

## AN IN VITRO STUDY OF THE ANTIMICROBIAL ACTIVITY OF THE ROOT EXTRACT OF *CALOTROPIS PROCERA* AND *MORINGA OLEIFERA*

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### Abstract

The crude root extract of *Moringa oleifera* and *Calotropis procera* were investigated for antimicrobial activity on three organisms; *Neisseria gonorrhoeae*, *Staphylococcus aureus* and *Escherichia coli*. The disc plate diffusion method was used to determine the sensitivity of these organisms. Various concentrations of aqueous and 1 % potash extract at 60 %, 50 %, and 40 % were used. Extract from the root of *Calotropis procera* presented better inhibitory effect on the test organisms. *Neisseria gonorrhoeae* appeared most susceptible to the antimicrobial effect of the root extracts. Evidence of antimicrobial activities of the plants extracts underscores their therapeutic utilization traditionally.

**Keywords:** In vitro, disk plate diffusion, susceptibility test, antimicrobial activity, ethnopharmacological, *Calotropis procera*, *Moringa oleifera*

### 1. Introduction

Before the advent of modern medicine, people all over the world depend on herbs for the treatment of various ailments (Osunde *et al.*, 1998). Abelson in Eboatu (1995) reported that over 21 clinically useful prescription drugs worldwide (including morphine, quinine, atropine, and digitanin) are derived from higher plants. More importantly however, is that 74 % of these drugs came to the attention of pharmaceutical industries because of their use in traditional medicine.

Various scientific researches have shown that some plants and plant extracts have antimicrobial activities. Osbon (1943) in George (1987) reported the presence of antimicrobial agents in 440 species out of 2300 species of plants surveyed. Such scientific study has led to the isolation of substances with therapeutic properties many of which have found use as modern drugs while others have served as substances for synthesis of drugs. Omenka and Osuoha (2000) showed that ethanol, petroleum ether, glycerine and water extracts of grape fruit clearly inhibited the growth of *Escherichia coli*, *Salmonella typhi*, *Aspergillus niger* and *Candida albicans*. Extract from *Spondia mombin*, *Carica papaya*, and *Viscum album* exert inhibitory effect on *Staphylococcus aureus*, *Salmonella typhi* and *Klebsiella pneumonia* (Osunde and Isibor, 1998).

*Calotropis procera* is a shrub up to 5.5 m high and occasionally branchless to a height of 2.5 m. All parts of the plant exude white latex when cut or broken. *Calotropis procera* belongs to the family Asclepiadaceae. It enjoys a wide chemotherapeutic value and is used to cure several ailments among local inhabitants. A decoction of the root bark mixed with red potash is used by Fulani in Yola to cure sexually transmitted disease (Ijoma *et al.*, 1997). The latex is used for treating guinea worm blisters, venereal sore, ophthalmic disorder and as an antidote against scorpion sting (Jurgne von May dell, 1990). The plant taste bitter and it is poisonous in high dosage. It is called Tumfafiya in Hausa and Babambe in Ffulde.

*Moringa oleifera* (drum stick) is a member of the family Moringaceae. It is a small graceful tree with sparse foliage often planted in compound or used as fence in northern Nigeria. *Moringa oleifera* is known as Zogalle in Hausa; Ewe-Igbala in Yoruba; Okwe-Oyibo in Igbo and Kabije in Fulfulde. The leaves are eaten as food and also used for treating conjunctivitis. Root and stem bark are used for medicinal purposes and seeds for purifying water by local inhabitants. Eboatu (1995) stated that tropical Africa is rich in plants of potential pharmaceutical importance, and many of these plants

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have been tagged through their use in traditional medicine. Besides, the development of resistance by microorganism to most of the existing drugs has led to the active research in pharmacognosy where newer drugs are sought for from plants by testing various extracts for antimicrobial activity. All communities in Nigeria have peculiar herbs, plants, roots etc which are used in some ways for the treatment of symptoms and diseases varying from simple skin rash to cancer (Eboatu, 1995). Although the efficacy of many of these crude preparations have not been verified, but the fact that they still survive in use till date suggest that they are effective to some degree. In view of the foregoing statement, this study is aimed at establishing an in vitro scientific justification to a claim by traditional medical practioners in Yola that root of *Moringa oleifera* and *Calotropis procera* treated with potash could be used in the treatment of urinary tract infection, specifically gonorrhoea.

## 2. Materials and Methods

### (a) Collection of plant materials and preparation of extract

Root back of *Calotropis procera* and *Moringa oleifera* were collected from the premises of Federal University of Technology Yola. The samples were washed, dried at room temperature and then crushed using mortar and pestle. The crushed samples were wrapped in brown paper and stored separately in a bottle containing silica gel. Extracts from the root samples were prepared with distilled water and 1 % potash solution. Three different concentrations; 60 %, 50 % and 40 % of the extracts were made by dissolving equivalent amounts of the crushed root samples in 100 mls of each solvent. The suspended solutions were left to stand for 48 hours and then filtered in bijou bottles. The filtrates were labeled appropriately and stored at room temperature.

### (b) Test Organisms

Test organisms: *Neisseria gonorrhoeae*, *Staphylococcus aureus*, and *Escherichia coli* were obtained from the Microbiology laboratory Department of Federal medical center Yola. They were checked for purity and viability and then maintain on agar slant. The slants were stored at 4°C.

### (c) Preparation of media

Nutrient and Chocolate media were used for the purpose of this research. Nutrient media were prepared according to manufacturer specifications and chocolate media by the addition of 5 % blood to molten nutrient medium.

### (d) Susceptibility test

The disk plate diffusion method was used. 6 mm diameter disks were made from whatman No. 1 filter paper. The disks were placed in the prepared crude extracts and allowed to stay for 10 minutes after which they were removed and allowed to dry at room temperature. Standard broth culture of the test organisms were prepared by sub-culturing five colonies of an over night culture into 5 ml of culture broth and allowed to stand for four hours at 35 °C (Baker *et al.*, 1993). Sterile cotton swab was used to transfer inoculum from the standardized bacteria suspensions to the entire surface of the prepared medium. The agar surface was allowed to dry for about five minutes, and then extract impregnated disks were placed on it using the smooth end of an inoculating needle. The plates were placed in 35 °C incubator for 18 hours after which the diameters of zones of inhibition were measured in mm (Prescott *et al.*, 2003). For each organism and extract, the experiment was performed in triplicate. Distilled water and 1 % potash solution were used as control while commercial antibiotic disk containing gentamycin 10 µg, septrin 25 µg and ciproxin 10 µg were used as standard.

## 3. Results

The antimicrobial activities of aqueous and 1 % potash root extracts are on Tables 1 and 2 respectively. Table 1 shows the effect of aqueous root extract on the test organisms. *Neisseria gonorrhoeae* appears to be most susceptible of the test organisms while *Escherichia coli*, resistant to the root extracts.

Table 2 shows effect of 1 % potash extract of root samples on test organisms and unlike the aqueous extract, the 1 % potash extract shows a better inhibitory activity on the test organisms.

Table 1. Effect of aqueous root extracts on test organisms.

Test organism	<i>Escherichia coli</i>			<i>Staphylococcus aureus</i>			<i>Neisseria gonorrhoeae</i>		
	60%	50%	40%	60%	50%	40%	60%	50%	40%
<i>Calotropis procera</i>	0.00	0.00	0.00	3.08	1.97	0.25	3.43	2.23	0.75
<i>Moringa oleifera</i>	0.00	0.00	0.00	0.30	0.00	0.00	0.51	0.21	0.00
<i>Calotropis procera</i> & <i>Moringa oleifera</i>	0.00	0.00	0.00	2.05	1.30	0.00	2.40	1.40	0.25

Table 2. Effect of 1% potash extract of root samples on test organisms

Test organism	<i>Escherichia coli</i>			<i>Staphylococcus aureus</i>			<i>Neisseria gonorrhoeae</i>		
	60%	50%	40%	60%	50%	40%	60%	50%	40%
Concentration of extract	60%	50%	40%	60%	50%	40%	60%	50%	40%
<i>Calotropis procera</i>	0.00	0.00	0.00	3.13	2.15	0.75	3.68	2.60	1.00
<i>Moringa oleifera</i>	0.00	0.00	0.00	0.35	0.07	0.00	0.53	0.21	0.00
<i>Calotropis procera</i> & <i>Moringa oleifera</i>	0.00	0.00	0.00	2.38	1.70	0.00	2.65	2.33	0.50

Table 3. Effect of solvent used for extraction (control) and commercial antibiotic disk on test organisms.

Test organism	Commercial antibiotic disc			Control	
	CN	SXT	CPX	water	1% (p)
<i>Escherichia coli</i>	3.40	4.91	0.00	0.00	0.00
<i>Staphylococcus aureus</i>	6.63	3.37	2.90	0.00	0.00
<i>Neisseria gonorrhoeae</i>	8.43	5.47	0.00	0.00	0.00

KEY: CN – Gentamycin, SXT – Septrin, CPX – Ciproxin  
 1%(p) – 1 % potash solution.  
 Zone of inhibition in “mm”

Table 3 shows result of antimicrobial activity of solvent used for extraction (control) and standard commercial antibiotic disks. Also placed in a column in Table 3 is result of 1 % potash extract of *Calotropis procera* which is the extract with highest antimicrobial activity.

**4. Discussion**

Results obtained from this research validate the antimicrobial activities of the plants root extracts and thus authenticate their therapeutic use by traditional medical practitioners. The root extract of *Calotropis procera* presents a better inhibitory effect on the test organisms than *Moringa oleifera*. This could be attributed to the presence of the active substance causing the inhibitory effect, in higher concentration in the root extract of *Calotropis procera*.

Tables 1 and 2 illustrate an increasing inhibitory effect of the roots extracts as the concentration increases. This implies that antimicrobial activity of a substance is concentration dependent, which is in concordance with the finding of Oboh and Abulu (1997), that antimicrobial activity is a function of the concentration of the active ingredient reaching an organism. Besides, a better antimicrobial activity is observed when the root of *Calotropis procera* is used single than when it is used in combination with the root of *Moringa oleifera*. This may be due to cross reaction of the active substances in both root samples which may have led to reduction in activity; or a dilution of the activity of *Calotropis procera* since *Moringa oleifera* showed infinitesimal sign of activity on test organisms when used alone. In the same vein, a higher inhibitory activity of the root

extract of *Calotropis procera* is observed on *Neisseria gonorrhoeae* than *Staphylococcus aureus*. While *Escherichia coli* appeared resistant to all the plant extracts.

Comparing results in Tables 1 and 2, a better antimicrobial activity is observed in 1 % potash extract (Table 2). The increase in antimicrobial activity could be attributed to the presence of potash in the extract or the alkaline pH (9.71) of solvent used for extraction which may have aided extraction of the active ingredient. This finding agrees with the report of Gunnar *et al.* (1991), that different extracts of plant show different antimicrobial activities on an organism.

Comparing results from the standard antibiotic disks to 60 % of the 1 % potash extract of *Calotropis procera*, a significant level of activity is observed with the crude root extract which gives credence to its ethnopharmacological use as remedy for urinary tract infection caused by *Staphylococcus aureus* and *Neisseria gonorrhoeae*. Results obtained from control, solvent used for extraction, shows that both solvents (distilled water and 1 % potash solution) do not have antimicrobial activity on the test organisms. This validates the fact that antimicrobial activities of the roots extracts could not have been due to the solvents used for extraction.

**5. Conclusion**

The inhibitory effect expressed by the roots extracts particularly 1 % potash extract of *Calotropis procera* supports the claim by some Fulani herbalist in Yola that the root of *Calotropis procera* treated with potash could be used to cure gonorrhoeae (Ijomah

*et al.*, 1997). The sensitivity demonstrated by the organisms is an indication that the plants contain active substances that could be identified, extracted, purified and used as a therapy against diseases caused by these organisms.

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#### REFERENCES

- Baker, C.N. and Thornbery, C.H., 1983. Inoculum standardization in antimicrobial susceptibility test evaluation of the overnight agar culture. *Journal of Clinical Microbiology*, 17, 450-457.
- Eboatu, A.N., 1995. Drugs from plants. Symposium on chemical biotechnology for national development. Sheda Science and Technology Complex, Abuja, pp. 210-211.
- George, M.S., 1987. Antimicrobial agents: *Encyclopedia of Science and Technology*. McGraw Hill, 1, 786.
- Gunnar, S., Mohammed, H.F., Per, C., Mekonan, H., Mats, T., Olov, H., Amed, M., Abudirizak, O. and Abdukadir, H., 1991. Inventory of plants used in traditional medicine in Somalia. *Journal of Ethnopharmacology*, 38, 1-9.
- Hans-Jurgen, von Maydel, 1990. *Tree and Shrubs of the Sahel: their characteristics and uses*. Germany, pp. 212-214.
- Ijomah, J.U., Ido, M. and Umar, H.A., 1997. Medicinal plants in used by Fulani traditional herbalist in Yola North and South local government areas of Adamawa state. *Journal of Applied Science and Management*, 1(1), 59-63.
- Oboh, P.A. and Abulu, E.O., 1997. The antimicrobial activities of extracts of *Psidium guajava* and *Citrus auratifolia*. *Nigerian Journal of Biotechnology*, 8(1), 25-27.
- Omenka, C.A. and Osuaha, J., 2000. Antimicrobial potency of grape fruit seed extract on five selected pathogens. *Nigerian Journal of Microbiology*, 14(2), 39-42.
- Osbon, 1943. In George, M.S., 1987. Antimicrobial agents: *Encyclopedia of Science and Technology*. McGraw Hill, 1, 786pp.
- Osunde, M.I. and Isibor, O.N., 1998. In vitro antimicrobial activities of extracts of *Spondia mombin*, *Carica papaya* and *Viscum*. *Journal of Medical Laboratory Science*, 3, 16-20.
- Prescott, L.M., Harley, P.J. and Klein, D.A., 2003. *Microbiology* (5th ed.). McGraw-Hill companies. New York, 809pp.