wjpmr, 2017,3(11), 09-13

SJIF Impact Factor: 4.103

WORLD JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.wjpmr.com

Research Article

ISSN 2455-3301

WJPMR

EFFECTIVENESS OF EXTRACT OF *MORINGA OLEIFERA* IN TREATMENT OF INFANTILE DIARRHEA

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Article Received on 24/09/2017 **Article Revised on 15/10/2017** **Article Accepted on 05/11/2017**

ABSTRACT

Study to determine the antibacterial effect of ethanol extract of leaf, seed and seed coat of Moringa oleifera on bacterial agents of infantile diarrhea was carried out. This was determined using disc diffusion and well-in-Agar antimicrobial screening methods. A total of sixty nine suspected infants (0-5years) were screened using standard microbiological methods. Bacterial agents isolated and identified were *E-coli* and *Salmonela sp.* While *E.coli* was isolated from 17(60.71%) subjects, *salmonella sp* was isolated from 11(39.2%) subjects. Results showed that more infants of three years old had diarrhea while those at the age of five years were the least. Male infants were affected more than the female infants. Well in agar antimicrobial screening methods showed appreciable inhibitory effect on both E. coli and *Salmonella sp* than disc diffusion method. Furthermore, ethanolic extract from the leaf exhibited more antimicrobial action on the two test isolates followed by extract from the seed, then extract from the root. While extract from the seed coat only showed that alkaloids and terpenoids were absent in the leaf and root saponin was absent in the seed, tannin was only present in the leaf. Above results indicate that extract from most parts of *Moringa oleifera* have curative hence can be used for the cure of diseases such as diarrhea.

KEYWORD: Antibacterial, extracts, Moringa oleifera, Bacterial and infantile Diarrhoea.

INTRODUCTION

Moringa oleifera is the most widely cultivated species of the genus Moringa, which is the only genus in the family Moringaceace (NRC, 2006). English common names include moringa, nezolive tree, west indian. It is also known as drumstick tree, from the appreance of the long slender, triangular seed pods, horseradish tree, from the taste of the roots which resembles horseradish, or oil tree, from the oil derived from the seeds (NRC, 2006). The tree itself is rather slender, with dropping branches that grow to approximately 10cm in height. In cultivation, it is often cut back annually to 1-2 meters and allowed to regrow so the pods and leaves remain within arms's reach (Makkar *et al;* 2007).

In developing countries, Moringa has potential to improve nutrition, boast food security, foster rural development, and support sustainable land care. It may be used as forage for livestock or a micronutrient liquid (Makkar *et al;* 2007).

Diarrhea is derived from the Greek word "diarrhoea" meaning "flowing through" (Ethlberg *et al;* 2006) further defined diarrhea as frequent bowel evacuation or the passage of abnormally soft or liquid feaces. World Health Organisation (WHO, (2000) defined dairrhoeas as the passage of loose or liquid feaces. World Health Organisation (WHO), (2000) defined diarrhea as the passage of loose or liquid stools more frequently than is normal for the individual.

Diarrhea commonly results from gastrentririts caused by bacteria toxin (Willey *et al;* 2006). In malnourished indivdiuals, diarrhea can lead to severe dehydration and become life threatening without treatment (Valentiner *et al;* 2003). Diarrhoea is a leading cause of childhood mortality in the developing world (WHO 1997). It causes 1.5-5.1 million infant deaths per year in the whole world (Bern *et al*; 1992). In addition, it contributes to malnutrition and retarded physical growth and mental development (Bagin *et al;* 1993). A research conducted in southeast Asia and Africa, proved that diarrhea is responsible for 8.5% and 7.7% of all deaths, respectively annualy (WHO, 2000).

According to Bern *et al;* (1992), bacterial agents that cause diarrhea in infants and children are *Escherichia coli*, *Campylobacter jejuni*, *Shigella sp*. *Salmonella sp*. *Enterobacter sp*. *Vibrio cholera* and *Yerisinia entercolitica*.

The practical importance of this study is that it will provide health planners, researchers and healthcare providers in the country with information to assess the status of the infection among infants, the bacteria causative agents and infantile diarrhea, the anti-microbial ability of *Moringa Oleifera* to those agents. Additional objective of this study is to assess an alternative way of treatment of infantile diarrhea, using natural occurring plant specimen such as *Moringa Oleifera*.

MATERIALS AND METHODS

Sample Collection

Stool Sample: Stool samples were collected from different heath institutions in Owerri, Nigeria using clean wide mouth specimen bottles. Approval was obtained from hospital ethical committee prior to collection and informed consent was obtained from the parents or care givers of the infants (subjects) engaged in this study.

Sixty nine (69) children aged 0-5 years with diarrhea were recruited consecutively as they presented to the health facility from February to October 2013. Parameters such as Age, Sex, social, economic and nutritional status of the subjects were matched. A pretested questionnaire was used to obtain information on age, sex, breastfeeding status, occupation/education status of the parents.

The social class was determined using classification of social class as proposed by Oyedegi (1995). This classification used the parental occupation and educational attainment to determine the social class. The nutritional status was determined using the modified well come classification (Hendrickse et *al*, 1991).

Moringa Olifera Sample: Fresh leaf, seed coat and root of *Moringa Oleifera* samples were collected from Springfield integrated Farms, Owerri, Nigeria. The plant materials were identified and authenticated by plant Taxanomists at the plant science and bio-technology department, Imo State University, Owerri, Nigeria. The samples were processed according to the method adopted by WHO. This was carried out by drying them in the laboratory for four weeks and then ground into powdered forms, using a mortar and pestle, and stored in a sterile bottle for future use.

Laboratory Analysis

Stool appearance and macroscopy: Physical appearance of each stool sample which include consistence, colour was critically determined. Also, presence of blood and worms were noted (Cheesbrough, 2002). Smear of the stool samples were prepared on clean grease free glass slides following the methods described by fawole and Ose (2004).

Cultural Analysis of Stool Samples

Cultural analysis of stool samples was carried out using methods as described by cheesbrough (2002), Suleman and Ibarhim (2002), Fawole and Ose (2004) and Prescott *et al*; (2007). Stock cultures of isolates were maintained on nutrient agar slant for further identification (Suleman *et al*; 2002). The density of suspension on the media for susceptibility test was determined using Mcfarland standardization method.

Identification of Bacterial Isolates

Bacterial isolates were identified using colonial and cellular characteristics, then biochemical properties. Biochemical tests carried out include, gram staining, urease test, citrate utilization test, oxidase test, indole test, methyl-red test, vogas –proskauer test, coagulase test, catalase test and sugar fermentation test.

Extraction of Plant Materials

The method of Ogbonna *et al;* (2010) was adopted for extraction of the plant materials.

Assays for Antimcrobial Activity

Antimicrobial activity of extracts of moringa oleifera on the isolates was carried out using well in agar and Disk diffusion sensitivity pattern as described by Oluma *et al;* (1984) and Doughari *et al;* (2007).

Disc Diffusion method: Sterile Whattman No 1 filter paper disc were soaked in different concentration of different *moringa oleifera* plant parts extracts to be impregnated with the extract. With the aid of a sterilized forcep, the paper disc impregnated with the concentration of plant extracts were placed on the surface of the nutrient agar plates previously inoculated with standardized inoculum of different bacterial isolates. Single disc containing 30mg of chloramphenicol was placed on the nutrient agar surface as positive control. All plates were then incubated at 37^0C for 18-24hours. After the incubation period, the antimicrobial activity was determined through measurement of the diameter of zones of inhibition (mm) around each of the extracts and the antibiotics.

Well- in Agar Method: A sterile coke borer was used to make five wells (5mm diameter) in plants inoculated with standardized inoculums of different bacterial isolates, Half millitre of different concentration of the extract from different parts of the plant was then introduced into the well using sterile Pasteur pipettes. Well containing chloramphenicol (30mg) was included as control. All plates were then incubated at 37^0C for 18-24 hours. After the incubation period, the antimicrobial activity was determined through measurement of the diameter of zones of inhibition (mm) around each of the extracts and the antibiotic.

Determination of Minimum Inhibitory Concentration: The minimum inhibitory concentration of ethanol extracts from the leaves, seed-coat, seed and root of *Moringa oleifera* was determined as described by Akinpelu and Kolawole (2004).

Determination of Minimum Bactericidal Concentration (MBC): The minimum bactericidal concentration (MBC) of the plant extract on the clinical bacteria isolates was carried out as described to Ajaiyeoba *et al*, (2003).

Phytochemical Analysis

Phytochemical analysis was performed following the method of Sankar (2012). Analysis of Phytochemicals from solvent free extract of *Moringa oleifera* was individually performed using different quantitative tests for alkaloids, carbohydrates and glycosides, flavonoids, steroids, terpenoids, saponin and tannin.

RESULTS

Isolation of Enteropathogens

Out of sixty nine diarrhoearic children examined, 28 (40.58%) diarrhea stools that contained recognizable enteropathogens isolates were subjected to cultural, morphological and biochemical analysis and this revealed them to belong to two bacteria groups; *Escherichia coli* and *Salmonella species. E. coli* were found to be implicated in 17 (60.71%) subjects while *Salmonella sp* was isolated from 11 (39.29%) subjects as shown in the table 1.

Table 1: Overall prevalence of bacterial agents of infantile diarrhea in the sample.

Isolates	Percentage occurrence (%)
Escherichia coli	60.71
Salmonella sp	39.29
Total	28 (100)

Age Distribution of Infants (Children) With Diarrhea

Table 2 Shows the age distribution of children with acute diarrhea according to the bacteria isolated. These comprised children with positive culture and those with negative stool culture. The result shows that the children between the age group of 13-36 months accounted for 57% of the total bacteria isolated. Children in the age group of 49-60 months had lowest (10.41%) incidence of bacteria associated with diarrhea.

Table 2: Age Distribution of children with Diarrhoea.

Age (months)	Subjects with Positive Stool Culture (%)	Subject With Negative Stool Culture (%)	Total No. (%)
2-12	5(17.86)	8(19.51)	13(18.84)
13-24	9(32.14)	11(26.83)	70(28.99)
25-36	7(25.00	9(21.95)	16(23.19)
27-48	4(14.29)	8(19.51)	12(17.39)
49-60	3(10.41)	5(12.20)	8(11.59)
Total	**28(99.7)**	**41(1000**	**69(100)**

This result showed that more males had positive stool culture 60.71% than females 39.29%. this is shown in table 2.

Distribution of Antibiotics Use Prior to Hospitalization

Table 3 shows the distribution of antibiotics treatment prior to hospitalization in children with diarrhea. From the table, it shows that among those with positive stool culture, 32.14% received antibiotics and 67.86 did not receive antibiotics. While among those with negative stool culture, 41.46% received antibiotics treatment prior to hospitalization and 58.54% did not.

Table 3: Distribution of antibiotics use prior to hospitalization in children with diarrhoea.

Antibiotics	Subjects with Positive Stool Culture No (%)	Subject With Negative Stool Culture No (%)	Total (%)
Yes	9(32.14)	17(41.46)	26(37.68)
No	19(67.86)	24(58.54)	43(62.32)
Total	28(100)	41(100)	69(100)

Phytohemial Analysis of Ethanoli Extrat of *Moringa Oleifera*

Table 4: Phytochemical analysis of *moringa oleiferaethanolic* extract.

	Plants Part			
Phytochemicals Components	**Leaf**	**Seed**	**Seed Coat**	**Root**
Alkaloid	+	+	+	+
Glycoside	+	+	-	+
Flavonoid	-	+	+	-
Steroid	+	+	-	+
Terpenoid	+	+	-	+
Saponin	+	-	+	+
Tannin	+	-	-	-
Anthraquinone	+	+	+	+

Key: + = Presence - = Absence

Different phytochemical components of *Moringa oleifera* are presented in table 4. The leaf, seed root and seed coat of *Moringa oleifera* etholic extracts contained a number of phytochemicals such as alkaloids, flavonoid, Glycoside, sterile, terpnoids, saponin, tannin and anthraquinone. However, the result revealed that the Ethanolic leaf extract lack flavonoid. Ethanol extract of seed, seed coat and root were found to be lacking tannin, while only the seed coat lacked glycoside, steroid and terpenoids.

Antimicrobial Assay of Extracts

Table 5: Well in Agar Antimicrobial activities of Ethanolic extract of parts of *Moringa Oleifera.*

Test org	Leaf 250 125 62.5 31.3	Seed 250 125 62.5 31.3	Seed Coat 250 125 62.5 31.3	Root 250 125 62.5 31.3
E. coli	40 31 25 18	21 16 11 9	0 0 0 0	30 22 18 15
Salm. Sp	29 21 16 11	15 14 11 6	0 0 0 0	25 21 18 13

Table 5 shows the results of antimicrobial activities of ethanolic extracts of *Moringa oleifera* parts (leaf, seed, seed coat and root) against then test isolates (*E. coli and salmonella sp*) using well-in-Agar method. The results showed that most of the isolates were highly resistant to the seed coat. The result also revealed that many of the *E.coli*was more susceptible than *Salmonella sp* with varying degree of zone of inhibition.

Table 6: Disk-diffusion method of Antibacterial activities of ethanolic extract of parts of *Moringa oleifera.*

Test org	Leaf 250 125 62.5 31.3	Seed 250 125 62.5 31.3	Seed Coat 250 125 62.5 31.3	Root 250 125 62.5 31.3
E. coli	20 18 15 12	15 13 10 6	11 6 0 0	21 16 14 12
Salm. Sp	91 17 13 9	13 11 9 0	0 0 0 0	19 17 15 12

Table 3.9 shows the results of antimicrobial activities of ethanolic extracts of *Moringa oleifera* parts (leaf, seed coat and root) against the test isolates were also sensitive to some parts (leaf, seed and root) but when compared with that of Well-in Agar method, their respective zones of inhibition were seen to have smaller resistance to the seed coat extract.

Table 7: Minimum Inhibitory Conc. Of ethanolic extract of parts of *Moringa oleifera* (mg/ml).

Test org	Leaf	Seed	Seed Coat	Root
E. coli	31.3	62.5	0	62.5
Salm. Sp	31.3	62.5	0	62.5

Table 8 shows the result of minimum inhibitory concentration (MIC) of all the extracts on the test isolates. The minimum inhibitory concentration ranged 31.3mg/ml-62.5mg/ml with one of the extracts (seed coat) not being determined. This is because ethanolic extract of *Moringa*seed coat is not active against two organisms.

Table 8: Minimum Bactericidal Concentration (%) of ethanolic extract of parts of *Moringa oleifera (mg/ml).*

Test org	Leaf	Seed	Seed Coat	Root
E. coli	62.5	250	0	125
Salm. Sp	125	0	0	125

DISCUSSION

The result shows that *E. coli* is one of the major indicators of bacterial contamination while *Salmonella sp* are moderate.

Pinfold (1990) discussed the advantages of *E. coli* as an indicator of faeco-oral contamination and disease. This is because during child bearing, infants are being exposed to food-borne germs for the first time and they are losing the protection of breast milk which has anti-infective properties.

Out of the bacteria isolated, *E. coli* recorded the highest of 60.71% followed by *Salmonella sp* of 39.29%. These show that there are high significant rates of *E. coli* due to contamination of foods or from the mothers' hands. Mothers' hands are important sources of coliform contamination than the infants as food can be contaminated through contact with dirty hands of food handlers. This again emphasizes the importance of personal hygiene, particularly frequent hand-washing of mothers and their infants. The similar view was reflected in earlier studies. Shalid *et al;* (1996) and Kha MU (1982) partially breastfed children are at high risk of *E. coli* and *Salmonella sp* than the exclusively breast-fed children. The reason is that breast milk is the ideal food for infants as it remains a major source of nutrients and meets the full term infant's complete nutritional needs and has low risk of bacterial contamination Fashey *et al;* (2000). The immunological benefits of breastfeeding are: reduces the risk of infectious diseases such as Gastroenteritis, respiratory infections and ear infections because material antibodies are passed to the infant (Fashey *et al;* 2000) and also reduces the risk of food allergy and the risk of sudden infant death syndrome (SIDS).

The growing resistance of microorganisms to convectional antimicrobial agents is a source of concern to clinical microbiologists all over the world. As a result,

efforts are being made to develop antimicrobial agents from local sources for better chemotherapeutic effects. The demand for more natural anti-microbial activities of plants extracts (Nasar-Abbas *et al;* 2004).

According to Mathabe *et al;* (2006) and Alumad *et al;* (2007), plant extracts are promising natural antibacterial agents with potential applications in pharmaceutical industries for controlling the pathogenic bacteria. Most antibacterial medicinal plants (example *M. oleifera*) attack Gram-positive strains while few are active against gram-positive strains while few are active against Gram-negative bacteria Srimvassa *et al;* (2001) and Jabeen *et al;* (2008).

In this study, the result showed that well in Agar method of antimicrobial sensitivity test of ethanol extracts of *M. oleifera* leaf were more active against all active and *Salmonella sp.*

The result shows that *M. oleifera* leaf extract (using well in Agar test) is more Antibactericidal than others (seeds, seed coat and roots). The antibacterial activity of seeds of *M. oleifera* has been investigated (Jabeen*et al*, 2008). Also the antibiotic principles of *M. oleifera* have been isolated by others Eilert (1976) and Faizi*et al*, (1994).

Phytochemically, the study revealed the presence of certain phyto consistuents. Meanwhile, several authors have characterized and reported several chemical compounds present in the leaf, seed, seed coat and roots. Faizi*et al*, (1994) reported the isolation of two nitrile glycosides from the ethanolic extracts of *M. oleifera* leaf. It is important for health workers to work with local communities to identify and encourage safe bearing practices and to improve infants' motion to increase their resistance to infection such as diarrhea.

Proper hand wash should be adopted. This should be observed by both the mother and the infant regularly. The leaf extracts of *M. oleifera* well-in Agar sensitivity test method showed an appreciable inhibitory activity against the two isolated bacteria (*E. coli* and *Salmonella sp.*). Hence from this study, well-in-Agar method of antibacterial sensitivity test of ethanolic leaf extract of *M. oleifera* is highly recommended for treatment of infantile diarrhea caused by bacterial agents.

Infantile diarrhea is one of the killer diseases among the children. The infection is widely perceived to be contracted through unhygienic condition of the mother, the child or the environment. The search for most curable ways of treating infantile diarrhea caused by bacterial agents (*E. coli* and *Salmonella sp*) mostly using well-in-agar as an Antimicrobial sensitivity/screening method.

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