

Effects Of Moringa Oleifera Leaf Tea On Salmonella Typhi And Escherichia Coli

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Abstract: The choice of medicinal plant by traditional medical practitioners was not based on knowledge of the plant constituents or phytochemicals, but the search for safer and cheaper remedies. The effect of *Moringa oleifera* leaf tea bag extracts on *Salmonella typhi* and *Escherichia coli* from stool sample was investigated using the agar well diffusion method. Five different extracts were obtained from the *Moringa oleifera* leaf tea bag. The result shows that the first extraction has the widest zone of inhibition on the two test organisms of 8mm and 3mm respectively. Only the first extraction has effect on *Escherichia coli* with the zone of inhibition of 3mm. The phytochemical analysis of the tea bag extract showed that it contains saponins, tannins, phenols, glycosides, alkaloids, flavonoids and the absence of reducing sugar. Thus extract of the plant can be used in treating infections caused by these test organisms.

I. Introduction

The art of using herbs in the treatment of diseases must have come too early in an unscientific way (1). The choice of medicinal plant by traditional medical practitioners was not based on knowledge of the plant constituents or phytochemicals, but the search for safer and cheaper remedies.

Moringa oleifera is a plant of high medicinal and nutritional values as well. It is also called magic tree because of its enormous benefits to mankind. The leaf is a natural antihelmintic, antibiotic, detoxifier, outstanding immune builder used in some countries for the treatment of malnutrition and malaria (2). *Moringa oleifera* leaves contain phytochemicals having anticancer and hypotensive and are considered full of medicinal properties as medicine. Other important medicinal properties of the plant include antispasmodic, diuretic, antihypertensive, hepatoprotective (3) Antibacterial and antifungal activities (4).

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 (5) 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 (6).

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 is renewed interest in tea because of growing consumer awareness of health benefits derived from tea consumption (7). Tea therefore belongs to a rapidly expanding market of "wellness beverages" (8).

Salmonella typhi is a genus of gram negative, motile rods shaped bacteria which causes typhoid fever and gastroenteritis in humans (9). The World Health Organization (WHO) estimated annual rate of 12.6 million typhoid fever infections with nearly 600,000 deaths every year (10). In Africa, poor hygiene conditions and inadequate water supply and poverty is further aggravating an increase in causes of typhoid infections and acute gastroenteritis diarrhea due to non typhoidal *Salmonellas* (11).

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.

Moringa is a native to the Western and sub – Himalyan region, India, Pakistan, Asia Minor, Africa and Arabia (12).

Plants remain the primary sources of many important orthodox medicines, currently in the market it will be absurd, morally unreasonable, culturally unrealistic and scientifically naive to ignore traditional medicines. This work is aimed at producing tea bags from *Moringa oleifera* leaves extracts to determine its effect on *Escherichia coli* and *Salmonella typhi* isolates.

II. Materials And Methods

Collection of the Leaf Sample

Fresh leaves of *Moringa oleifera* were collected from healthy and uninfected tree in Gwallemaji area of Bauchi metropolis and was transported in a polyethene bag to the laboratory. The leaves were identified and confirmed by a botanist for its authenticity.

Preparation of the leaves

Only the healthy and uninfected leaves were hand-picked from the stalks and first washed under running tap water to eliminate dust and other foreign particles and was rinsed thoroughly with distilled water, drained and were air-dried in the laboratory at room temperature (32-35°C) to constant weight over a period of five (5) days. The dried leaves were then crushed using a sterile mortar and pestle after which it was sieved with 600µm spore size mesh to obtain a uniform particle size and was then stored in an air tight container for future use.

Preparation of tea bags

The tea bags material was made using heap fill tea bags filter paper. The filter paper was cut with a pair of scissors into rectangular shapes of 12 x 10cm after which it was folded and the two sides was then sealed with an electrical hand heat sealing machine and then sterilized using the hot air oven at the temperature of 160°C for 60 minutes and was then ready to be refilled with the crushed dried *Moringa oleifera* leaves and then the top was also sealed by passing a thread (piece of string) that served with a paper label attached to the tea bag as a handle for deeping in the water after filling with 2g of it.

Test Organisms

The test organisms *Salmonella typhi*, and *Escherichia coli* were clinical isolates collected from the Microbiology Laboratory of the Abubakar Tafawa Balewa University (A.T.B.U) 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) media 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. After 24hours, *Escherichia coli* were identified on the *Salmonella shigella* Agar with a pinkish colony while *Salmonella* were present as colourless. In DCA, *Salmonella* were also identified with a colourless colony.

Hot water Extraction

One of the prepared *Moringa oleifera* tea bags was then dipped into 100ml of distilled hot water of 100°C in a beaker of 250ml capacity to extract (infused) for five (5) minutes. This procedure was repeated for four (4) times using the same tea bag and the same quality and quantity of water in different beakers.

Preliminary Phytochemical Analysis

Preliminary phytochemical test to determine the presence of alkaloids, tannins, phenols, glycosides, saponins and flavonoids were carried out in each of the tea bag extract using the method described by (13).

III. Results

Table 1 illustrates the qualitative phytochemical analysis of the *Moringa oleifera* tea bag extracts. The screening revealed the presence of saponins, tannins, phenols and alkaloids in the five extracts. Glycoside was also presence in the first, second, third and forth extracts with the exception of the fifth extract. Flavonoid was also present in the first, second and third extracts. Reducing sugar component was not detected at all in the five extracts. The cultural, morphological and physiological characteristics of the test organisms revealed the suspected test organisms. *Salmonella typhi* and *Escherichia coli* both gave a typical colonial characteristic. They were gram – negative rod shape and motile (Table 2). The biochemical reaction of the test organisms and their confirmation are showed in table 3. It revealed that *Salmonella typhi* and *Escherichia coli* were both positive to catalase and methyl red test and are both negative to indole test. *Salmonella typhi* was positive to hydrogen sulphide while *Escherichia coli* was negative to it. *Salmonella typhi* was non – lactose fermenter but ferments glucose with the production of acid and gas. It also ferments manitol with the production of acid only. *Escherichia coli* fermented lasctose with the

production of acid only. It also fermented manitol and sucrose with the production of acid as well, but fermented glucose with the production of acid and gas.

The result of the antimicrobial effect of hot water (100°C) of *Moringa oleifera* leaf tea bag extracts against the test isolates is represented in table 4. The first extract showed the highest effect (8mm) against *Salmonella typhi* and the lowest zone of inhibition was (3mm) from the third extract. There was no zone of inhibition observed at all in the fourth and fifth against *Salmonella typhi*. The first extraction had the highest effect (3mm) against *Escherichia coli* while the other extractions had no zone of inhibition at all.

TABLE 1: Qualitative Phytochemical Analyses Of Each of The Extracts of *Moringa oleifera* Leaves Tea Bag

Solvents used for extraction	No. of extraction	Saponins	Tannins	Phenols	Glycosides	Alkaloid	Flavonoid	Reducing Sugar
Hot Water	1	+	+	+	+	+	+	-
	2	+	+	+	+	+	+	-
	3	+	+	+	+(trace)	+	+	-
	4	+(trace)	+(trace)	+(trace)	+	+(trace)	-	-
	5	+(trace)	+(trace)	+(trace)	-	+(trace)	-	-

KEY:

(+) Indicates presence of the components.

(-) Indicates absence of the components.

TABLE 2: Morphological and Gram Reaction of the Test Organism

Test Organism	Colonial Characteristics	Gram Reaction	Shape	Motility
<i>Samonella typhi</i>	Pale Pigments	-ve	R	+ve
<i>Escherichia coli</i>	Pinkish pigments	-ve	R	+ve

KEY:

-ve = Negative

+ve = Positice

R = Rod.

TABLE 3: Biochemical Reaction of the Test Organism

Biochemical reaction	<i>Salmonella typhi</i>	<i>Escherichia coli</i>
Catalase	+ ve	+ve
Methyl red	+ ve	+ve
Indole	- ve	-ve
Hydrogen Sulphide	+ ve	-ve
Sugar Fermentation		
Glucose	AG	AG
Manitol	A	A
Sucrose	-ve	A
Lactose	-ve	A

KEY:

+ve = Positive.

-ve = Negative.

A = Acid production.

AG = Acid and Gas production.

TABLE 4: The Antibacterial Activity of the *Moringa oleifera* Leaf tea bag extracts on the test Organisms

Test Organism	Diameter of the inhibitory inhibition zone in (mm)				
	1 st d	2 ⁿ d	3 ^r d	4 th	5 th
<i>Salmonella typhi</i>	8	6	3	-	-
<i>Escherichia coli</i>	3	-	-	-	-

KEY:

1. = First Extraction

2. = Second Extraction
3. = Third Extraction
4. = Fourth Extraction
5. = Fifth Extraction

IV. Discussion

The *Moringa oleifera* leaf tea bag extracts exhibited different degrees of antibacterial effect on *Escherichia coli* and *Salmonella typhi*. And this was based on its traditional use in treatment of some clinical diseases. *Escherichia coli* and *Salmonella typhi* are known to cause intestinal tract infections.

The extracts showed more considerable effects on the *Salmonella typhi* than *E.coli*. This contradicts the work of (14) who reported the effect of *Moringa oleifera* aqueous extracts on *Salmonella typhi*, *S.aureus*, *P. Aeruginosa* and *Escherichia coli* to be sensitive at concentration of 200mg/l

The antimicrobial assay of hot water *Moringa oleifera* tea bag extract effect against *Salmonella typhi* of 8mm zone inhibition of the first extract agrees with the findings of (15) and (16) who noted that aqueous extract of *Moringa oleifera* leaf possess significant antimicrobial activity against both Gram negative and Gram positive bacterial organisms from wounds, thus signaling its broad spectrum of antibacterial activity.

Moreover, aqueous extract inhibited the *Salmonella typhi* from the first, second and third extract, thus indicating that water is a good solvent for extraction for the leaf, more so, that most people who use the leaf for traditional means of treatment of various skin ailments and other diseases make use of water – base extract of the leaf. Yamaji (17) noted that water has maximum polarity water soluble flavonoids have no antimicrobial significance and water soluble phenolics are only important as antioxidant compounds. The presence of the photochemical constituents noticed in this work has been reported to account for the excretion of antimicrobial acting by plants (18). The effective extracts had the presence of saponins, tannins, phenols, glycoside, alkaloids and flavonoid which were not reported by (14).

The reason why the extracts 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 agrees with (19) who confirmed a variation in the minerals content of green leafy vegetable cultivated on soil fortified with different chemical fertilizer.

This is also in agreement with (20) who reported that plants occur in different habitats a great magnitude of variation in the concentration and composition of photochemical ingredients in the different parts of such plant is expected. Moreover, (21) response, to perceived threats by the plants, therefore variation exist in the production of these photochemical depending on the type and amount of threat encountered by the plant.

V. Conclusion

The demonstration of antibacterial effect of *Moringa oleifera* leaf tea bag extracts against *Salmonella typhi* and *Escherichia coli* has provided a scientific basis for its local usage as a medicinal plant in the treatment of the diseases usually caused by these organisms. The five different extracts obtained has shown an intermediate significant effect on the test organisms with the first extract showing a higher effect on *S.typhi* than *E.coli* in terms of zone of inhibition. There was the presence of saponins, tannins, phenols, alkaloid, and flavonoid in the *Moringa oleifera* leaf tea bag extracts. The presence of these substances revealed the importance of natural product indeed a promising development of antibiotic without side effects. The fact that the plants are very common makes it a cheaper alternative for drug development for human consumption. *Moringa oleifera* leaves tea bag extract can be used to treat common medical condition. Presence of photochemical indicates possible preventive and curative properties of *Moringa oleifera* leaves.

References

- [1]. Kafaru, E. (1994). "Immense help for nature" workshop elikaf health service limited.
- [2]. Thilza, I.B., Sanni, S., Isah, Z.A., Sanni, F.S., Talle, M. and Joseph, M.B.(2010). In vitro antimicrobial activity of water extract of *Moringa oleifera* Leaf stalk on bacteria normally implicated in eye diseases. *Academia Arena*. 2(6):80-82.
- [3]. Reuckmani, K., Kavimani, S., Anandan, R. and Jaykar, B. (1998). Effect of *Moringa oleifera* Lam. On paracetamol-induced hepatotoxicity. *Indian journal pharmaceutical Science*. 60:33-35.
- [4]. Nickon, F, Saud, Z.A, Rehman, M.H. and Haque, M.E. (2003). In vitro and microbial activity of the compound isolated from chloroform extract of *Moringa oleifera* lam, *Pakistan Journal of Biological Sciences*. 6(22):1888-1890
- [5]. Schmidt, M., Schmitz, H.J., Kreuter M.H. (2005). Toxicity of green tea extracts and their constituents in rat hepatocytes in primary culture. *Food chemical toxicology*. 43:307-314.
- [6]. International Tea Committee (1998). Supplement to Annual bulleting of Statistics. Pp 103-146.
- [7]. Mckay, D.L. and Blumberg, J.B. (2002). The role of tea in human health: an update. *Journal of the American College of Nutrition* 21:1-13

- [8]. Byun, J.O. and Han, J.S. (2004). A study perception and actual status of utilization for green tea. *Korean Journal of Food Culture*. 18:184-192.
- [9]. Le, M., Dworkin, M., Falkow, S. and Resenberg, E. (2003). The genus *Salmonella*. In the prokaryotes, an evelong electronic resource for the microbiological community, electronic release 3.14.4th Edition, springer-verlag New York, USA.
- [10]. WHO. (2003). Manual for the laboratory identification and antimicrobial susceptibility testing of bacteria pathogens of public health importance in the developing world. Pp 103-162.
- [11]. John, A. C., Fouad, G.Y., Stephen, P. Maha, T., Saidu, A. O. and Frank, Y.M. (2003). Estimating the incidence of typhoid fever and febrile illness in developing countries. *Emergency infections Diseases*. 9(5):539-544.
- [12]. Mughal, M.H., Ali, G., Sirvata., P.S. and Igbal, M. (1999). Improvement of drumstick (*Moringa Pterygosperma gaertii*) a unique source of food and medicine through tissue culture. *Harmda medicine*. 42:37-42
- [13]. Odebiyi, A., and Sofowara E.A. (1999). Phytochemical screenings of *Nigeria* medicinal plants part II *Lyodia* 44:234-246.
- [14]. Napoleon, P., Amitha, J. and Emilin, R.R. (2009). Isolation, analysis and identification of photo chemicals of antimicrobial activity of *Moringa oleifera* Lam. *Current Biotica*. 3(1): 33-37.
- [15]. Majorie, M. C. (1999). Plant products as antimicrobial agents. *Clinical microbiology review*. 12(4): 564-582.
- [16]. Srinivasan, D., Perumalsamy, L. P., Nathan, S. and Sures, T. (2001). Antimicrobial activity of certain Indian medicinal plant used in folkloric medicine. *Journal of Ethanopharmacology*. 94: 217-222.
- [17]. Yamaji, K., Ishimoto, H., Usui, N. and Mori, S. (2005). Organic acids and water soluble phenolics produced by paxillus species. *Mycorrhiza*. 15(1): 17-23
- [18]. Pretorius, C. J. and Watt, E. (2001). Purification and identification of active components of *Carpobrotus edulis* L. *Journal of Ethno pharmacology*. 76: 87-91.
- [19]. Reedy, N. S. and Bhatti, G. (2001). Contents of minerals in green leafy vegetable cultivated on soil fortified with different chemical fertilizer. *Plant Food & Human nutrition* 5: 1-6.
- [20]. Farooq, A., Sajid, L., Muhammad, A. and Anwarul, H. G. (2007). *Moringa oleifera* a food plant with multiple medicinal uses, *Phytotherapy Research*. 21:17-25.
- [21]. Waller, G. R. & Nowactki, E. K. (1978). Role of alkaloids. *Alkaloids Biology and metabolism in plants*. Plenus press. New York. Pp 249