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Full Length Research Paper

Comparative antimicrobial activities of some plant extracts and commercial antibiotics against some selected pathogens of food origin

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The antimicrobial activities of three plants (*Momordica charantia*, *Morinda lucida*, and *Hunteria umbellata*) were investigated. These plants were extracted using methanol and ethanol as solvents. These extracts were used to evaluate the growth of five food borne bacteria namely; *Bacillus* sp, *Proteus vulgaris*, *Streptococcus* sp., *Shigella* sp., *Lactobacillus* sp., and the research revealed zones of inhibition ranging from 0 to 36 mm. The minimal inhibitory concentration (MIC) of the extracts ranged from 20 to 100 mg/ml; that of the ethanolic extract of *Momordica charantia* against *Bacillus* sp. being the lowest (20 mg/ml) and most effective and that of *M. lucida* being the highest (100 mg/ml) and least effective. Most of the extracts were merely inhibitory against the organisms except ethanolic extracts of *H. umbellata* and *M. charantia* against *Bacillus* sp., *Streptococcus* sp. and *Lactobacillus* sp. with the ethanolic extract of *H. umbellata* showing the lowest MBC of 40 mg/ml. The activity indices of the extracts were calculated and the highest activity index (2.818) was that of the ethanolic extract of *M. charantia* against co-trimazole using *Bacillus* sp. as the test organism. *H. umbellata* and *M. charantia* showed more promising potential as antimicrobial agents than *M. lucida* against foodborne pathogens and hence against enteric diseases. These effective plants should be purified and further assayed for toxicological activity and possible use as drugs or preservatives.

Key words: Antimicrobial activity, foodborne pathogens, *Momordica charantia*, *Hunteria umbellata, Morinda lucida*, commercial antibiotics, activity index.

INTRODUCTION

Antibiotics are naturally occurring or synthetic organic compounds which inhibit or destroy selective bacteria, generally at low concentrations (Brooks et al., 2007). In spite of the fact that there is a wide range of current antibiotics available for treatment of bacterial infections, there are still some challenges to be met in microbial chemotherapy, some of which are the development of resistance by the microbes to chemotherapeutic agents due to abuse of use, high cost, limited effective lifespan and undesirable side effects of certain antibiotics (Cowan, 1999; Okemo et al., 2003; Reuter, 2005).The establishment and discovery of these has led to the search of new antimicrobia l agents mainly among plant extracts with the goal to discover new chemical struc-tures, which overcome these disadvantages (Bouamama et al., 2006).

The use of plants as source of medicine in treating disease is an ancient practice. People in all continents have long applied poultices, and imbibed infusions of hundreds, if not thousands of indigenous plants, dating back to pre-history (Cowan, 1999). The epidemiology of food borne diseases is changing and reports from different parts of the world indicate that strains of resis-tant food borne pathogens have emerged as public health problem. Over the last two decades for examples, bacterial infections caused by *Salmonella enteritidis, Staphylococcus aureus, Escherichia coli* and newer food borne pathogens have become increasingly resistant to empirical antimicrobial agents (Slutsker et al., 1998). The search for new antimicrobial natural products from plant materials is essential in order

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to curb the menace of multiple antibiotics resistant pathogens. In fact, plants produce a diverse range of bioactive molecules, making them rich sources of different types of medicines (Nair et al., 2005). Some of the advantages of the use of plants as antimicrobial agents include, among others, reduced cost, relative lower incidence of adverse reactions com-pared to modern conventional pharmaceuticals (Karachi, 2006), and ready availability. 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; the stem, bark and leaves of *Anthocliesta dialonensis* are used as antipyretic and in the treatment of stomach ache, gonorrhoea and fever (Azoro, 2002).

'*Abeere'* is the Yoruba name for *Hunteria umbellata* seed. The use of this plant in herbal medicines has been reported (Bevan et al., 1967; Sofowora, 1993). The phytochemical analysis of the crude medicinal plant revealed the presence of saponins, steroids, tannins, volatile oils, phenols and copious amount of alkaloids. *Momordica charantia*, called bitter melon or bitter gourd in English, is a tropical and subtropical vine of the family *Cucurbitaceae*, widely grown in Asia, Africa, and the Caribbean for its edible fruit, which is among the most bitter of all fruits. Popularity of *M. charantia* (MC) in various systems of traditional medicine for several ailments (antidiabetic, abortifacient, antihelmintic, contraceptive, dysmenorrhea, eczema, emmenagogue, antimalarial, galactagogue, gout, jaundice, abdominal pain, kidney (stone), laxative, leprosy, leucorrhea, piles, pneumonia, psoriasis, purgetive, rheumatism, fever and scabies, etc.), has made it a suitable test plant for this report. Bitter melon contains an array of biologically active plant chemicals including triterpenes, proteins, and steroids.

Morinda lucida is a medium sized tree with a crooked hole and rather short twisted branches. It belongs to the family *Rubiaceae.* It is referred to as the brimstone tree in English language and as '*oruwo*' in Yoruba language. Generally, it is used in agri-horticulture, traditional medicine against venereal diseases and even for religious, superstitious and magical uses. In medicine, it is used against cutaneous and mucocutaneous infections, as febrifuges, laxatives and industrially to produce inks, dyes, stains and mordants. All parts of the plant are used medicinally. The parts are generally active against leprosy, rheumatism, arthritis, tumors and cancers. Its phytochemical composition includes saponins, anthraquinones, cardenolides, and alkaloids.

MATERIALS AND METHODS

Collection of plant material

Three different plants (*H. umbellata* seeds, *M. charantia* stem and *M. lucida* stem) that were free from disease were properly selected and bought at Jegele market in Ilorin, Kwara State, Nigeria.

Preparation of plant extracts

The fresh plant samples were washed thoroughly and sun-dried and the stems were ground to fine powders using an electric blender. The solvents used for extraction during this research work are ethanol and methanol, both at 70% concentration. Appropriate amount of the dried plant samples were weighed and mixed with the appropriate quantity of solvents to get required concentration. For *H. umbellata,* the concentration used was 400 mg/ml while that of *M. charantia* and *M. lucida was* 300 mg/ml. After 72 h, the preparations were thoroughly shaken one at a time and filtered appropriately by passing them through Whatman No. 1 filter paper on a clean funnel. The extracts were stored in sterile covered sample bottles to keep them from evaporating and then getting more concentrated before use.

Collection and maintenance of test organisms

The pure cultures of the test organisms were obtained from the culture collection center of the Department of Microbiology, Public Health Laboratory Unit, University of Ilorin. The test organisms collected are *Bacillus* sp., *Streptococcus* sp., *Lactobacillus* sp., *Shigella* sp., *and Proteus vulgaris*. They were collected in slants in McCartney bottles containing nutrient agar and incubated at 37°C. They were then stored as stock cultures until required for analysis. Further subcultures were carried out at 2-weekly intervals to maintain the viability of these organisms.

Antimicrobial assay of extracts

The antimicrobial activity of the different plant species were evaluated by agar well diffusion method as described by Kela and Kufeji (1995). Sensitivity agar was used for the assay. The microorganisms were activated by inoculating a loopful of the strain in the nutrient broth (25 ml) and incubated at 37°C for 24 h. Sterile sensitivity test agar was dispensed into sterile Petri dishes at 45°C. The plates containing sensitivity agar were seeded with inoculum obtained as described earlier.

After inoculation, 4 wells (6 mm in diameter) were punched in the agar medium using sterile stainless cork borer. Each well was filled with a different plant extract and the fourth well served as the control containing either ethanol or methanol depending on the extract being assayed. Plates were left on bench for one hour before incubation at 37°C for 24 h to allow pre-diffusion of the ex-tracts (Esimone et al., 1998). The resulting zones of inhibition were measured using a ruler.

Antimicrobial assay of known antibiotics

The method used here is described by Bauer et al. (1966). The test organisms from the stock cultures were swabbed on the surface of the solidified sensitivity agar using sterile swab sticks to obtain a lawn swab. Two types of antibiotic discs were used depending on the Gram staining property of the test organism under observation, that is, Gram positive or Gram negative. The plates were slightly opened and an appropriate antibiotic disc is placed inside. The plates are not turned upside down but they are incubated at 37°C for 24 h. After incubation, the zones of inhibition around each antibiotic disc were measured.

Minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of the extracts

The MIC of both the ethanolic and methanolic extracts were determined using the test-tube dilution method (Spooner and

Table 1. Antibiotic sensitivity test against test organisms.

Test organism	GEN	COT	CHL	AUG	AMX	ERY	TET	CXC	CFL	NAL	NIT
Bacillus sp.	+++10	+++12	++5	+4	+3	++7	++9	0	+++11	+3	++6
Streptococcus sp	+++11	+++10	+++11	0	+2	+++12	++8	0	++9	+++13	+++10
Lactobacillus sp	++ 9	++7	++9	+4	0	++8	+5	+++10	+++12	+++11	+++14
Shigella sp	0	+++14	++8	0	+++13	0	+++12	++6	++9	++8	+++12
Proteus vulgaris	++9	+++16	++8	0	0	++6	+++11	0	0	+++12	+++13

Key: GEN- gentamycin; COT- co-trimazole; CHL- chloramphenicol; AUG- augmentin; AMX- amoxillin; ERY- erythromycin; TET-tetracycline; CXC-cloxacillin; CFL-ciproflaxin; NAL- nalidixic acid; NIT- nitrofurantoin; NA- not applicable.

Table 2. Zones of inhibition indicating the antimicrobial activity of the extracts on the test organisms.

Test organism	Gram property	Diameter of zones of inhibition (mm)					
		***Hunteria Umbellata* (400 mg/ml)**		***Morinda lucida* (300 mg/ml)**		***Momordica charantia* (300 mg/ml)**	
		Ethanol	**Methanol**	**Ethanol**	**Methanol**	**Ethanol**	**Methanol**
Bacillus sp	+ve	10	19	10	0	31	14
Streptococcus sp	+ve	30	17	7	10	36	25
Lactobacillus sp	+ve	21	0	15	10	13	7
Shigella sp	-ve	20	15	5	0	13	19
Proteus vulgaris	-ve	19	0	0	0	15	0

Sykes, 1972). The least concentration that prevented visible growth was made the minimum inhibitory concentration. About 1 ml of broth was taken from the test tubes that showed no growth and inoculated into a nutrient agar plate. The plates were incubated for 24 h at 37°C and proper dilutions were made where necessary. The least concentration of the extract in which no growth was observed on the nutrient agar medium after 24 h was taken as the minimum bactericidal concentration of the extract.

RESULTS

The antibiotic sensitivity analysis of the test organisms was shown in Table 1. Of the 5 organisms, it was observed that *Proteus vulgaris* was resistant to more antibiotics. The least zone of reaction noted was 0 mm which is for *Bacillus* sp. against cloxacillin, *Streptococcus* sp. against augmentin and cloxacillin, *Lactobacillus* sp. against amoxillin, *Shigella* sp. against gentamycin, augmentin, and erythromycin and *Proteus vulgaris* against augmentin, amoxillin, cloxacillin and ciproflaxin. Even though *P. vulgaris* showed resistance to four different antibiotics, it also showed the highest sensitivity to an antibiotic- co-trimazole giving the highest zone of inhibition of 16 mm. Of the 11 different antibiotics used, co-trimazole showed the highest activity. The zones of inhibition ranged from 0 to 16 mm.

Table 2 shows the result of the antimicrobial susceptibility test of the extracts on the test organism based on the zones of inhibition observed and measured after incubation. Overall, the ethanolic extracts showed the highest activity and are considered the most effective. Again, *P. vulgaris* showed the least susceptibility and hence the highest resistance to the extracts of both methanolic and ethanolic. The highest activity was shown by the action of the ethanolic extract of *M. charantia* while the least activity was shown by the action of the methanolic extract of *M. lucida*. The most susceptible organism to the extracts was *Streptococcus* sp. while *P. vulgaris* was the least susceptible.

Table 3 shows the results of the minimum inhibitory concentration (MIC) determination of the extracts against the test organisms. The MIC ranged from 0 to 20 to 100 mg/ml. The lowest and hence most effective MIC was that of the ethanolic extract of *M. charantia* against *Bacillus* sp. and was 20 mg/ml, while that of the ethanolic extract of *M. lucida* against *Bacillus* sp. which was 100 mg/ml. Table 4 shows the result of the minimum bactericidal concentration of the extracts against the test organisms. It was observed that most of the extracts were merely inhibitory and not bactericidal. Table 5 shows the activity index of the extracts:

Activity index = Inhibition zone (mm) of extract / inhibition zone of standard (Menghani et al., 2011)

The highest zone of inhibition in the antibiotic susceptibility testing was taken as the standard for each organism.

Table 3. Minimum inhibitory concentration (MIC).

Test organism	MIC (mg/ml)					
	Hunteria umbellata		***Morinda lucida***		***Momordica charantia***	
	Ethanol	**Methanol**	**Ethanol**	**Methanol**	**Ethanol**	**Methanol**
Bacillus sp.	50	60	100	-	20	40
Streptococcus sp.	20	70	60	70	30	40
Lactobacillus sp.	40	-	80	60	50	70
Shigella sp.	50	50	70	-	50	70
Proteus vulgaris	70	-	-	-	60	-

Table 4. Minimum bactericidal concentration of extracts against test organism.

Test organism	MBC (mg/ml)					
	Hunteria umbellata		***Morinda lucida***		***Momordica charantia***	
	Ethanol	**Methanol**	**Ethanol**	**Methanol**	**Ethanol**	**Methanol**
Bacillus sp.	-	-	-	-	50	-
Streptococcus sp.	40	-	-	-	50	-
Lactobacillus sp.	50	-	-	-	-	-
Shigella sp.	-	-	-	-	-	-
Proteus vulgaris	-	-	-	-	-	-

Table 5. Activity index of the extracts against standards.

Test organism	Standard used/ ZI	Zone of inhibition (mm) / Activity index					
		Hunteria umbellata		***Morinda lucida***		***Momordica charantia***	
		Ethanol	**Methanol**	**Ethanol**	**Methanol**	**Ethanol**	**Methanol**
Bacillus sp.	CFL	10	19	10	-	31	14
	11	0.909	1.727	0.909	0	2.818	1.273
Streptococcus sp.	NAL	30	17	7	10	36	25
	13	2.308	1.308	0.538	0.769	2.769	1.923
Lactobacillus sp.	CFL	21	-	15	10	13	7
	12	1.75	0	1.25	0.833	1.083	0.583
Shigella sp.	COT	20	15	5	-	13	19
	14	1.43	1.07	0.36	0	0.93	1.36
Proteus vulgaris	COT	19	-	-	-	15	-
	16	1.19	0	0	0	1.07	0

DISCUSSION

The data presented in this research work has shown that plants serve as a very effective source of new antimicrobial agents for use in pharmaceutical industries and medicine. Ethanolic extracts of these extracts showed more promising antimicrobial potentials against selected test bacteria. The main aim of this study is to validate and authenticate the antimicrobial potentials of certain plants and simultaneously, justify their use in the daily diet to cure mankind from certain ailments. The presence of bioactive substances has been reported to confer resistance to plants against bacteria, fungi, and pests, and therefore, explains the demonstration of antimicrobial activity by the plants used in this study.

The activity index of the extracts against commercial antibiotics showed that the plant extracts (at the concentrations used) were more effective against the test

organism than the commercial antibiotics. Also, the activity of the extracts especially those of *H. umbellata* and *M. charantia* against both Gram positive and Gram negative bacteria is indicative of the possibility of the use of these plants to produce drugs with a broad spectrum of activity. This observation is very significant because of the possibility of developing therapeutic substances that will be active against multi-drug resistant organisms. Further determination and purification of the active ingredients should also be considered in the future.

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