically active. These non nutrient plant chemical compounds or bioactive components are often referred to as phytochemicals or phyto constituents that are responsible for protecting the plant against microbial infections or infestations by pests (Nweze et al*.,* 2004).

Herbal plants as the major remedy in traditional system of medicine have been used in medical practices since antiquity. In addition to their ancient historical uses, the therapeutic efficacy of many indigenous plants for various diseases has been described by traditional herbal practitioners. Plants are the richest source of natural antimicrobial agents. Traditional healers claim that some plants are more efficient to treat infectious diseases than synthetic antibiotics. From ancient times, different parts of plants have been used to cure specific ailments. The plants are widely used because of their easy availability and cost effectiveness (Cordell, 2000).

The antimicrobial activities of plant decoction may reside in a variety of different components, including aldehyde and phenolic compounds. The types of plants and methods of application vary from locality to locality with 80% of rural dwellers relying on them as means of treating various diseases. For example, the use of bearberry and cranberry juice to treat urinary tract infections is reported in different manuals of phyto-therapy, while species such as lemon balm, garlic and tea tree are described as broad-spectrum antimicrobial agents (Heinrich et al .,2004).

A single plant may be used for the treatment of various disease conditions depending on the community. Several ailments including fever, asthma, constipation, oesophageal cancer and hypertension have been treated with traditional medicinal plants (Duraipandiyan et al*.,* 2006). The plants are applied in different forms such as poultices, concoctions of different plant mixtures, infusions as teas or tinctures or as component mixtures in porridges and soups administered in different ways including oral, nasal (smoking or steaming), topical (lotions, oils or creams), bathing or rectal (enemas). Different plant parts and components (roots, leaves, stem barks, flowers or their combinations) have been employed in the treatment of infectious pathologies in the respiratory system, urinary tract, gastrointestinal and biliary systems, as well as on the skin (Adekunle and Adekunle, 2009). Phytochemicals are natural compounds and they form the base of modern drugs as we use today (Cordell, 2000). Phytochemicals are basically divided into two groups; primary and secondary metabolites. Primary metabolites consist of alkaloids, flavonoids, tannins etc. (Kumar et al*.,* 2005). Phytochemicals like flavonoids and phenols are strong antioxidants and have an important role in the health care system (Sofowora, 1993). Antibiotics are one of our most important weapons in fighting microbial infections and have greatly benefited the health-related quality of human life (Fransworth, 1993). Although, antibiotics have been widely used in last decades, the development of microbial resistance to them is also increased (Cohen, 1992). In order to overcome this problem, scientists deviated their research towards anti-microbial compounds of medicinal plants as an alternative solution. The demand for herbal medicine is due to their wide biological activities, higher safety compared to synthetic drugs and low cost (Grabley and Thiericke, 1999).

With the upsurge in the use of these herbal plants, there is a need for a thorough scientific evaluation to validate the supposedly therapeutic effects of these plants. Therefore, this study aimed at to determine the antibacterial activities, mineral content and phytochemical properties of “Aju Mbaise” decoction administered to nursing mothers.

MATERIALS AND METHODS

Sample collection, identification and preparation

The fresh leaves of the plants (“Aju Mbaise”) *sphenocentrum jollynum, Cnestis ferruginea, Psidium guajava.L, Jatropha curcas, Diodia sarmentosa* and *Heterotis rotundifolia* were collected from Mbaise and was identified and authenticated at the Herbarium Unit, Botany Department, Abia State University Uturu, Nigeria. The samples were air dried for seven days under shed, at room temperature, to avoid loss of active compounds and then grounded into powder using a hand milling machine. These were stored in an air tight glass bottles for further use.

Extraction process

Aqueous extract

The extraction process used was hot-water method

(decoction) following the procedure of Handa et al. (2008). 50 g of the powdered sample "Aju Mbaise" were soaked in 500 ml of distilled water and boiled for about ten minutes. After boiling, the sample was double-filtered using cheese cloth and collected in a conical flask and allowed to cool. It was put in plastic containers and stored at 4°C until required for analysis

Preparation of the test organism

The isolates identified were *Salmonella spp, Staphylococcus aureus* and *E. coli* were then sub-cultured on sterile Nutrient agar and Nutrient broth. The microbial cultures were diluted with Peptone water until the final suspension that contained about 1.0x108Cfu/ml of the isolates was obtained. The cell densities obtained were in accordance with 0.5 McFarland's standard which was used in all the investigations.

Preparation of Nutrient Broth

14 g of Nutrient broth powder was suspended in 1000 ml of distilled water. It was boiled to dissolve completely. The medium was dispensed and sterilized by autoclaving at 121°C for 15min and was allowed to cool to 45°C.

Preparation of nutrient agar

24 g of Nutrient agar powder was dissolved in 1000 ml of distilled water; it was gently heated to dissolve the medium completely and was sterilized by autoclaving at 121°C for 15min and was allowed to cool to 45°C.

Preparation of peptone water

16 g of peptone was dissolved in 1000ml of distilled water and was heated gently to dissolve the medium completely. It was sterilized by autoclaving at 121°C for 15min and was allowed to cool to 45°C.

Preparation of paper discs

Some filter papers were perforated in order to get several paper discs of about 6mm in diameter. The paper discs were sterilized in a hot air oven at about 120°C for 25-30 min.

Antibacterial sensitivity testing of "Aju Mbaise" decoction

Disc diffusion method as described by Osadebe and Ukwueze, (2004) was used for this study to determine the antibacterial activity of the decoction. 0.2 ml aliquot of the extracts was dropped on a sterile filter paper disc of 6mm in diameter and allowed to get absorbed before they were placed into solidified Nutrient agar plate, which was inoculated with each of the test organisms and appropriately labelled. Discs impregnated with chloramphenicol were used as control in each case. The Nutrient agar plates were than incubated at 37°C for 24 h. The zones of growth of inhibition were measured with a meter ruler.

Determination of minimum inhibitory concentration (MIC) of the decoction

One gram of the extract was dissolved in 4ml of Nutrient broth which gave 250 mg/ml. 0.8 g of the same extract was also added in 4 ml of Nutrient broth for concentration of 200 mg/ml. However, two fold dilutions were done from the 200 mg/ml concentration. This was done by putting 2 ml of the 200 mg/ml concentration to 2 ml of nutrient broth contained in a test tube and was homogenized. After obtaining different concentrations, 3 drops of overnight broth cultures of the test organisms were inoculated into the concentrations. Each of the organisms was inoculated into different tubes containing the different concentration of decoction. The tubes were then incubated at 37°C for 24 h. The lowest concentration of the decoction that inhibited the growth of the test organisms was recorded as the minimum inhibitory concentration (MIC) of the decoction.

Determination for the minimum bactericidal concentration (MBC) of the decoction

Tubes without any visible growth from the MIC test were subculture on sterile Nutrient agar plates. They were then incubated at 37°C for 24 h. The lowest concentration of the extracts that shows no growth was recorded as the minimum bactericidal concentration (MBC) of the decoction.

Phytochemical screening of the plant "Aju Mbaise" test for alkaloid

2 g of the plant sample was added in 5 ml of 1% aqueous hydrochloric acid was added and was placed in a water bath for about 3 min. 3 drops of Mayer's reagent was added. A white precipitate was an indication of a positive test while none indicates a negative test (Trease and Evans, 1989).

Test for flavonoids

5 ml of dilute ammonia was added to a portion of an aqueous filtrate of the plant. 1 ml of concentrated

Table 1. Zones of growth inhibition produced by decoction of “Aju Mbaise” on isolates at different concentration in (mg/ml).

Test Organisms	Concentration of extracts (mg/ml) Crude extract							
	Crude extract	250mg/ml	200mg/ml	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	Control
E. coli	9.0	7.5	7.5	-	-	-	-	18
Salmonella spp	8.5	8.0	6.5	-	-	-	-	30
Staphy aureus	-	-	-	-	-	-	-	19

Key: - No zone of inhibition.

Table 2. Minimum inhibitory concentration (MIC) of the decoctionof “Aju Mbaise” plant on isolate at different concentration at (mg/ml).

Test Organisms	Concentration of extracts (mg/ml)						
	250mg/ml	200mg/ml	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	MIC (mg/ml)
E. coli	-	-	+	+	+	+	200
Salmonella spp	-	-	+	+	+	+	200
Staphyaureus	R	R	R	R	R	R	R

Key: - = No growth, + = Growth, R = Resistance.

Table 3. Minimum bactericidal concentration (MBC) of the decoction of “Aju Mbaise” plant on isolate in different concentration at (mg/ml)

Test Organisms	Concentration of extracts (mg/ml)						
	250mg/ml	200mg/ml	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	MBC (mg/ml)
E. coli	-	-	+	+	+	+	200
Salmonella spp	+	+	+	+	+	+	>250
Staphy aureus	R	R	R	R	R	R	R

Key: - = No growth, + = Growth, R = Resistance

tetraoxosulphate (IV) acid was then added. A yellow coloration that disappears on standing indicates the presence of flavonoids.

Test for saponins

10 ml of distilled water was added to two gram (2 g) of the sample in a test tube, shake vigorously and was heated. The persistent frothing even after heating is an indication of the presence of saponins.

Test for tannins

2 ml of 1% ferric chloride was added in 1gm of the sample. A colour change was an indication of the presence of tannins.

Test for cyanogenic glycoside

1 g of the plant sample was added to 2 ml of chloroform. Then 2 ml of concentrated tetraoxosulphate (VI) acid was added to form a lower layer. A reddish brown colour at the inter phase shows a positive test.

RESULTS AND DISCUSSION

The preliminary phytochemical screening and Quantitative analysis of “Aju Mbaise” plants results shows the presence of important phytochemicals which include alkaloids (6.343 %), saponins (7.600 %), tannins (3.240%), flavonoids (0.366 %), and cyanogenic glycoside (0.068%), which corresponds with a previous report by Rejesh et al. (2008) who stated that plants contained active components which are active against microorganisms (Tables 1-5). These natural metabolites are vital as potential antimicrobial agents (Dwivedi et al., 2011).

These natural metabolites play important role in cancer prevention and treatment (Bonyadi *et al.,* 2009). Alkaloids often have pharmacological effect when administered to woman. Plants containing alkaloids have been known to possess antidiarrheal activities and are known to be the largest groups of secondary metabolites in plants (Obasi et al., 1990). Pure plant isolated alkaloids can be used as basic medicinal agents for analgesic, antispasmodic and bactericidal effects (Stray, 1998). Flavonoids are antioxidants and free radical scavengers which prevent

Table 4. Preliminary phytochemical screening of "Aju Mbaise" plant

Secondary metabolites	Signs	Degree of Presence
Alkaloids	++	Medium Conc.
Tannins	++	Medium Conc.
Saponins	+++	High Conc.
Flavonoids	+	Low Conc.
Cyanogenic glycoside	+	Low Conc.

Table 5. Quantitative phytochemical screening of the plant

Secondary metabolites	% per gram
Saponins	7.600
Alkaloids	6.343
Tannins	3.240
Flavonoids	0.366
Cyanogenic glycoside	0.068

Table 6. Mineral content of "Aju Mbaise" decoction.

Mineral	Degree of Presence	Per 100 ml
Mn	Trace	0.05 mg
Na	Low Conc.	4.60 mg
K	High Conc.	130.30 mg
Ca	Medium Conc.	12.43 mg
Fe	Low Conc.	2.15 mg
Cu	Trace	0.07 mg
Mg	Medium Conc.	10.40 mg
Cr	Trace	0.06 mg
P	Low Conc.	0.90 mg
Zn	Low Conc.	2.23 mg

oxidative cell damage; it has strong anticancer activity and protects the cells against all stages of carcinogenesis (Okwu and Iroabuchi, 2004). Flavonoids in the intestinal tract lower the risk of heart disease (Okwu, 2005). It has been discovered in various studies that flavonoids contains hypolipidemic potential (Narender et al*.,* 2006).

Tannins have been reported to possess astringent properties that hasten the healing of wound and inflamed mucus membranes (Okwu, 1998). Tannins have been found to form irreversible complexes with prolin-rich protein (Shimada, 2006) and these results in the inhibition of cell protein synthesis. Plants that contain tannins as major constituents are used for the treatment of intestinal disorders like diarrhoea and dysentery (Dharmanda, 2003). It has also been revealed that tannins react with proteins to provide tanning effect which helps in the treatment of inflamed/ulcerated tissues (Parekh, 2007). Plants that contain tannins as major constituents are used for the treatment of intestinal disorders like diarrhoea and dysentery (Dharmanda, 2003).

Phenolic compounds are synthesized in plants as secondary metabolites. They have several biological activities which include anti-oxidant, anti-inflammatory, anti-aging and inhibitory properties. It is believed (Dwivedi et al*.,* 2011). The presence of these phenolic compounds in "Aju Mbaise plant" reveals its antimicrobial activity. Therefore, the antibacterial activities of "Aju Mbaise" decoction may be connected with the presence of these phenolic compounds present in them.

The decoction contained essential minerals (Table 6). Minerals are required for normal growth, activities of muscles and skeletal development (Calcium), cellular activities and oxygen transport (Copper and Iron), chemical reaction in the body and intestinal absorption (Magnesium), fluid balance and nerve transmission (Sodium and Potassium).This corresponds to Oluyemi et al. (2006) which reported that Iron is useful in prevention of anaemia and other related diseases.

Indrayan et al*.* (2005) reported that sodium (Na) play a role in ionic balance of the human body and maintain tissue excitability. Because of the solubility of salts,

sodium is essential in the transport of metabolites. Magnesium is an activator in enzyme systems which maintain an electrical potential in nerves, while sodium and potassium influences osmotic pressure and contribute to normal PH equilibrium. Magnesium as well plays a role in energy production and in supporting the immune system (Muhammad et al., 2011). Zinc insufficiency can lead to stunted growth in children and to change in their appetite, taste, smell and weight loss (Black et al. 2004).Calcium is an important constituent of body fluid and bone formation coupled with phosphorus Nutrients rich foods are vital both in adult and children. Deficiency of these minerals can lead to malfunction and health of humans (Merck, 2005).

Conclusion

This research work showed that the decoction contained appreciable amounts of minerals such as calcium, magnesium, potassium, sodium, zinc, copper, manganese, iron, chromium and phosphorus. The minerals are comparable to those found in food regarded as good dietary sources of these minerals which are required to improve health and growth performance in humans. The decoction possessed active principles that are known to inhibit Gram negative bacterial and this potency are attributed to the presence of the phenolic compounds found in them. Hence, the results of this study justify the use of “Aju Mbaise” decoction for therapeutic purposes.

Recommendations

“Aju Mbaise” decoction is mainly taken by nursing mothers; hence, there is a need for the comprehensive study of the side effect of this decoction to the mother and child. Also, the toxicity of the decoction, stability and the standard dosage of the decoction should be studied in details.

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