Antibiotic-Plant Synergy as a New Strategy for Combating Drug Resistant Bacteria

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Bacterial resistant to antibiotics is of great concern for treating bacterial diseases. Therefore, there is ever increasing need to research for better ways of curbing resistance from pathogenic bacteria. One of the ways to do this is to test for antibiotic-plant's extract combined activity (synergistic activity).This could provide a way out for bacterial resistant diseases. The synergistic effect from the association of antibiotic with plant extracts against resistant bacteria leads to new choices for the treatment of infectious diseases. This effect enables the use of the respective antibiotic when it is no longer effective by itself during therapeutic treatment. This chapter seeks to present a study that was carried out to investigate synergistic activity of methanolic extract of the plant *Adenium obesum* stem-bark (*Apocynaceae* plant family species) with oxytetracycline (an antibiotic) against some bacterial isolates. The objective of this chapter is to show, using synergy, a way to prepare a new anti-microbial combination for multidrug resistant diseases caused by pathogenic bacteria based on a combined activity of oxytetracycline and methanolic extract of *Adenium obesum*. The synergistic activity is verifiable using Kirby and Bauer techniques. Results obtained from the studies seem to be promising and may enhance the natural products uses showing synergistic potentiality of the stem-bark of *Adenium obesum* in the combating various infectious diseases caused by some bacterial isolates.

Keywords synergism; methanol; Adenium obesum; oxytetracycline

1. Introduction

Herbal medicine represents one of the most important fields of traditional medicine all over the world. To promote the proper use of herbal medicine and determine their potential as source of new drugs, it is essential to study medicinal plants which have folklore reputation in more intensified way [1].

Adenium obesum (*Apocynaceae*) is a plant found in northern Nigeria, commonly known as 'karya' in Hausa Language, a commonly spoken language across the northern part of the country [2].The English name is Desert rose. It is a Succulent shrub or small tree, up to (4–6)m tall, sometimes with a fleshy taproot; stem swollen at base up to (1–2) m in diameter; bark pale greyishgreen, grey or brown, smooth, with sticky, clear or white latex; branchlets glabrescent, pubescent at apex. Leaves arranged spirally, clustered at the end of branchlets, simple; stipules minute or absent; petiole up to 4 mm long; blade linear to obovate, 3–12(–17) cm × 0.2–6 cm, base cuneate, apex acute to rounded or emarginate, entire, slightly glaucous, dull green or pale green, leathery, pinnately veined with distinct or indistinct lateral veins [3].

The plant is important in traditional medicine. In the Sahel, a decoction from the roots, alone or in combination with other plants, is used to treat venereal diseases; a root or bark extract is used as a bath or lotion to treat skin diseases and to kill lice, while the latex is applied to treat septic wounds [4]. In Somalia, a root decoction as nose drops is prescribed for rhinitis [4]. In northern Kenya, the latex is rubbed on the head against lice and the plant's powdered stems are applied to kill skin parasites of camels and cattle[4]. The bark is chewed as an abortifacient [4].

Because of resistant of some bactaria to many antibiotics, the need to test for antibiotic-plant's extract combined activity (synergistic activity) should arise.This could serve as a way out for the problems associated with bacterial resistant maladies. Synergism from the association of antibiotic with plant extracts against resistant bacteria pave ways to investigations that could lead to treatment of diseases caused by resistant pathogenic bacteria.It booster the use of single antibiotic when it is no longer effective by itself when it is administered. This study was undertaken for the first time to investigate synergistic activity of methanolic extract of *Adenium obesum* stem-bark with oxytetracycline.

The objective of this study was to formulate new, cost effective anti-microbial combination for multidrug resistant diseases based on the synergistic activity of oxytetracycline and methanolic extract of *Adenium obesum (Apocynaceae)*, a tradomedicinal plant common in Nigeria. The synergistic activity was verified using Kirby and Bauer techniques.

2. Material and Methods

Collection and Preparation of Plant Material The stem-bark of *Adenium obesum* was collected from Samaru area of Zaria; Kaduna State, Nigeria. It was authenticated at the Herbarium, Biological Sciences Department, Ahmadu Bello University,Zaria,Nigeria where a specimen (voucher number 1836) was deposited. The bark was air dried and ground into powder using a porcelain mortar and pestle. It was then sealed in a polyethene bag and stored in a desiccator prior to evaluation.

2.1. Extraction

The powered stem-bark of *Adenium obesum* (500g) was packed in a thimble, placed inside a soxhlet extractor and extracted exhaustively, first with petroleum spirit (60-80) ^{o}C for 48 h and then methanol for 42 h . The extracts were concentrated *in vacuo* at 40 ^{o}C in a rotary evaporator to yield the dry extracts [5].

2.2. Phytochemical screening

The methanolic extract of the stembark of *Adenium obesum* was subjected to preliminary phytochemical (qualitative) tests to detect the presence of alkaloids, steroids, saponins, glycosides, anthraquinones, tannins,terpenoids, coumarins,carbohydrates and flavonoids using standard techniques [6-8].

2.3. Test organisms

Eight American Type Culture Collection (ATCC) strains bacterial strains were selected; *Escherichia coli* (ATCC 25922), *Klebsiella pneumonia* (ATCC 15380), *Pseudomonas aeruginosa* (ATCC 25619), *Salmonella typhi* (ATCC 9184), *Bacillus subtilis* (ATCC 6623), *Streptococcus pyogenes* (ATCC 10782), *Staphylococcus aureus* (ATCC 25923) and *Corynebacterium ulcerans* (ATCC 6939).The bacterial strains were maintained on nutrient agar and sub-cultured every three days. An inoculum of each bacterial strain was suspended in 5 ml of Mueller Hinton broth (MHB) and incubated overnight at 37 ºC. The overnight cultures were diluted with Normal Saline and adjusted to give a concentration of bacterial cells equivalent to a McFarland 0.5 standard of Barium Sulphate solution prior to the bacterial testing [9].

2.4. Susceptibility test (Zone of Inhibition)

The synergistic activity study was achieved by combining the extract with the antibiotic, oxytetracycline, using disc diffusion technique (Kirby and Bauer technique). Methanolic plant extract of *Adenium obesum*, 125µg/ml, was used in combination with oxytetracycline 62.5 µg/ml for incubation period of 24 h at 37°C [10].

Zones of inhibition were measured for oxytetracycline, stem-bark extract of *Adenium obesum* and in the end for combination of oxytetracycline and the plant stem-bark extract.

2.5. Minimum inhibitory concentration (MIC)

A series of culture tubes (microdilution assays) [11] were prepared all containing the same volume of medium inoculated with test microorganisms. The lowest concentration of sample at which the subculture from test dilution yielded no viable organisms was recorded as minimum inhibitory concentration [12]. Decreasing concentration of drug was added to the tubes; a step wise dilution (two fold serial dilutions) was used starting from highest to lowest concentrations (20mg/ml, 10mg/ml, 5mg/ml, 2.5 mg/ml and 1.25 mg/ml). One tube was left without drug to serve as positive control and other without drug and inoculum to serve as negative control. The cultures were incubated at 37 °C for 24 h. The tubes were inspected visually to determine the growth of organisms by the presence of turbidity and the tubes in which drug is present in minimum concentration sufficient to inhibit the bacterial growth which remains clear was noted as MIC of the drug. In experimental terms MIC is the concentration of the drug present in the last clear tube, which is the tube having the lowest drug concentration in which growth is not observed. The MIC was determined for oxytetracycline alone,then for the stem-bark extract of *Adenium obesum* and finally combination of oxytetracycline and methanolic stem-bark extract of the plant (1:1).

3. Results and Discussion

Preliminary phytochemical screening of the plant methanolic extract revealed the presence of alkaloids, steroids, saponins, glycosides, anthraquinones, tannins and flavonoids. However, coumarins were absent in the extract (Table 1). The MIC results for oxytetracycline alone, stem-bark extract of *Adenium obesum* only and that of combination of oxytetracycline and methanolic stem-bark extract of *Adenium obesum* (1:1), antibiotic-plant, were presented in Table 3. The MIC values were found to be small with oxytetracycline alone and it was found to be smaller with the methanolic extract of *Adenium obesum* . However, the MIC was found to be the least with combination of oxytetracycline and methanolic stembark extract of *Adenium obesum*. Moreover, the therapeutic efficacy was found to be higher even in low concentration with the antibiotic-plant combination. This clearly exhibits the advantage of the combination of oxytetracycline and methanolic extract of the plant over the other two individual forms.Therefore, this clearly showed that oxytetracycline presented good synergism with methanolic extract of *Adenium obesum*.

In these findings, *Streptococcus pyogenes* (ATCC 10782) shows higher sensivity to synergistic combination indicated by higher zone diameter (37 mm), lowest synergistic effect was observed against *Pseudomonas aeruginosa* (ATCC 25619) (24mm) and Salmonella typhi (24 mm). No synergistic activity was observed against *Klebsiella*

pneumonia (ATCC 15380).Out of 8 different bacteria, both Gram positive and Gram negative tested, 87.5% were sensitve to synergistic activity of the drug and the extract (Table 2).

Table 1 Phytochemical screening of Adenium obesum (stem-bark) methanolic extract

S/No.	Phytochemicals	Results methanolic extract
1	Carbohydrates	+
2	Steroids	+
3	Terpenoids	+
4	Saponins	+
5	Tannins	+
6	Anthraquinones	+
7	Cardiac glycosides	+
8	Alkaloids	+
9	Coumarins	-
10	Flavonoids	+

+/- = presence or absence of phytochemical tested

Table 2 Susceptibility Test (Zone of Inhibition)

Test organisms	Zone of inhibition (mm)		
	O	E	EO
Escherichia coli (ATCC 25922)	25	20	27
Klebsiella pneumonia (ATCC 15380)	6	0	0
Pseudomonas aeruginosa (ATCC 25619)	20	18	24
Salmonella typhi (ATCC 9184)	17	19	24
Bacillus subtilis (ATCC 6623)	27	25	32
Streptococcus pyogenes (ATCC 10782)	29	26	37
Staphylococcus aureus (ATCC 25923)	26	26	30
Corynebacterium ulcerans (ATCC 6939)	21	19	26

O= Oxytetracycline, E = Methanolic extract of *Adenium obesum*, EO= Methanolic extract of *Adenium obesum* + oxytetracycline.

Table 3 Minimum inhibitory concentration (MIC)

Test organisms	MIC of O (μg/ml)	MIC of E (μg/ml)	MIC of EO (1:1) (μg/ml)
Escherichia coli (ATCC 25922)	125	500	62.5
Klebsiella pneumonia (ATCC 15380)	1500	2000	1500
Pseudomonas aeruginosa (ATCC 25619)	500	1000	125
Salmonella typhi (ATCC 9184)	1000	1000	500
Bacillus subtilis (ATCC 6623)	500	1000	62.5
Streptococcus pyogenes (ATCC 10782)	500	500	125
Staphylococcus aureus (ATCC 25923)	1250	1250	125
Corynebacterium ulcerans (ATCC 6939)	1000	1250	500

O= Oxytetracycline, E = Methanolic extract of *Adenium obesum*, EO= Methanolic extract of *Adenium obesum* + oxytetracycline.

The results of the synergism study depicted that the Gram positive bacteria (*Bacillus subtilis* -ATCC 6623, *Streptococcus pyogenes*-ATCC10782, *Staphylococcus aureus*-ATCC25923 and *Corynebacterium ulcerans*-

ATCC6939) were those that exhibited higher sensivity to synergistic effect than the Gram negative ones (*Escherichia coli*-ATCC25922, *Klebsiella pneumonia*-ATCC15380, *Pseudomonas aeruginosa*-ATCC25619 and *Salmonella typhi*-ATCC 9184).This is because Gram-negative bacteria were reported to have higher intrinsic resistance to most antimicrobial agents [13].In general, the antibiotic-plant combination is synergistically very active against the bacteria. This high activity could be ascribed to the presence of the secondary metabolites available in the plant.There are tannins which are reported to have various physiological effects like anti-irritant, antisecretolytic,antiphlogistic, antimicrobial and antiparasitic effects. Phytotherapeutically tannin-containing plants are used to treat nonspecific diarrhoea, inflammations of mouth and throat and slightly injured skins [14-15]. Steroids are also there,which are important drugs used as hypotensives, cardiac depressants, sedatives and anti-dysenteric agents [16]. Glycosides which are used as laxative and carthatic drugs were also confirmed. Alkaloids that act as antimalarial,anti-amoebic agents, astringents were present [16].

The test organisms used in this study are associated with various forms of human infections.From a clinical point of view, *Streptococcus pyogenes* (ATCC 10782) cause a wide spectrum of diseases, including tonsillitis, erysipelas, impetigo, scarlet fever, rheumatic fever, septicaemia and acute glomerulonephritis [17]. *Escherichia coli* (ATCC 25922) causes septicemias and can infect the gall bladder, meninges, surgical wounds, skin lesions and the lungs especially in debilitate and immunodeficient patients [18]. Infection caused by *Salmonella typhi* (ATCC 9184) is a serious public health problem in developing countries and represents a constant concern for the food industry [19].

4. CONCLUSION

The demonstration of synergistic activity by the antibiotic-plant against both Gram negative and Gram positive bacteria is an indication that the plant can be a source of bioactive substances that could possess broad spectrum of activity most especially when it is combined with antibiotic.Thus, there is increasing need for researchers to investigate the synergistic capacity of plants or other natural products, independent of the antimicrobial activity they have. These findings also suggest that the need for understanding of synergism mechanism is fundamental to development of pharmacological agents to treat diseases by various bacteria using medicinal plants in combination with antibiotics.

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