

EFFECT OF *MORINGA OLIEFERA*, *PHEONIX DACTYLIFERA*, *ZINGIBER OFFICINALE* LEAF TEA ON *SALMONELLA TYPHI*, *ESCHERICHIA COLI* AND *STAPHYLOCOCCUS AUREUS*.

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ABSTRACT

The choice of medicinal plant by traditional medicinal practioners was not based on knowledge of the plant constitutes or phytochemicals but the search for safer and cheaper remedies. The tea bags were prepared by weighing 1g of *Moringa oliefera*, 0.5g of *Pheonix dactylifera*, and 0.5g *Zingiber officinale* transferred into the teabag and sealed with a hot sealing machine. The effect of *Moringa* leaf tea bag extracts on *Salmonella typhi*, *Escherichia coli* which was gotten from stool and *Staphylococcus aureus* was gotten from urine sample and was investigated using paper disc diffusion method. Five different extracts were obtained from the tea bag. The result shows that the first extraction had the widest zone of inhibition on the three organisms of 9mm for *Salmonella*, 6mm for *Escherichia coli* and 5mm for *Staphylococcus aureus* respectively. Both the first and second extraction had effect on *Escherichia coli* and *Staphylococcus aureus* with the zone of inhibition 6mm and 5mm. but for the *Salmonella* from the first to the third

Introduction:

Herbs are plants used for food, flavoring, medicine, or fragrances for their savory or aromatic properties. The art of using herbs in the treatment of disease must have come too early in an unscientific way (Kafaru, 1994). The choice of medicinal plants by traditional medical practitioners was not based on knowledge of the plant constituents or photochemical, but for the search of cheaper and safer remedies to man.

Over the years, plants have been used as valuable sources of natural product for maintaining animal and human health. Plants have been reported to contain large varieties of chemical substances that posses important preventive and curative therapies (Nascrimento *et al*, 2000).

extraction showed zone of inhibition. The phytochemical analysis of the tea bag extract showed that it contains Alkaloid, tannins, saponins, phenols, and cardiac glycoside and the flavonoid was absent. Thus extract of the plants can be used in treating infections caused by these test organism.

Keywords: *Moringa, Oliefera, Pheonix, Dactylifera, Zingiber.*

Despite the presence of various approaches to drug discovery, plants still remain reservoir to natural medicine (Mohamed and Ojewole, 2006). *Moringa oliefera* is a fast growing tree. It is the most widely cultivated specie of the genus *moringa*, which is the only genus in the family *moringaceace*. English common names include *moringa* and drumstick tree. *Moringa oliefera* is a plant of high medicinal and nutritional value as well. It is also called the magic tree because of its enormous benefits to mankind.

The leaf is a natural antibiotic, detoxifier outstanding immune builder used in some countries for the treatment of malnutrition and malaria (Thilza, *et al.*, 2010). *Moringa oleifera* leaves contain phytochemicals and medicinal properties. Other properties include antibacterial and antifungal activities (Nickon *et al.*, 2003).

Date palm "*Phoenix dactylifera*" is one of the oldest fruits in the Arab world and it is extensively cultivated for its edible sweet fruit. (El-shibli and Korelainen, 2009).

Ginger (*Zingiber officinale*) is a flowering plant ginger root or simply ginger is widely used as a spice or folk medicine. Ginger has enormous benefits to mankind. Ginger contain phytochemical having anticancer and are considered full of medicinal properties as medicine.

The drinking of tea begun in China centuries ago and has over the years become an inseparable part of most cultures worldwide. Tea is currently the most widely consumed beverage in the world. (Schmidt *et al.*, 2005) and therefore ranks as an important world food product. About one tenth of the world production volume of tea is supplied by Kenya which is Africa's largest producer of tea. (International Tea Committee 1998).

Tea is generally consumed for its attractive aroma and taste as well as the unique place it holds in the culture of many societies. In recent times there has been renewed interest in tea because of growing consumer awareness of health benefits derived from tea consumption. (McKay and Blumberg, 2002). Tea

therefore belongs to a rapidly expanding market of "wellness beverages" (Byun and Han, 2004).

Salmonella typhi is a genus of gram negative motile rod shaped bacterium which causes typhoid fever and gastroenteritis in humans.(Le, *et al*, 2003). The World Health Organization (WHO) estimate 12.6million typhoid fever infections with nearly 600,000 deaths every year. In Africa, poor hygiene conditions. Inadequate water supply and poverty is further aggravating an increase in case of typhoid infections and acute gastroenteritis and diarrhea due to non typhoidal *Salmonella*, (John *et al*, 2003).

Escherichia coli are not always confined to the intestine and their ability to survive for brief period outside the body makes them an ideal indicator organism to test environmental samples for fecal contamination.

E.coli is a gram negative, facultatively anaerobic rod- shaped, coliform bacterium of the genus that is commonly found in the lower intestine of warm blooded organisms (Singletun, 1999).

Staphylococcus aureus is a gram positive coccal bacterium often found in the skin and nostrils. (Kluytmans *et al*, 1997).

Plants remain the primary source of many important orthodox, medicines, currently in the market it will be absorb and scientifically native to ignore traditional medicine.

METHODOLOGY

Collection of Sample

The *Moringa oleifera* leaves were collected from healthy and uninfected tree in Rafin Zurfi area of Bauchi metropolis and then the Date palm (*Phoenix dactylifera L*) and Ginger (*Zingiber officinale*) were bought from Wutin market and transported in a polyethene bag to the laboratory.

Preparation of *Moringa oleifera* Leaves

Only the healthy and uninfected leaves were collected the leaves were handpicked from the stalks and washed under running tap water to eliminate dirty and other foreign particles. It was then drained and air dried in the laboratory at room temperature over a period of five days. The dried leaves were then crushed using a sterile mortar and pistol after which it was sieved with 600micrometer spore size mesh to obtain a uniform particle size and was then stored in an air tight container for future use.

Preparation of *Phoenix dactylifera* (date palm)

The Date palm fruits were collected, washed thoroughly in running tap water and shared dried for one week (7days) in an open air, crushed using mortar and pistol to reduce it to powder, sieved and stored in a bottle.

Preparation of *Zingiber officinale* (Ginger)

The Ginger was collected washed thoroughly in a running tap water rinsed in distilled water sliced and air dried for twenty days. It was then crushed using a sterile mortar and pistol, sieved and stored in a bottle.

Preparation of Tea Bags

The tea bag material was made using a heap fill tea bags filter paper. The filter paper was cut with a pair of scissors into rectangular shapes of 12×10cm after which it was folded and then the two sides were sealed with an electrical hand heat sealing machine and then will be sterilized using the hot air oven at the temperature of 160°C for 60min and then filled with the mixture of the three extract and the top was sealed by passing a thread (piece or string) that served with a paper label attached to the tea bag as a handle for dipping it in the water filling with 2g of it.

Test Organisms

The test organisms used for this study (*Salmonella typhi*, *Escherichia coli* and *Staph aureus*) were isolated from clinical samples at Abubakar Tafawa Balewa University Teaching Hospital they were maintained on MacConkey agar and preserved at 4°C.

Subculture Media and Cultural Examination

The Deoxychocolate Citrate Agar (DCA), *Salmonella Shigella* Agar (SSA) and Manitol Salt Agar (MSA).were prepared according to the manufacturer's specification for subculturing and proper identification of the organisms from the MacConkey media. The agar were poured into petri dishes and allowed to cool using a sterilized wire loop sample were taken from the positive MacConkey media and streaked across all the media. The petri dishes were then incubated at 37°C.

Hot water Extraction

One of the prepared tea bag was then dipped into 100 ml distilled hot water at 100°C in a beaker of 250ml capacity of extract for 5min. This procedure was

repeated for four times using the same tea bag and the same quantity and quality of water in different beakers.

Phytochemical Screening

Qualitative phytochemical screening was done to determine the following compounds Alkanoids, tannins, saponin, flavonoid, phenols, and glycoside using the method as described By Soforowa (1993).

Preparation of Antibiotic Disc

Whatman's No.1 filter paper was perforated and batches of 100disc was dispensed into a clean screw capped bottle and sterilized in a hot air oven at 160°C for one hour. One milliliter of the tea extract was introduced onto the already sterilized screw capped bottles containing the disc, the prepared antibiotic were stored in refrigerator.

Antimicrobial Testing

The method used was paper disc diffusion method described by Aida, *et al*, (2001). The test organism was streaked unto a solid nutrient agar plate. An already prepared disc containing the impregnated tea extract were picked with a sterile forceps and placed onto the cultured plates and was incubated at 37°C for 24hours. After which the zone of inhibition were checked and measured with a meter rule and recorded.

RESULTS

Table 1, shows that the qualitative phytochemical analysis of the *Moringa oliefera*, *Phoenix dactylifera*, and *Zingiber officinale* tea bag extracts. the screening revealed the presence of Alkaloid, Tannins, Saponins, phenols, and cardiac glucoside in the extract. Alkaloid, tannins and saponins were present in the first, second, third, fourth and fifth extract for phenols and glycoside which were present from the first to the third extract. while flavonoid was absent in all the extract.

Table 2, shows the cultural, morphological and physiological characteristics of the test organism revealed that suspected test organisms *Salmonella typhi*, *Escherichia coli* are gram negative organism, rod shape and motile while *Staphylococcus aureus* is gram positive, cocci shape and non motile. and the biochemical reactions of the test organisms and their confirmation are showed in here which revealed that *Salmonella typhi*, *Escherichia coli* and *Staphylococcus aureus* were positive to catalyze and methyl red test and were negative to indole test. *Salmonella typhi* was positive to hydrogen sulphide

while *Escherichia coli* and *Staphylococcus aureus* were both negative to Hydrogen sulphide. *Salmonella typhi* was non sucrose and lactose fermenter but ferment glucose with the production of acid and gas and it ferment maltose with the production of Acid only. *Escherichia coli* ferment Sucrose, Lactose and Maltose with the production of acid only and it also ferment sucrose, lactose and maltose with the production of acid only and it also ferment glucose with the production of acid and gas.

Staphylococcus aureus ferment sucrose, glucose, lactose and maltose with the production of acid only.

Table 3: The result of the antimicrobial effect of hot water (100^{0c}) of *Moringa* leaf tea bag extracts against the test organism is represented in table iii. The first extract showed the highest effect of (9mm) against *Salmonella typhi* which was followed by (6mm) against *Escherichia coli* and (5mm) against *Staphylococcus aureus*. and the lowest zone of inhibition was (2mm) from the third extract on *Escherichia coli*. There was no zone of inhibition observed at the fourth and fifth of *Salmonella typhi* and *Escherichia coli* while in *Staphylococcus aureus* there was no zone of inhibition from the third extract.

TABLE 1: Qualitative Phytochemical Composition of Successive Extracts of *Moringa oliefera* Tea bag

	No of extraction	Alkaloid	Tannins	Saponins	Flavonoid	Phenols	Glycoside
Hot water	1	+	+	+	-	+	+
	2	+	+	+	-	+	+
	3	+	+	+	-	+	+
	4	+	+	+	-	-	-
	5	+	+	+	-	-	-

Key:

+ = Present

- = Absent

TABLE II: Morphological And Gram Reaction Of The Test Organism

Colonial characteristic	Gram reaction	Shape	Motility	Catalase	Methyl-red	Indole	H ₂ S	Citrate utilization	Sucrose	Glucose	maltose	Lactose	Probable organism
Pale pigments	-ve	R	+ve	+ve	+ve	-ve	+ve	-ve	-ve	AG	A	-ve	<i>Salmonella typhi</i>

Pinkish pigments	-ve	R	+ve	+ve	+ve	-ve	-ve	-ve	A	AG	A	A	<i>Escherichia coli</i>
Pale pigments	+ve	C	-ve	+ve	+ve	-ve	-ve	+ve	A	A	A	A	<i>S.aureus</i>

Key:

- ve = Negative

+ ve = Positive

R = Rod

C = cocci

A = Acid Production

AG=Acid and Gas production

TABLE 3: Antibacterial Activity of in *Moringa oliefera*, *Phoenix dactylifera* And *Zingiber officinale*

Tea Bag extract on the Test Organisms.

Test organisms zone in (mm)	Control (Gentamycin)	Diameter of the inhibitory inhibition zone in (mm)				
		1 st	2 nd	3 rd	4 th	5 th
<i>Salmonella typhi</i>	13	9	5	3	-	-
<i>Eschechia coli</i>	9	6	4	2	-	-
<i>Staphylococcus aureus</i>	11	5	3	-	-	-

Key:1st = First extraction2nd = Second extraction3rd = Third extraction4th = Fourth extraction5th = Fifth extraction**DISCUSSION**

The *Moringa oliefera*, *Phoenix dactylifera*, *Zingiber officinale* tea bag extract exhibited different degrees of antibacterial effect on *Salmonella typhi*, *Escherichia coli*, *Staphylococcus aureus*. And this was based on the traditional use in treatment of some clinical diseases, *Escherichia coli* and *Staphylococcus*

aureus are known to cause intestinal tract infection while *Salmonella typhi* are known to cause typhoid fever.

The extract showed more considerable effect on the *Salmonella typhi* than *E.coli* and *Staphylococcus aureus*. This contradicts the work of Napoleon *et al* (2009) who reported the effect of *Moringa oliefera* aqueous extract on *Salmonella typhi*, *S.aureus*, *P.aeruginosa* and *Escherichia coli* to be sensitive at concentration of 200mg/l. which signifies that the organisms were susceptible at that concentration.

The antimicrobial assay of hot water *Moringa oliefera*, *Phoenix dactylifera* and *Zingiber officinale* tea bag extract effect against the three organisms inhibition of the first extract agrees with the findings of (Marjorie,1991), (Srinivasam, 2001) and Saddiq and Bawazir(2001) as they reported antibacterial activity from aqueous extract against *E.coli* and *S.typhi*

The antimicrobial assay of hot water *Moringa oliefera* tea bag extract against *Salmonella typhi* shows the widest zone of 9mm for the first one extract which agree with the findings of Saddiq and Bawazir(2010),Majorie, (1999) and Srinivasam, (2001) who noted that aqueous extract of *Moringa* leaves, date palm and ginger possess significant antimicrobial activity against both gram negative and gram positive bacteria organism from wounds. thus signaling its broad spectrum of antibacterial activity.

Moreover, aqueous extract inhibited the *Salmonella typhi*, *Escherichia coli*, from the first, second and third extract and it inhibit *Staphylococcus aureus* from the first and second, thus indicating that water is a good solvent for the extraction of the three plants. more so that more people who use the plants for traditional means of treatment if various skin ailment and other disease can make use of water base extract of the plant.

The presence of the photochemical constituents noticed in this work has been reported to account for the excretion of antimicrobial acting by plants. Pretorius and Watt(2001).The effective extract had the presence of Saponins, tannins, phenols, glycoside and Alkaloids which were not reported by (Napoleon *et al*, 2009).

The reason why the extract did not have strong significant effect on the test organisms could be as a result of the type of soil the plant was grown this is also in agreement with Farooq *et al*(2007) who reported that plants occur in different habitat a great magnitude of variation in the concentration and composition of photochemical ingredients in the different parts of such plant is expected.

CONCLUSION

The demonstration of antibacterial effect of *Moringa oliefera*, *Phoenix dactylifera*, *Zingiber officinale* tea bag extracts against *Salmonella typhi*, *Escherichia coli*, and *Staphylococcus aureus* has provided a scientific basis for their local usage as a medicinal plant in the treatment of diseases usually caused by these organisms. The five different extract obtained have shown an intermediate significant effect on the test organism with the first extracts showing a higher effect on *S.typhi*, *E.coli*, *S.aureus* in terms of zone of inhibition. There was the presence of these substance Alkaloid, tannins, Saponins, glycoside, in the tea bag extracts the presence of this substance revealed the importance of natural products indeed a promising development of antibiotic without side effect. The facts that the plants are very common make it then cheaper alternative for drug development for human use. The tea bag extracts can be used to treat common medical condition.

RECOMMENDATION

More research should be carried out on the use of these three substrates to treat some other diseases caused by the bacteria.

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