IN – VITRO SYNERGISTIC ACTIVITY OF EUPHORBIA BALSAMIFERA ROOT EXTRACT AND AMPICLOX AGAINST SOME PATHOGENIC BACTERIAL ISOLATE

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Abstract
Euphorbia Balsamifera (root) was collected, identified, air-dried, ground and extracted by cool- extraction using mercerization method. Standard phytochemical tests of Trease and Evans was adopted for the phytochemical screening which revealed the presence of Terpenoids, Anthraquinones, Flavonoids, Saponins, Tannins, Alkaloids and Cardiac glycosides. The Minimum Inhibitory Concentration of methanolic extract in combination with Ampiclox using 3 different clinical pathogenic Bacterial isolates was found to be 15mg/ml to 34.7mg/ml. The highest synergistic activity was attained against Escherichia Coli. This result indicated strong antibacterial activity of the methanolic extract in combination with Ampiclox against the pathogenic Bacterial isolates. The spectra of antimicrobial activities displayed by the methanolic extract alone and methanolic extract in combination with Ampiclox could be attributed to the presence of these phytochemicals and this signified the potential of the plant to serve as a source of therapeutic agents.

Keywords: synergism, phytochemical, ampiclox, pathogenic bacterial isolates, in-vitro.

INTRODUCTION
A large portion of the population of developing countries uses traditional medicine alone or in combination with orthodox drugs to treat wide variety of ailments. It is estimated that about 40% of all drugs in developing countries are obtained from plants and 65% of the people living in developing countries still depend on traditional medicine as compared to orthodox medicine. To promote the proper use of herbal medicine and determine their potential as source of new drugs for Sustainable Development and Human Capacity Building, it is essential to study medicinal plants which have folklore reputation in more intensified way (Tijjani et al., 2011, Parekh 2007).

Euphorbia balsamifera is a plant found in Canary Islands, North America, West Africa, Somalia and South of the Arabian Peninsula (Dalziel 1956). Euphorbia is a succulent shrub or small tree, which grows to a height of 2 meters. The plant produce latex which is used externally as anti- venom on snake bites; it is also applied to guinea worm sores. The motive behind the choice of Euphorbia balsamifera is based on it is traditionally acclaimed to be effective in treatment of infections wounds in men and animals (Dweek 1996). Native Americans use the plant for many medicinal purposes including treatment of skin infections (Applied on the skin) and gonorrhea (internally). The roots and leaves of the plant are strong laxative as also reported by (Dalziel 1956).

Since some bacteria are resistant to many antibiotics, there is need to test for antibiotics plant extract combined activity (synergetic activity). This could provide an easy way out for bacterial resistant diseases. The synergetic effect of extracts against some resistant bacteria may leads to new choices for the treatment of infectious diseases. Therefore, the present study was under taken for the first time to investigate synergetic activity of methanolic extract of Euphorbia balsamifera root with Ampiclox. The objective of this study was to formulate new, cost effective anti-microbial combination for multidrug resistant diseases base on the synergistic activity of Ampiclox and methanolic extract of Euphorbia balsamifera root, a trado medicinal plant commonly use in west Africa. The synergetic activity was verified using Kirby and Bauer techniques.

MATERIAL AND METHODS
Collect and Preparations of Plant Material
The roof of euphorbia balsamifera was collected from Kazaure local government of Jigawa State, of Nigeria. It was confirmed and authenticated by a botanist at the department of biological science, Bayero University Kano, Nigeria. The root was properly washed with tap water, rinsed with distilled water and air dried. The dry sample was then pulverized using pestle and mortar. It was then preserved in desiccators before subsequent experiments.

Extraction
The powder root of Euphorbia balsamifera (400g) was soaked in 1600ml methanol (equivalent to 9:1 and water respectively) and allowed to stand for 72hours. The mixture of the root of Euphorbia balsamifera was filtered using Whatman no. 1 filter paper. The extracts were concentrated in vacuo at 40°C using rotary evaporator, after which the crude extracts were obtained from the solvents (Harbone 1984).

Phytochemical Screening
The methanol root extract of Euphorbia balsamifera was subjected to preliminary phytochemical test to detect the presence of Alkaloids, Anthraquinones, Cardiac Glycosides, Flavonoids, saponins, Tannins and Terponoids using standard techniques (Trease and Evans, 1989; Sofowora 1993).
Test Organisms
The clinical bacterial isolates used are *Escherichia coli*, *Klebsiella pneumonia* and *proteus* these were obtained from the department of microbiology, Aminu Kano Teaching Hospitals (AKTH) Kano, Nigeria. The bacteria isolates were maintained on nutrient agar and sub culture for three days. Inoculums of each bacterial strain was suspended in 5ml of Mueller Hilton broth (MHB) and incubated overnight at 37°C for three days. Inoculums of each bacterial strain was poured into Petri dishes and allowed to solidify. The discs were autoclaved at 121°C for 15 minutes, the molten agar were poured into Petri dishes and allowed to solidify.

Preparation of the Media
The Muller Hinton agar was prepared according to the manufactures instruction, 40g of the agar were weight and dissolve in 1000ml of distilled, the molten water, this were autoclave at 121°C for 15 minutes, the molten agar were poured into Petri dishes and allowed to solidify.

Preparation of Paper Discs
Stock solution of each extract fraction were prepared by weighing 1.0g of the extract and dissolving it in 1.0ml of dimethyl sulphur oxide (DMSO) to yield a concentration of 2.0ml from the stock solution to give concentrations of 0.1, 0.2 and 0.3mg/ml in separate bottles, disc of 6mm diameter were punched out from a filter paper (Whatman No.1) using puncher and then sterilized. The discs were impregnated into each of the above concentration.

SYNERGISTIC ACTIVITY
The synergistic activity study was achieved by combining the extract with the antibiotic, Ampiclox using disc diffusion technique. Methanol extract of *Euphorbia balsamifera* 0.1mg/ml was used in combination with 0.1mg/ml of Ampiclox in 1:1 ratio. The distance between the discs was maintained as standard conditions for 24 hours at 37°C and the zone diameter using a ruler (Betoni *et al.*, 2006).

MINIMUM INHIBITORY CONCENTRATION (MIC)
The MIC of the plant extracts were determined on solid medium (nutrient agar) using method reported by (Ferreira *et al.*, 2003). Standardized suspension of the test organism was inoculated into a series of sterile tubes of the extract and incubated at 37°C for 24 hours. The tubes were inspected visually to determined the growth of organisms for the presence of turbidity and the tubes in which antibiotic is present in minimum concentration sufficient to inhibit the microbial growth which remains clear was noted as MIC of the extract (Siddiqui and Ali 1997). In experimental terms MIC is the concentration of the drug present in the last clear tube, which is the tube having the lowest antibiotic concentration in which growth is not observed.

The minimum inhibitory concentration was determined for Ampiclox alone, *Euphorbia balsamifera* root extract alone and finally combination of Ampiclox and *Euphorbia balsamifera* root extract(1:1).

RESULTS
Preliminary Phytochemical Screening
The preliminary phytochemical screening of the root extract of *Euphorbia balsamifera* revealed the presence of Alkaloids, Anthraquinones, Cardiac glycoside, Flavonoids, Saponins, Tannin and Terpenoids as shwon on the table 1.

The result of the antimicrobial activities of the root extract of *Euphorbia balsamifera* on strains pathogenic bacteria were shown in (Table 2). Therefore synergism was possible with the antimicrobial drug tested. The MIC results for Ampiclox alone, root extract of *Euphorbia balsamifera* alone and combination of Ampiclox and methanol root extract of *Euphorbia balsamifera* (1:1) were presented on table 3.

### Table 2. Result of antimicrobial activity of *Euphorbia balsamifera* root methanol extract

<table>
<thead>
<tr>
<th>Organism</th>
<th>Methanol Extract of Euphorbia balsamifera (mm)</th>
<th>Ampiclox (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Methanol Extract of Euphorbia balsamifera</td>
<td>Ampiclox</td>
</tr>
<tr>
<td></td>
<td>1000mg/ml</td>
<td>2000mg/ml</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>9</td>
<td>16</td>
</tr>
<tr>
<td><em>Proteus</em></td>
<td>8</td>
<td>17</td>
</tr>
<tr>
<td><em>Klebsiella pneumonia</em></td>
<td>12</td>
<td>18</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>S/N</th>
<th>Test organism</th>
<th>MIC of E (mg/ml)</th>
<th>MIC of A (mg/ml)</th>
<th>MIC of E:A (1:1) (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Escherichia coli</em></td>
<td>500</td>
<td>125</td>
<td>60</td>
</tr>
<tr>
<td>2</td>
<td><em>Proteus</em></td>
<td>1000</td>
<td>500</td>
<td>125</td>
</tr>
<tr>
<td>3</td>
<td><em>Klebsiella pneumonia</em></td>
<td>1000</td>
<td>500</td>
<td>125</td>
</tr>
</tbody>
</table>

Key: E = Extracts of *Euphorbia balsamifera*, A = Ampiclox, E:A = *Euphorbia balsamifera*: Ampiclox

### Table 3: Minimum Inhibitory Concentration of *Euphorbia balsamifera* root methanol extract

<table>
<thead>
<tr>
<th>Test organism</th>
<th>MIC of E (mg/ml)</th>
<th>MIC of A (mg/ml)</th>
<th>MIC of E:A (1:1) (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>10.3</td>
<td>11.1</td>
<td>19</td>
</tr>
<tr>
<td><em>Proteus</em></td>
<td>15.8</td>
<td>18.0</td>
<td>16</td>
</tr>
<tr>
<td><em>Klebsiella pneumonia</em></td>
<td>17.5</td>
<td>16.1</td>
<td>24</td>
</tr>
</tbody>
</table>

### Table 4: Synergistic activity of methanol extracts of *Euphorbia balsamifera* root

<table>
<thead>
<tr>
<th>Test organism</th>
<th>Zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>A = Ampiclox, E = Extracts <em>Euphorbiabalsamifera</em> E:A = Extracts <em>Euphorbiabalsamifera</em>: Ampiclox</td>
</tr>
<tr>
<td><em>Proteus</em></td>
<td>10.3</td>
</tr>
<tr>
<td><em>Klebsiella pneumonia</em></td>
<td>15.8</td>
</tr>
<tr>
<td><em>Klebsiella pneumonia</em></td>
<td>17.5</td>
</tr>
</tbody>
</table>

Key: A = Ampiclox E = Extracts *Euphorbiabalsamifera* E:A = Extracts *Euphorbiabalsamifera*: Ampiclox

DISCUSSION
The result of the synergism exhibited higher sensitivity then that of the methanol root extract and Ampiclox drug alone. In general, the plant drug combination is synergistically very active against the microorganism
(Tijjani et al., 2011). This high activity could be ascribed to the presence of the secondary metabolites available in the plant and that of the Ampiclox drug combination. The activities of methanolic root extract alone and synergy mixture of methanolic root extract and Ampiclox with zone of inhibition of (11.1 mm) and (19 mm) respectively against the Gram negative E. coli is impressive because Gram negative bacteria tend to have higher intrinsic resistance to most antimicrobial agents (Ndukwe et al., 2005).

However, the demonstration of low MIC value for the synergy mixture of 50 mg/ml, 125 mg/ml and125 mg/ml against E. coli, Proteus and Klebsiella pneumonia respectively as shown on the Table 3 is an indication that the phytoconstituents of the plant in combination with Ampiclox have a better therapeutic potential than the methanolic root extract of the plant or Ampiclox alone.

There are Tannis which are reported to have various physiological effects like anti - irritant, antisecretolytic, antiphlogistic, antimicrobial and Antiparasitic effects (Tijjani et al., 2011). Phytotherapeutically tannin containing plants are used to treat nonspecific diarrhea, inflammations of mouth and throat and slightly injured skins (Westendarp 2006, Trease and Evan, 2000). As mentioned in the result the plant extract based on the different concentration raised shows a high zone diameter especially in E.coli and Klebsiella pneumonia.

CONCLUSION

The antimicrobial activity exhibited by both the methanolic root extracts alone and synergy of (1:1) of methanolic root extract and Ampiclox against clinical microbial isolates that are associated with various infectious diseases is a testimony that provides scientific justification for acclaimed ethno medicinal value of the plant and synergism is a way out to tackled bacterial resistance.

REFERENCES


Dweek, A. A. (1996); Plants for Africa, part 2, pp. 120-123. www.dweekdata.com.uk


Tijjani A., Sallau M.S., Sunusi, I., (2011) synergistic activity of methanol extract of Adenium obesum (Apocynaceae) stem bark and Oxytetracycline against some clinical bacterial isolates, Bayero Journal of pure and applied science, 4 (1); 79-82.

