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Full Length Research Paper

The antimicrobial activity of *Morinda lucida* leaf extract on *Escherichia coli*

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The inhibitory activity of the leaf extract of *Morinda lucida* on *Escherichia coli* was investigated both *in vitro and in vivo*. The effect of the extract *in vivo* was obtained by feeding albino rats with the extract. The *in vitro* experiment was carried out using the agar well diffusion method and results showed that 25 mg/ml of the extract inhibited *E. coli* with a zone of inhibition measuring 5 mm. Immunological indices observed in the albino rats showed that the extract was able to eliminate and control symptoms of *E. coli* infection in the experimental rats. Results of haematological indices in group A rats infected with *E. coli* revealed an increase in WBC (4900 to 5600 mm$^{3)}$ and neutrophil count (46 - 68%), while a decrease occurred in PCV (41 - 27%) and Hb (13.7 - 9.0 g). However, result for group B (rats infected with *E. coli* but treated with extract) showed a decrease in WBC (5800 – 3600 mm^3) PCV (31 - 29%), Hb (10.4 - 9.7 g) and neutrophil count (52 - 49%). At the same time, group C (rats fed with the extract alone) gave results as follow; WBC (5700 – 4200 mm^3), PCV (42 - 36%), neutrophil (58 - 63%) while group D, (Placebo administered with normal saline) had their WBC increased (5200 – 5400 mm^3); PCV (37 - 38%) and neutrophil count (52 - 54%). Results on urinalysis showed fluctuation in pH values for rats in groups A, C and D from before infection to after infection. These results have indicated that the extract has been able to control the establishment of *E. coli* infection in the rats. This has given a clue that the extract can be of use in the treatment of the symptoms of infections caused by enteropathogenic *E. coli*.

Key words: Antimicrobial activity, immunological indices, urinalysis, *Escherichia coli*, phytochemical screening, infection, albino rats.

INTRODUCTION

The use of medicinal plants for treatment of microbial diseases is well known and has been documented since ancient times. Plants synthesize many components, which act as defensive agents, helping to protect them from microbial infection and other predators. Those compounds are bioactive and can be medicinal, intoxicating or toxic depending on circumstances. Several plants species have been tested for antimicrobial properties, but the vast majority have not yet been adequately evaluated.

In different parts of Nigeria, different varieties of plants are used in the treatment of different types of diseases. Roots, barks or leaves of *Newboldia laevis* are used in the treatment of scrotal elephantiasis, dysentery, ringworm, syphilis, sore eyes and ear ache (Azoro, 2002). Also, the stem bark and leaves of *Anthocliesta dialonensis* are used as antipyretic and in the treatment of stomach ache, gonorrhoea and fever (Azoro, 2002). Shoots of *Phyllantus pentandorous* is used in treating boils and backache. Herbalists in Soha-Zaria use bark of the stem of *Jacaranda mimoisaides* in the treatment of diarrhoea (Azoro, 2002). Extracts from *Cassia alata* has antimicrobial activity against both fungi and bacteria (Azoro, 2002).

Different substances have been identified in medicinal plants which are believed to be the antimicrobial agents and these include; different forms of alkaloids, sesquiterpenes, diterpenes, triterpenes saponins, triterpen aglycous, flavonoids, sterols, coumarin, quinines, monoterpenes, different forms of other proteins as well as lipids and tannins (Sofowora, 1993).

Escherichia coli normally colonize an infant's gastrointestinal tract within 40 h of birth, arriving with food or water or with the individuals handling the child. In the bowel, it adheres to the mucus of the large intestine. It is the primary facultative organism of the human gastrointestinal tract (Todar, 2007). As long as these bacteria

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do not acquire genetic elements encoding for virulence factors, they remain benign commensals (Evans et al., 2007).

Virulent strains of *E. coli* can cause gastroenteritis, urinary tract infections, and neonatal meningitis. In rarer cases, virulent strains are also responsible for peritonitis, mastitis, septicemia and Gram-negative pneumonia (Todar, 2007).

Recently it is thought that *E. coli* and certain other foodborne illnesses can sometimes trigger serious health problems months or years after patients survived that initial bout (Todar, 2007).

Antimicrobial agents are substances that have the ability to kill microorganisms or inhibit their growth. Such substances may either be synthetic chemical or natural products. Those that kill microorganisms are referred to as cidal agents, thus we have bacteriocide, fungicide, virucide and algaecide while those that only inhibit or prevent microbial growth are called static agents, thus we have bacteriostatic, fungistatic (Prescott et al., 2008).

Antimicrobial agents vary in their selective toxicities. Some act in a rather non-selective manner and have similar effects on all types of cells. Others are far more selective and more toxic to microorganisms than animal tissues. Antimicrobial agents with selective toxicity are especially useful in chemotherapeutic agents in the treatment of infectious disease as they can be used to kill disease causing organisms without harming the host (Prescott et al., 2008). Most of the drugs and even antibiotics are no longer active against the targeted organisms. We therefore witness the occurrence of many antibiotic resistant organisms, and ineffective malarial drugs. In addition, majority of the orthodox drugs are both expensive and display dangerous side effects in the users. As a result, managing patients especially in developing countries is rather expensive.

Discovering and identifying new safe drugs without severe side effects has become an important goal of research in biomedical science. Hence, increasing the quality and quantity of chemical compounds tested in diverse biological systems should increase the chances of finding new leads for therapeutic agents.

In view of the serious problems emerging with old and new pathogens, a broad screen of natural product is urgently needed, and plants are the natural and underexploited source of novel compounds.

MATERIALS AND METHODS

Collection of sample

Fresh leaves from *Morinda lucida* plants found growing naturally on the grounds of the Federal University of Technology, Akure were harvested. The plant was authenticated by Mr. Aduloju of the Crop Production Department, Federal University of Technology, Akure.

Preparation of extracts

The leaf samples of *M. lucida* were air dried until well dried and ground into powder. About 900g of the dried leaves were soaked into methanol. The mixture was allowed to stand for 72 h, after which it was filtered using first, muslin cloth then No.1 Whatman filter paper. The filtrate was concentrated in vacuo using the rotary evaporator. Typed culture of *E. coli* (NCIB 68) was collected at the Microbiology Department, Obafemi Awolowo University, Ile-Ife.

Laboratory animals used

The Swiss albino rats were obtained at the Pharmacy Department of Obafemi Awolowo University, Ile-Ife.

Infectivity dosage and standardization of inoculum

Standard inoculum of the test organism was prepared by inoculating 10 ml of sterile nutrient broth with the organism. This was then incubated for 18 h at 37°C. The culture medium was then centrifuged at 300 rpm for 20 min. The supernatant was discarded and the cells were washed twice with normal saline. Finally, 10 ml of saline was added and kept in the refrigerator. This served as the stock.

Serial dilution was prepared from the stock culture up to the sixth dilution. 1 ml from each dilution was orally administered unto the rats and watched for symptoms of infection. The dilution that established infection in the rat which showed by the symptoms was used as infectivity dosage for the rats throughout the infection.

Antibacterial sensitivity Assay

Exactly 25 mg/ml of the extract was tested for inhibitory activity (*in vitro*) against *E.coli* using the agar well diffusion method.

About 1ml of 18 h broth culture of the test organism was introduced into a separate sterile petridish. Exactly 20 ml of sterile molten nutrient agar was poured into the petridish containing the test organism. The agar was allowed to set and holes were bored into the plate using sterile cork borer of 7 mm in diameter aseptically. The wells bored on the plate were filled with the crude extract at a concentration of 25 mg/ml. A control experiment was set up the same way, however instead of the extract; sterile water was introduced into the hole bored. The Petri dishes were incubated upright at 37°C for 18 - 24 h. The relative susceptibility of the organism to the extract is indicated by clear zones of inhibition around the wells which were observed, measured and recorded in millimeters.

In vivo analysis

The rats were acclimatized for two weeks on basal diet and weighed. They were then divided into four groups (A, B, C, D) and given oral administration of organism, organism and extract, extract alone and normal saline (placebo) respectively.

The inhibitory effect of the extract *in vivo* was determined by observing the faecal samples of rats in groups A and B for the presence of *E. coli.*

Haematological test

Blood samples were collected from the rats before and after the experiment. They were analysed for white blood cell count (WBC), packed cell volume (PCV), haemoglobin (Hb) levels, erythrocyte sedimentation rate (ESR), and differential count.

The WBC was done using Turk's solution, haemacytometer and counting chamber (Ogwumike, 2002). The PCV was measured using a Hawksley haematocrite centrifuge, spinning for 5 min at 12

Table 1. Antimicrobial activity of extract of *Morinda lucida* against *E. coli.*

Organism	Zone of Inhibition
E. coli	5 mm

Table 2. Phytochemical compounds of methanol extract of *M. lucida.*

Phytochemicals	Absence or presence in extract
Saponins	+ve
Tannins	+ve
Anthraquinones	+ve
Phlobatannins	-ve
Alkaloids	+ve
Liebermans Test	+ve
Salkowski	-ve
Keller Killiani	-ve
Legals Test	-ve

Key: +ve: present.
-ve: absent.

rev/s before reading through a microhaematocrit reader (Ogwumike, 2002).

Haemoglobin levels were measured colorimetrically by the oxyhaemoglobins method using Reichert's haemoglobinometer (Ogwumike, 2002).

ESR was done using Winchester tube and differential count was carried out using Leishman's stain and viewing under the microscope (Cheesebrough, 2000).

Urinalysis

The urine macroscopy was carried out using a combi 9-test strip which measured the value of pH, glucose, ascorbic acid, ketone, nitrite, protein, bilirubin, urobilinogen and blood in urine (Cheesebrough, 2000).

RESULTS AND DISCUSSION

The extract inhibited the growth of *E. coli in vitro* recording a zone of inhibition of 5 mm (Table1).

The phytochemical components of *M. lucida* revealed the presence of saponins, tannins, anthraquinones and alkaloids. This is similar to the report of Nwinyi et al. (2008) and Adejumobi, et al. (2008).

The WBC of the infected rats increased from 4900 – 5600 mm^3 after infection with the organism. This is a signal that an infection has been established and there is the production of more white blood cells by the animal to fight the antigen *E. coli*. In the treated rats where WBC dropped from 5800 – 3600 mm^3, it shows evidence that the *E. coli* was prevented from establishing infection. In the differential count, neutrophil was higher in the group A rats and reduced in group B after treatment. Since neutrophil is majorly responsible for phagocytosis of pathogenic organisms during the first few hours after their entry into tissues, increase in the value of neutrophil is a confirmation of the establishment of an infection while a decrease indicates no infection. The decrease of neutrophil in group B therefore supports the fact that the extract inhibited the test organism *in vivo* thus, disallowing it to form an infection.

In the infected rats, the values of PCV, HB and RBC strongly decreased after oral dosing with the organism which is suggesting that the infection is haemolytic (Table 2). The group B rats in comparison recorded a slight reduction in the values of PCV, Hb and RBC. This is suggestive of a low ability to cause anaemia. A high level of Hb, PCV and RBC is an indication that the rats are not anaemic while a lower level is a sign of anaemia.

Moreover, reduction in body weight of infected rats recorded on Table 3 which may be due to the anaemic condition of the rats explains the reduction in their PCV value obtained after infection in Table 4. However in group B rats, there was increase in body weight. This supports the fact that there was no visible anaemic condition and most probable the cause of anaemia in the rats was eliminated.

The extract also showed the ability to act as immunostimulatory substance as group C rats that was given the extracts that recorded a boost in their neutrophil after the administration. This ability of extracts to serve as immunostimulatory substances have been reported by Ezekwesili et al. (2005).

A look at the result of the urine macroscopy showed the presence of protein and nitrite in infected rats, whereas they were absent in the rats infected but treated with the extract (Table 5). Cheesbrough (2000) wrote that in a urinary infection there will be the presence of protein and nitrite. This therefore has confirmed that an infection was established in the group A rats, and was prevented in the group B rats by the administration of the extract.

Conclusions

Medicinal plants are cheaper than orthodox medicine which are not only very expensive but are also not readily available to the rural farmers (Atata et al., 2005).

It is observed that *M. lucida* inhibited the growth of *E. coli* both *in vitro* and *in vivo* as it was able to prevent the establishment of *E. coli* infection in albino rats. In addition to this ability, it did not cause an anaemic condition in the experimental rats.

In conclusion, *M. lucida* has stimulatory effect on the experimental animals and also possess bioactive components which if properly harnessed could be used in the treatment of *E. coli* infections.

Purification of the extract is therefore recommended in other to obtain the pure bioactive components for Industrial use. Furthermore, toxicological analysis of the extract is essential so as to examine its toxicity level on sensitive organs of mammals.

Table 3. Average body weight of the rats.

Group	Before Infection	During Infection			After Infection
	Day 1(g)	Day 2(g)	Day 3(g)	Day 4(g)	Day 5(g)
A	101 ± 1.00	93 ± 0.57	90 ± 5.0	95 ± 2.0	95 ± 0.75
B	105 ± 0.57	118 ± 2.0	123 ± 2.85	123 ± 3.0	125 ± 3-0
C	120 ± 1.7	120 ± 2.0	130 ± 5.0	139 ± 1.0	141 ± 1.0
D	110 ± 0.00	115 ± 2.0	118 ± 0.57	120 ± 1.0	120 ± 1.0

KEY: A- Rats infected with *E. coli,* B- Rats infected with *E. coli* and treated with extract, C- Rats fed with extract only, D- Control.

Table 4. Effect of leaf extract of *Morinda lucida* on immunological indices in albino rats.

	BEFORE INFECTION									AFTER INFECTION								
Group	Hb (g)	PCV (%)	WBC (mm3)	ESR (mm/h)	RBC (x10^6)	N (%)	L (%)	E (%)	M (%)	Hb (g)	PCV (%)	WBC (mm3)	ESR (mm/hr)	RBC (x10^6)	N (%)	L (%)	E (%)	M (%)
	13.9	41.0	4900	4.8	4.0	46	56	-	-	9.0	27	5600	9	2.7	68	32	-	1
A	13.7	40.0	4800	4.9	3.8	44	54			8.5	25	5400	7	2.5	67	30		1
	13.5	39.0	4700	5.3	4.2	48	52			9.5	29	5800	11	2.9	69	34		1
	10.6	29.5	5800	5.4	3.4	51	46	-	1	9.7	29	3600	7	3.0	49	50	1	-
B	10.7	33	5500	5.2	3.2	52	47		1	9.5	31	3500	6	3.1	50	51	1	
	9.8	30.5	5300	4.4	3.6	53	48		1	9.9	27	4700	8	2.9	48	49	1	
	14.5	38.0	5700	4.5	3.9	60	40	1	1	12.0	36	4200	5	3.8	63	32	2	-
C	14.3	41	5600	4	4.0	58	41	1	1	11.8	35	4000	7	3.6	65	31	2	
	13.6	44	5800	3.5	4.3	56	39	1	1	11.6	37	4400	9	4.0	61	30	2	
	12.5	36.0	5200	5.6	3.7	52	48	-	-	12.7	38	5400	6	4.0	54	46	-	-
D	12.8	35	5000	5.2	3.5	52	49			12.2	39	5500	5	3.9	55	44		
	12.2	40	5400	4.7	3.9	52	47			13.2	37	5300	7	4.1	53	48		

A - Rats infected with E. coli
B - Rats infected with E. coli and treated with extract
C - Rats fed with extract only. D - Control

Table 5. Effect of extract of *M. lucida* on the urine biochemical indices.

	Before Infection				After Infection			
Group	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
pH	6	7	6	6	9	7	7	7
Protein	-	-	-	-	+	-	-	-
Glucose	-	-	-	-	-	-	-	-
As. Acid	-	-	-	-	-	-	-	-
Ketone	-	-	-	-	-	-	-	-
Nitrite	-	-	-	-	+	-	-	-
Bilirubin	-	-	-	-	-	-	-	-
Urobilino	Norm	Norm	Norm	Norm	Norm	+	+	Norm
Blood	-	-	-	-	-	-	-	-

A- Rats infected with *E. coli*
B- Rats infected with *E. coli* and treated with extract
C- Rats fed with extract only
D- Control.

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