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Comparative Evaluation of Antimicrobial Activities of Leaf Extract of *Mirabilis jalapa* and Microbial Toxins on Some Pathogenic Bacteria

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Abstract: The ethanolic extract of the leaf of *Mirabilis jalapa* was tested for antimicrobial activity against five pathogenic bacterial strains: *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Bacillus cereus* and *Klebsiella pneumoniae*. Antagonistic activities of toxins from nine strains of bacteria and four fungi isolated from refuse dumps was also tested on the pathogens. The isolates producing the toxins were identified as *Pseudomonas* sp., *Acinetobacter* sp., *Corynebacterium* sp., *Actinomyces* sp., *Clostridium* sp., *Bacillus* sp., *Shigella* sp., *Proteus* sp. *Enterobacter* sp., *Penicillium* sp., *Aspergillus flavus*, *Aspergillus niger* and *Aspergillus repens*. The agar ditch diffusion method was used was used for the *in vitro* antimicrobial bioassay. The leaf extract was found to have a higher antimicrobial efficacy than the toxins from the organisms as shown in the growth inhibition indices. The highest zone of inhibition of leaf extract was 13.0 mm and the least 4.0 mm. The highest zone of inhibition of bacteria isolates was 9.0 mm and the least 3.5 mm while the highest zone of inhibition of fungi isolates was 13.0 mm and the least 2.0 mm. Fungi isolates produce toxins with broad spectrum of activity. However the commercial antibiotics was found to be more potent than the toxins or the extract; But with narrower spectrum of activity. Phytochemical screening of the extract revealed the presence of tannins, saponins, alkaloids and cardiac glycosides. The toxins and the plant extract possess' antimicrobial activities comparable to conventional antibiotics; and can thus be a good source of agents for biocontrol and chemotherapy.

Key words: *Mirabilis jalapa*, toxins, antimicrobial, phytochemicals

INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine. Medicinal plants are of great importance to the health of individuals and communities. Herbal medicines serve the health needs of about 80% of the world's population, especially for millions of people in the vast rural areas of developing countries (WHO, 2001).

Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids and flavonoids which have been found to have *in vitro* antimicrobial properties (Edeoga *et al*., 2005). The medicinal value of these plants lies in some chemical substances that produce definite physiological action on the human body. The relatively lower incidence of adverse reactions to plant preparations compared to modern conventional pharmaceuticals, coupled with their reduced cost, consequently encouraging both the consuming public and national health care institutions to consider plant medicines as alternatives to synthetic drugs (Nair *et al*., 2004). Undoubtedly, medicinal plants are relevant in both developing and developed nations of the world as sources of drugs or herbal extracts for various chemotherapeutic purposes (Alanins *et al*., 2005). In as much as is being carried

out on the usefulness of medicinal plants, some organisms have been discovered also to contain potential antimicrobial agents capable of eliciting effect on other pathogenic organisms. Such organisms have been isolated from various sources such as food, soil, water and so on.

A medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs (Razaq *et al*., 2003). Medicinal plants also include those used in galenical preparations such as decoctions; Plants used for extraction of pure substances either for direct medicinal use or for hemi-synthesis of medicinal compounds; Foods and spices. Plants are the basic sources of knowledge of modern medicine. In fact, plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines. Higher plants, as sources of medicinal compounds have continued to play a dominant role in the maintenance of human health since ancient times (Anwarul and Atta-ur-Rahman, 2005). Over 50% of all modern clinical drugs are of natural product origin and natural products play an important role in drug development programs in the pharmaceutical industry (Nair *et al*., 2004).

Mirabilis jalapa belongs to the family Nyctaginaceae. It is a perennial herb that reaches a height of 50-100 cm from a tuberous root. Some cultivated hybrid can grow up to a meter in height. It is a popular ornamental plant grown worldwide for the beauty of its flowers which can be white, red, pink, purple or multicoloured and their sweet fragrance.

Mirabilis jalapa has been used in traditional medicine (Nair *et al*., 2004) which may be due to the presence of some biomolecules of pharmacological importance (Edeoga *et al*., 2005). However, there have not been any comparative studies on the antimicrobial activity of the plant and toxins from refuse isolates on certain pathogenic organisms. The present investigation is therefore designed to isolate, characterize and identify microorganisms associated with refuse dump site and assay the antagonistic effect of their toxins on some pathogens. Comparative evaluation of the antimicrobial activities of the crude extracts from *Mirabilis jalapa* on the pathogenic organisms as well as screening for the phytochemicals of pharmacological importance also determined.

MATERIALS AND METHODS

Isolation and Characterisation of Organisms from Refuse Dump Sites

Samples from different refuse dump sites around Akure metropolis, Nigeria; were collected in January, 2006 in into sterile bottles. The micro floral was isolated using standard methods (Prescott *et al*., 2005). The microbial analysis was carried out in the Research Laboratory, Department of Microbiology, Federal University of Technology and Akure, Nigeria.

Extraction and Fractionation of *Mirabilis jalapa* Leaf

Fresh leaves of the plant were collected, sun dried and ground into uniform powder. About 244 g of the dried powdered sample was soaked in 1300 mL of 60% ethanol until super saturation for 72 h. The sample was filtered with the aid of muslin cloth. The extract was concentrated in vacuo using rotary evaporator. The extract was dissolved in 0.1 M Tris-HCL buffer (pH 7.0, 5 mL) and applied to a column (5×85 cm) of Sephacryl S-300 HR, pre-equilibrated and developed with the same buffer. Fractions corresponding to the peak were pooled together concentrated and freeze dried. The powder was dissolved in water and applied to a Sephadex G-25, column (1.5×50 cm), then eluted with water and fractions were collected. The eluate obtained was concentrated and lyophilized.

Detection of Antagonistic Activity

The antagonistic activities of the refuse isolates against the pathogenic organisms were determined using the modified agar diffusions assay described by Schillinger and Lucke (1989). Old culture of the

refuse bacterial (isolates on slant) was inoculated aseptically into the tubes of fresh nutrient broth and incubated at 37°C for 24 h. After 24 h incubation, the culture supernatant of broth was obtained by centrifugation.

Wells were made with an 8 mm cork borer in plates containing solidified nutrients agar already seeded with 0.1 mL of the pathogenic isolates from 24-h-old culture. Then, few drops of culture supernatant of actively growing refuse isolates were introduced into the wells with the aid of sterile syringes. The plates were incubated at 37°C for 24 h after which zones of inhibition around each well were examined.

Antimicrobial Screening of the Plant Extract

The antimicrobial study of the plant extract was carried using the agar well diffusing method described by Tortora *et al*. (2005).

Phytochemical Screening of Leaf Extract

The phytoconstituents were assay for using standard methods described by Sofowora; 1993.The assay was carried out in the Biochemistry Department, Federal University of Technology and Akure, Nigeria.

RESULTS AND DISCUSSION

A total of nine toxin producing bacteria strains were isolated and identified as *Pseudomonas* sp., *Acinetobacter* sp., *Corynebacterium* sp., *Actinomyces* sp., *Clostridium* sp., *Bacillus* sp., *Proteus* sp. and *Enterobacter* sp. The identities of the four isolated fungi are *Aspergillus niger*, *Aspergillus flavus*, *Penicillium* sp. and *Aspergillus repens*. The toxins produced were found to be antagonistic to the growth of the pathogenic bacteria (Table 1 and 2). The susceptibility of the pathogens varies among the different genera. The observed variation may be due to difference in genetic composition of the organism which may be chromosomal or plasmid coded (Prescott *et al*., 2005). Another factor for the observed trend may result from the difference in the nature of the cell wall which differs in chemical composition and structural integrity. The antimicrobial potency of the toxin produced also differs. This may result from virulence and toxigenicity of the species resulting from phylogenicity. The fact that some organisms through unidirectional process release specific compounds that have negative effect on another at different proportion had been reported (Prescott *et al*., 2005; Tortoral *et al*., 2005).

Table 1: Antibacterial activity of refuse bacteria isolates

	Zone of inhibition (mm)								
Test bacteria	A	B	C	D	E	F	G	H	I
Bacillius cereus	-	-	-	6.0	-	-	9.0	-	-
Escherichia coli	-	-	8.5	7.0	-	4.0	7.0	5.0	9.0
Klebsiella pneumoniae	-	-	-	-	-	-	-	-	-
Salmonella typhi	-	-	-	3.5	-	-	-	-	-
Staphylococcus aureus	-	5.5	4.0	6.5	-	-	9.0	6.0	6.5

- = Nil

Table 2: Antibacterial activity of fungi isolates

	Zone of inhibition (mm)			
Test bacteria	FA	FB	FC	FD
Bacillius cereus	11.0	3.0	11.0	4.0
Escherichia coli	2.0	3.0	11.0	3.0
Klebsiella pneumoniae	3.0	2.0	12.0	2.0
Salmonella typhi	3.0	4.0	12.0	2.0
Staphylococcus aureus	4.0	4.0	13.0	4.0

Table 3: Antimicrobial bioassay of leaf extract of *M. jalapa*

	Concentrations and zone of inhibition (mm)			
Test bacteria	500 mg mL^{-1}	100 mg mL^{-1}	200 mg mL^{-1}	300 mg mL^{-1}
Bacilli us cereus	Nil	Nil	Nil	Nil
Escherichia coli	Nil	Nil	Nil	13.0
Klebsiella pneumoniae	Nil	Nil	Nil	6.0
Salmonella typhi	Nil	Nil	Nil	Nil
Staphylococcus aureus	Nil	Nil	Nil	4.0

Nil = Not effective

Table 4: Antibiotic Sensitivity of Pathogenic Bacteria Isolates

		Zone of inhibition (mm)									
Pathogens		OIF	C	CF	AM	GN	N	CIP	TE	NF	AX
Gram negative	*Escherichia coli*	17.0	9.0	NI	NI	4.5	2.0	17.0	7.0	12.0	2.0
	Klebsiella pneumoniae	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
	Salmonella typhi	16.0	10.0	NI	4.0	4.0	2.0	18.0	8.0	12.0	5.0
		FX	AU	OF	E	CIP	CD	GN	CX	CI	AP
Gram positive	*Bacillus cereus*	NI	NI	14.0	23.5	23.5	4.0	NI	NI	NI	NI
	Staphylococcus aureus	NI	NI	25.0	NI	NI	NIN	IN	NI	NI	NI

OF = Ofloxacin, C = Chloranphenicol, CF = Cefunoxine, AM = Ampicillin, N = Nitrofurantione, CIP = Ciprofloxacin, TE = Tetracycline, NF = Narfloxacin, TE = Tetracyclin, AX = Amoxicillin, FX = Floxapen, AU = Augumentin, E = Erythromycin, CD = Clidamycin, GN = Gentamycin, Cx = Cephaplexin, CO = Cotrimoazole, AP = Cloxacilin, NI = No Inhibition

Table 5: Phytochemical screening of leaf extracts *Mirabilis jalapa*

Chemical constituent	
Saponins	+
Tannins	+
Phlobatannin	+
Flavonoids	+
Alkaloids	+
Steroids	+
Terpenoids	+
Glycosides	+

+ = Positive

In comparison with the bacterial toxins, the fungi toxins showed more antagonistic activity against the pathogens (Table 2). Fungi had been known to be excellent and better producers of antimicrobial agents than bacteria.

The phytochemical screening of the leaf extract of *Mirabilis jalapa* revealed the presence of bioactive components such as alkaloids, tannins, flavonoids and phenolic compounds (Table 5). These compounds are said to be responsible for antimicrobial activities in plants (Edeoga *et al*., 2005). The observed antimicrobial activities of the extract may be traced to these bioconstituents.This confirms the ability of the extract to inhibit *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus* (Table 3). The susceptibility of the pathogenic bacteria to the extract (Table 4) or their resistance to extract at varying concentration might be ascribed to the differences in the morphology of the cell structures and chemical composition between these organisms as well as variation in permeability and osmotic potential (Hailu *et al*., 2005).

The ethanolic extract at lower concentrations of 50, 100 and 200 mg mL^{-1} was not effective in inhibiting the growth of the pathogen but at a higher value of 300 mg mL^{-1} it prevents the proliferation of the organisms. The inference is that the activity of the extract is concentration dependent. This is in agreement an earlier report that an increase in the concentration of an anti microbial agent might result in increase in effectiveness (Aspen, 2000).

The commercial antibiotics showed more potency in activity than the extract (Table 5). This may be attributed to the methods of production, purification, concentration as well as quality control and assurance of the commercial antibiotics. The presence of impurities in extract might be responsible for the lower values of zones of inhibition exhibited against the tested micro organisms (Sayed *et al*., 1987).

CONCLUSIONS

The comparison of the antimicrobial activities of the toxins from refuse isolates with that of leaf extract of *Mirabilis jalapa* showed that the former produced antagonistic substances but was were milder in activity than the latter. However the conventional antibiotics is more potent than both the toxins and the extract but with narrower spectrum of activity. The presence of the antinutritional factors in the extract was responsible for the antimicrobial activity of the extract and hence justifies the use of the plant in ethno medicine.

RECOMMENDATION

The future of medicinal plants as potential source for new drugs is promising and that the refuse dump site offers an enormous potential as source of lead molecules that can be of immense importance in chemotherapy. It is therefore recommend that further studies into isolation and characterization, purification of these toxins and biomolecules using HPLC, GC, NMR and spectral studies for structural elucidation of biomolecules of pharmacological importance. Toxicological studies are also necessary before possible formulation into dosage and *in vivo* trials.

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