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Application of Addawa'ul Humma Preparation for the Treatment of Typhoid Fever

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ABSTRACT

The study assessed the efficacy of Addawa'ul humma for the treatment of typhoid fever. Addawau'l humma is a name given to a herbal preparation consisting of (Mango, Neem, Orange, Lemons, and Guava extract). The preparation was tested and compare with the standard drug Ciprofloxacin as control experiment. Results of phytochemical screening showed the presence of Alkaloid, Flavonoid, Tannins, Glycosides, Anthraquinone, Saponins and Steroid. There is no significance difference between Addawa'ul humma and the standard drug Ciproflxacin ($P<0.05$). However, the physicochemical analysis result of Addawa'ul humma shows that it has the highest concentration of phosphate and sulphate (0.72 mg/kg and 0.2 mg/kg respectively). Results of the FT-IR indicated that the preparation consists of unsaturated with Alkanol functional group plus additional carbonyl group as shown by the FT-IR Spectra.

1. Introduction

Addawa'ul-humma exhibits more anti-typhoidal activity with a minimum of 8 mm zone of inhibition and 22.4 mm maximum zone of inhibition that is even similar with the standard drug ciprofloxacin (100 mm/dose) that give a zone of inhibition of 28.1mm. The MIC of Addawa'ul-humma and ciprofloxacin was found at 20 % concentrations. Addawa'ul-humma, and ciprofloxacin shows no statistical differences at $P>0.05$.

Typhoid fever is among the water-borne infections (Singh and Mcfeters, 1992) characteristic of environments with poor sanitation and hygiene. Human infection with *Samonella* is mainly by the oral route through ingestion of faecal contaminated food and water, unclean hands, flies and meat from infected animals (Carol *et al*., 1989). Typhoid and paratyphoid germs are passed in the faeces and urine of infected people. People become infected after eating food or drinking beverages that have been handled by a person who is infected or by drinking water that has been contaminated by sewage containing the bacteria.

In recent past, attention has been directed towards medicinal plant research to substantiate the claims of cure made by traditional healers thus providing scientific basis for their efficacy (Olukoya *et al*., 1993). These medicinal plants; *V. doniana* (root), *C. tora* (Leaf), *A. boonei* (bark), *S. jamaicensis* (leaf), and *C. papaya* (leaf) have been claimed by traditional medical practitioners in Ebonyi State to be effective when used for the treatment of fevers, particularly typhoid fever. Typhoid fever (enteric fever) caused by *Salmonella typhi* is an endemic disease in the tropic and sub-tropic and has become a major public health problem in developing countries of the world with an estimated annual incidence of 540 per 100,000. The annual incidence of typhoid is estimated to be about 17 million cases worldwide (WHO, 2008). It is often encountered in tropical countries including Nigeria where they constitute serious sources of morbidities and mortalities (Ibekwe *et al*., 2008). Typhoid and paratyphoid fevers are infections caused by bacteria, which are transmitted from faces to ingestion. Clean water, hygiene and good sanitation prevent the spread of typhoid and paratyphoid.

An herb is a plant or plant part used in it's entirely, while a drug is a synthesized copy of one chemical component, such as a component found in an herb. Herbal preparations have been used in the treatment of many diseases including malaria, jaundice, menstrual pain, waist pain, piles, delay in ejaculation, hypertension, rheumatism, and many others in Nigeria. Herbal preparations are not only used in Nigeria but also in developed countries such as UK, China, United States, India, for the treatment of mood disorders, particularly depression; for relief of anxiety and stress, insomnia; treatment of urinary tract infection, decrease kidney stone; to lower cholesterol levels and blood pleasure, as immune stimulants that help increase resistant to cold, relief from migraine headache and arthritis, healing of wounds; burns; skin ulcers; heart failure; hypertension (CSIR (TTC), 1992; Gulla *et al*., 2001).

2. Material and Methods

2.1 Study area

The study area is Gombe, Gombe state. Gombe is a city in north eastern Nigeria and a Local Government Area. It is located between latitude 10° 17 N and 11.10 °E and longitude 10.283 °N and 11.167 °E. The LGA has a total area of 52 km^2. It is the capital city of Gombe State and has an estimated population of 24268,000 (Population Census, 2006). The city is the headquarters of the Gombe Emirate, a traditional state that covers most of Gombe State (Gombe State Online Nigeria Daily, 2010).

2.2 Sample Collection

Five different samples of herbal preparations were collected from different Manufacturers within Gombe metropolis. Clinical specimens from teaching hospital Gombe was collected and processed by according to methods of Cheesbrough, (2016). Microbiological analysis of herbal preparations was carried out. Herbal preparations collected were tested for the bacterial and fungal load by pour plate method. All the microbial contaminants were characterized at least to genera level (Cheesbrough, 2016). Each container will be inspected or packaging unit for conformity with pharmacopoeia monographs or other requirements regarding packaging and labeling. Any defects that may influence the quality or stability of the contents (physical damage, moisture) will be inspected.

Table 1: Samples Composition

Name of Sample	Composition/Ingredient
ADDAWA'UL HUMMA(STEAMING)	Treated Water. Guava leaves, Mango leaves, Neem tree leaves, Bitter Orange tree leaves, Umbrella tree leaves

Chemical analysis

Organoleptic tests

- **(i) Colour:** The sample was examined under diffuse daylight or artificial light source with `wavelengths similar to those of daylight may be used. The colour of the sample was compared with that of a reference sample.
- **(ii) Odour:** Small portion of the sample was placed in to a beaker of suitable size, and slowly and repeatedly inhaled the air over the material. If no distinct odour is perceptible a gentle pressure will be applied to the beaker to confirm the odour. The sample's odour strength was determined (none, weak, distinct, strong) and then the odour sensation (aromatic, fruity, musty, mouldy, rancid, etc.). A direct comparison of the odour with a defined substance or reference substance was used (e.g. peppermint should have an odour similar to menthol, cloves should have an odour similar to eugenol).

Determination* of *physicochemical properties

(i)Total ash*:* About 2 – 4 g of the ground air-dried sample were accurately weighed, in a previously ignited and tared crucible (usually of platinum or silica). the weighed sample will be Spread the material in an even layer and ignite it by gradually increasing the heat to 500–600 °C until it is white, indicating the absence of carbon. Cool in a desiccator and weigh.

(ii) Acid insoluble ash: To the crucible containing the total ash, 25 ml of hydrochloric acid will be added and cover with a watch-glass and was boiled gently for 5 minutes. The insoluble matter on an ashless filter-paper was collected and wash with hot water until the filtrate is neutral. Filter-paper containing the insoluble matter will be transferred in to the original crucible, dry on a hotplate and ignite to constant weight. Allow the residue to cool in a suitable desiccator for 30 minutes, then weigh without delay. Calculate the content of acid-insoluble ash in mg per g of air-dried material.

Macroscopic and Microscopic Examination

(i) Surface and touch characteristics: Each of the sample was examined with a magnifying lens (6x to 10x). The dry residue of each of the sample was touched to determine if it is soft or hard; bend and rupture it to obtain information on brittleness and the appearance of the fracture plane – whether it is fibrous, smooth, rough, granular, etc.

Inspection by microscopy

The following equipment were used:
A microscope equipped with lenses providing a wide range of magnification and a sub-stage condenser, a graduated mechanical stage, objectives with a magnification of 4×, 10× and 40×, and colour filters of ground glass, blue-green; high eye point, eyepieces were preferred for wearers of spectacles; a lamp, either separate or incorporated into microscope.

Phytochemical screening

Each of the herbal drugs selected were analyzed for its phytochemical constituent without any extraction with relevant solvent.

Test for Flavonoids*: A 5 ml of the herbal drug were added to a concentrated Sulphuric acid (1 ml) and 0.5 g of Mg. A pink or red coloration that disappear on standing (3 min) indicated the presence of flavonoids.*

Test for Tannins*: Two methods were used to test for tannins. First, about 1 ml of the drug was added in 2 ml of water in a test tube, 2 to 3 drops of diluted ferric chloride solution were added and observed for green to blue-green (cathechictannins) or blue-black (garlic tannins) coloration. Second, 2 ml of the aqueous extract was added to 2ml of water, a 1 to 2 drops of diluted ferric chloride solution was added. A dark green or blue green coloration indicates the presence of tannins.*

Test for saponins: To 1 ml of aqueous extract were added few volumes of distilled water in a test tube. The solution was then be shaken vigorously and observed for a stable persistent froth for 20 min.

Test for alkaloids: Three methods were used to test for alkaloids.

(i) A 10ml of the extract will be evaporated under water bath to obtain the dry residue, follow by the addition of 1.5 ml HCl (2 %) acid solution. After that, 1 to 2 drops of Mayer's reagent and Wagner was added, a yellow- white precipitate indicates the presence of the alkaloidal base.

(ii) A 10 ml of the extract was evaporated under water bath to obtain the dry residue; it will then be dissolved in 5 ml of HCl (2 N) and filtered. A few drops of Mayer's reagent and Wagner's were added; the presence of precipitate indicates the alkaloids.
(3) A 15 ml of the aqueous extract was added 2 ml of NH_4OH a 10 % (pH =7). The alkaloid was extracted 3 times with 10 ml chloroform. The chloroform layer was then washed 3 times with 2 ml of HCL (10 %). This were divided into two portions. Mayer's reagent was added to one portion and Wagner's reagent to the other. The formation of a brown or white precipitate was regarded as positive for the presence of alkaloids.

Test for anthraquinone: Eight (8) ml of the extract was treated with the Bornträger reagent, a positive test is revealed on the appearance of a bright color change from orange red to purple.

Test for sterols and steroids: Sterols and steroids were sought by the reaction of Liebermann. Ten (10 ml) ml of extract will be evaporated. The residue was dissolved in 0.5 ml of hot acetic anhydride follow by the addition of 0.5 ml of the filtrate chloroform. The mixture was then treated with the Liebermann-Burchard. The appearance, at the interphase, a ring of blue-green, showed a positive reaction.

Test for the carbohydrate – Reducing sugars: Two methods were used to test for reducing sugars. First, the ethanol extract (1 ml) was added to 1ml of water and 20 drops of boiling Fehling's solution (A and B) in a test tube were added too. The formation of a precipitate red-brick in the bottom of the tube will indicates the presence of reducing sugars. Secondly, 2 ml of aqueous solution, 5-8 drops of boiling Benedict's solution. A red-brick precipitate showed the presence of reducing sugars.

Determination of functional groups

FT-IR was used to identify all the functional group present in the sample.

Microbiological analysis

Plate count

For bacteria, petri dishes of 9 – 10 cm in diameter were used, 1 ml of the herbal material was inoculated on a plate count media and incubated at a temperature not exceeding 45 °C for 48 – 72 hours. It was then diluted to obtain an expected colony count of not more than 300. Colonies formed was calculated using the plate with the largest number of colonies, up to a maximum of 300. For fungi Petri dishes of 9 – 10 cm in diameter was used, 1 ml of the herbal material were inoculated on the 15 ml of liquefied Sabouraud glucose agar with antibiotics incubated at 22 ^{0}C for 72 hours. The number of colonies formed was calculated the results using the dish with not more than 100 colonies.

Biochemical identifications

Inoculation of Klingler Iron Agar

Three characteristic colonies from the plating media will be inoculated into Kligler Iron Agar (KIA) as follows: the media will be stabbed to the butt and then the slant was streak with a zigzag configuration. The test tube was incubated overnight. On the following morning, reactions were examined in the KIA tubes. Tubes suspicious for *Salmonella* had an acid (yellow) but an alkaline (red) slant. They produced gas (bubbles or cracks in the agar) and/or produce hydrogen sulfide (black along the stab line).

Standardization of inoculums

The density of suspension inoculum on the media for susceptibility test will be determined using the McFarland standard Cells corresponding to $10^6 \times 10^8$ CFU/mL

Antibacterial assay

The sensitivity of each herbal product will be determined using agar well diffusion technique with modifications. Wells will be bored into the already gelled nutrient agar medium which has been previous seeded with the test organism using the spread plate method. The 6 mm diameter wells were bored using a sterile cork borer. The wells were then filled with 0.2 mL of each of the herbal product extracts (500, 400, 250, 100 and 50 mg/ml) and care was taken not to allow the solution to spill to the surface of the medium. The plates were allowed to stand on the laboratory bench undisturbed for 1 hour to allow proper absorption into the medium before the plates was incubated at 37 °C for 18 h. The plates were later observed for the zone diameter of inhibition (ZDI). The effects of the herbal extracts on the test organism will be compared with that of a standard antibiotic, amoxicillin as a control, using 12 mm as indicative of sensitivity according the guidelines of the Clinical and Laboratory Standards Institute of 2013.

Minimum inhibitory concentration (MIC) of the extract on Salmonella

The MIC of the herbal extract will be determined using methods of Bukar et al. with modification. Plant extracts of 100 %, 80 %, 60 %, 40 %, 20 concentrations will be prepared. One milliliter of the different concentrations of each herbal extract was added to 9 mL of the nutrient broth in test tubes and 1 mL of the standardized inoculum of the test organism was also be added. The control will be also set up, but amoxicillin was used instead of the herbal extracts. The activity was determined by visual method and increase in turbidity of the test tubes using spectrophotometer.

Minimum bactericidal concentration (MBC) of the extracts on Salmonella

The MBC of the extracts was determined using the method of Eldeman *et al.* (1986) with modifications. Samples was taken from tubes with no change in turbidity in the MIC assay and sub cultured onto freshly prepared nutrient agar plates and incubated at 37 °C for 18 h. The lowest concentration of the extract that did not allow any increase in number of viable cells or bacterial growth on the surface of the agar plates were taken as the MBC.

Statistical analysis

The results were expressed as mean ± SD. The two-way ANOVA test was used to compare results among and within groups for any significant difference in antibacterial activity of the extracts and the control.

3. Results

The findings from this study are presented and interpreted as follows:

Antimicrobial susceptibility pattern of Salmonella isolates

The salmonella isolates according to antimicrobial susceptibility pattern shows that (table 1) highest zone of inhibition was found against Ciprofloxacin (27 ± 2.0), followed by Amoxycillin (21 ± 2.0) and Gentamycin (19 ± 2.0).

Phytochemical characteristics of herbal preparations

The screening for phytochemical components of herbal preparations in this study (table 2), indicated that all the compounds are present in small to moderate amounts in all the samples tested, with yellow cassia as the highest, followed by Med Bunch, where alkaloids was found in high amount.

Microbiological quality of the herbal preparations

The assessment of microbiological quality of the herbal preparations (table 3) revealed that only Ma'u Shifa recorded a total colony count of 2 ± 1 and no faecal coliforms was found in the samples

Antibacterial efficacy of the Herbal Samples on the Salmonella isolates

Antibacterial activity of the Herbal Samples on the Salmonella isolates from this study (table 4) shows a highest zones of inhibition with respect to highest concentration (100%) across all the herbal samples. This trends were also observed for the Minimum Inhibitory Concentration (MIC) in 100% and 80% concentrations.

Minimum Bactericidal Concentration (MBC) the herbal samples

The lethal effects of the samples on Salmonella isolates in this study indicated a highest concentrations (MBC) of 10% in Med Bunch and Ma'uShifa preparations.

Table 2: Antimicrobial susceptibility pattern of *Salmonella* isolates from this study

Antibiotics (µg)	**Number and susceptibility of isolates**	
	Sensitive (Mean ± S.D)	**Resistant**
Ampicillin (10)	17 ± 4.0	00
Amoxycillin (30)	21 ± 2.0	00
Cefuroxime (30)	-	00
Ciprofloxacin (5)	27 ± 2.0	00
Cotrimoxazole (25)	9 ± 2.0	00
Erythromycin (25)	13 ± 1.0	00
Gentamycin (10)	19 ± 2.0	00
Pefloxacine	13 ± 1.0	00
Rocephin (10)	-	00

Table 3: Phytochemical Screening of herbal preparations used in this study

Phytochemicals	**A. Humma**
Alkaloids	+
Anthraquinone	+
Flavonoid	++
Glycosides	+
Saponins	+
Steroids	+
Tannins	++

Key: +++ = Present in high amount, ++ = Present in moderate amount, + = Present in small amount, - = Absent

Table 4: Microbiological quality of herbal preparations used in this study

Herbal Samples	Total coliform count	*E.coli* count
Addawa'ul humma	0	0

Table 5: Anti-salmonella Activity of the Herbal Samples on the Salmonella isolates from this study

Herbal Samples	Concentrations (%) and Susceptibility/Zones of Inhibition (mm)				
	20	**40**	**60**	**80**	**100**
Addawa'ul humma	8.0 ± 1.3	11.2 ± 1.0	13.4 ± 2.	18.1 ± 1.31	22.4 ± 1.1
Ciprofloxacin (10µg/disc)	0	0	0	0	28.1 ± 2.4

Values are expressed as Means ± Mean Deviation

Table 6: Minimum Inhibitory Concentration (MIC) of Herbal Samples

Herbal Samples	Concentrations (%) and Inference				
	20	**40**	**60**	**80**	**100**
Addawa'ul humma	0	1	1	1	1
Ciprofloxacin (10µg/disc)	0	1	1	1	1

Key: 0 = Turbid 1 = Clear

Table 7: Minimum Bactericidal Concentration (MBC) of Herbal Samples

Herbal samples		Herbal preparations and Test					
		M.B	**A.M**	**M.S**	**Y.C**	**A.H**	**Ciprofloxacin (10 µg/disc)**
MBC (%)	10	1	10	1	1	0.01mg/ml	

Key: M.B = Med-Bunch A.M = Al-Muwafaqa M.S = Ma'u Shifa,

Y.C = Yellow cassia A.H = Addawa'ul humma

Organoleptic properties of the Herbal preparations

The screening for organoleptic characteristics of the herbal preparations in this study (table 7) revealed that the samples fruity, turbid or clear, brown, green and bitter in taste. But Ma'ushifa and yellow cassia are offensive and choking.

Physicochemical properties of the herbal samples

The assessment of physicochemical properties of the herbal preparations (table 8) showed that Ma'ushifa and yellow cassia are acidic, while med bunch, Al'mawafaqa and Addawa'ul Humma are alkaline, with some quantity of phosphate (1.02-4.20mg/kg).

4.9 FT-IR representation of functional groups in the herbal samples

In this study, the common functional groups associated with the herbal preparations are; –OH, $–CH_3$, C ≡ C and C = O, but the methyl group ($–CH_3$) was absent in med bunch, Al'mawafaqa.

Table 8: Organoleptic Properties on the Herbal Preparations used in this study

Herbal samples	Organoleptic Properties				
	Odour	**Taste**	**Touch**	**Color**	**Clarity**
Addawa'ul Humma	Fruity	Bitter	Soft	Dark green	Turbid

Table 9: Physicochemical properties of the herbal samples used in this study

Herbal samples	Physicochemical Properties				
	Sulphate mg/Kg	**Phosphate mg/Kg**	**Acidity (mg/L)**	**Alkalinity (mg/L)**	**pH**
Addawa'ul Humma	0.72	4.2	0.51	0.92	7.52

Table 10: FT-IR representation of functional groups in the herbal preparations used in this study

Herbal Samples	Functional groups			
Addawa'ul humma	–OH	$-CH_3$	C ≡ C	C = O

4. Discussion

Use of traditional medicine in the treatment of typhoid in Nigeria has been gaining remarkable success, despite the side effects and shortcomings. In this study, highest zone of inhibition was found against Ciprofloxacin, followed by Amoxycillin and Gentamycin, which are some of the common drugs used clinically in the treatment of typhoid in this area.

The compounds are present in small to moderate amounts in all the samples tested, with yellow cassia as the highest, followed by Med Bunch, where alkaloids were found in high amount. All the herbal preparations use in this report for the anti-typhoidal, were found to contain the most important secondary metabolite such as alkaloid and flavonoids but med-bunch happens to contained more alkaloid than the rest of the herbal samples. Addawa'ul-humma contain more flavonoids, components exhibit their antimicrobial effects by destroying the cell wall of antigen and hence render it death. Steroid was also found in sufficient quantity in all the five herbal preparation. Research has shown more than 65 % of Nigerians in the rural areas rely heavily on locally made herbal products (WHO, 2008).

Addawa-ul humma shows 100 % inactivity at fungi. The assessment of physicochemical properties of the herbal preparations (table 8) showed that Addawa'ul Humma are alkaline, with some quantity of phosphate (1.02-4.20mg/kg), which is an important essential mineral element and part of nucleotide.

According to the traditional healers, medicines prepared by combining two or more plants are more potent than those prepared with single plants. This has been attributed to the additive effects of the plants (Addo-Fordjour *et al*. 2008, Okello and Ssegawa 2007) where the combination of several medicinal plants increases the quality and efficacy of medicine. Similar observations have also been recorded amongst the Kani communities in India (Ayyanar & Ignacimutum, 2005). There is the general belief that health care delivery system in Nigeria is very poor (W.H.O 2008). Various reasons have been adduced for this state of affairs and they include inadequate supply of health professionals, poor distribution of health facilities with concentration of the available few, in the urban centres, poor access to safe drinking water, poor harnessing of all available medical and health systems and poor infrastructural development, among others (*Farmacopea Argentina, 2008)*. This has made Nigeria to lag behind many other developing countries because a large proportion of Nigerians especially in the rural areas can still not access affordable health care. However, to a large majority of the populace their main source of health care is traditional medicine which is available, accessible and affordable to them (Erinoso, 1998).

This is the ancient medical practice that has sustained them over the centuries and which, in spite of government lukewarm attitude towards it, continue to wax stronger. For a practice which more than 80 per cent of the population rely upon for care

and cure, it deserves to be fully developed and sustained by all stakeholders (Pharmacopoeia, 2005). Government should therefore create the enabling environment for the development of traditional medicine and its eventual integration into the health care delivery system of the country and for the benefit of the people. Both the herbal preparations prove to contain no contamination, Addwa'ul humma, has the strong anti-typhoid activity

5. Conclusion

This study revealed that, Addawa ul humma preparation has a lot of phytochemical with functional group necessary to confer anti-typhoid activity.

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