**REPORT**

# *IN VITRO* ANTIMICROBIAL ACTIVITIES OF EXTRACTS OF *CARPOLOBIA LUTEA* ROOT

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**ABSTRACT**

Several plants are used in folk medicine to treat infections. *Carpolobia lutea*, G.Don (Polygalaceae) is a medicinal plant commonly used by herbalists in Southern Nigeria against dental and genitourinary infections. The study was to evaluate the *in vitro* antimicrobial activities of n-hexane, chloroform, ethyl acetate and methanol extracts of *Carpolobia* lutea root. Four typed cultures of bacteria namely, *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis* and two clinical strains of fungi, namely *Candida albicans* and *Tinea capitis* were analyzed using agar well diffusion method. The extracts that showed antimicrobial activity were then tested to determine the Minimum Inhibitory Concentration for each bacterial or fungal sample.The ethyl acetate extract gave the widest zone of inhibition (21.0mm) followed by chloroform when tested on *E. coli*. No inhibition was observed with *E.Coli*. None of the extracts showed any inhibitory effect against *Pseudomonas aeruginosa* and the fungal strains of *Candida albicans* and *Tinea capitis*. The most potent of these extracts was Chloroform extract with Minimum Inhibitory Concentration (MIC) of 25mg/ml for bacteria. The phytochemical screening of the root of *C. lutea* revealed the presence of saponins, anthraquinones, flavonoids, cardiac glycosides, simple sugar and terpenes.

**Keywords**: Antimicrobial, *Carpolobia lutea*, extract.

## INTRODUCTION

The plant *Carpolobia lutea* G. Don (Polygalaceae) is a shrub or small tree up to 15ft high (Hutchinson and Dalziel,1954) and is widely distributed in West and Central areas of Tropical Africa (Mitaine - Offer *et al*.,2002). It is known by many common names such as cattle stick (English), Abekpok Ibuhu (Eket), Ikpafum, Ndiyan, Nyayanga (Ibibio), Agba or Angalagala (Igbo) and Egbo oshunshun (Yoruba).

Because of its shrubby and smallish stems, it is popularly used as sweeping material or broom for compounds in rural areas among the Eket and Ibibio tribes of Akwa Ibom State, Nigeria. Herbalists from these tribes use the root decoction in locally-made alcohol as an aphrodisiac and also for the treatment of genitourinary infections, gingivitis and waist pains. The root decoction is also said to be useful in the treatment of internal heat.

The plant *Carpolobia lutea* has been reported to possess anti-inflammatory and anti-arthritic properties (Iwu and Anyanwu, 1982), contain three new triterpene saponins (Mitaine-Offer *et al*.,2002) and possess antimicrobial activities (Ajibesin,2005; Philip *et al*., 2005), as well as antidiarrhoeal and anti-ulcerogenic properties (Nwafor and Bassey,2007). The root is used to facilitate childbirth, treat sterility, headache, worm infestation and also has aphrodisiac and stimulant properties (Mitaine-Offer *et al*., 2002).

This study therefore sought to determine the antimicrobial properties that support the use of the roots of *Carpolobia lutea* by the local people in the treatment of various infections, since earlier studies were focused on its leaves and stem-bark.

## MATERIALS AND METHODS

### *Plant materials*

The fresh root (1.7kg) of C. lutea, G. Don was collected in April, 2005 from Ekpene Obo in Esit Eket Local Government Area of Akwa Ibom State, Nigeria. The plant was identified and authenticated as *Carpolobia lutea*, G. Don (polygalaceae) by Dr. (Mrs.) Margaret Bassey of the Department of Botany and Ecological Studies, University of Uyo and a herbarium specimen voucher number UUH 126 was assigned to it and deposited in the same department of Botany.

### *Test organisms*

Six organisms used in this study as test organisms were four typed cultures of bacteria: *Eschericia* coli (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 25923) and *Bacillus subtilis* (NCTC 8853) and two clinical isolates of fungi (*Candida albicans* and *Tinea capitis*) obtained from the

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Pharmaceutical Microbiology Unit of the Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, University of Uyo, Uyo. The typed cultures of bacteria and clinical isolates of fungi were sub-cultured on Nutrient agar (Oxoid) and Saboraud dextrose agar (Oxoid) slants respectively and stored at 4$^{o}$C until used.

***Antimicrobial agents***

The following chemotherapeutic agents were included in the test as positive controls: streptomycin 0.4mg/ml and Nystatin 50,000 I.U/ml.

***Preparation of extract***

The plant root was oven-dried at 45$^{o}$C and pulverized using pestle and mortar into fine powder and sequentially extracted by cold maceration for 72h using 2.5L of n-hexane, chloroform, ethyl acetate and methanol as solvents. These were then filtered and the filtrates were concentrated under reduced pressure by the use of rotary evaporator at 40$^{o}$C. These yielded dry extracts of n-hexane (16g), Chloroform (10g), ethyl acelate (6g) and methanol (25g) respectively. These extracts were thereafter kept in sterile bottles at -4$^{o}$C until used. The dry weight of the extracts was obtained by allowing the evaporation of the solvent to take place and was then used to determine the concentration in mg/ml.

***Phytochemical screening***

Phytochemical screening of the extracts was carried out to determine the presence of chemical constituents in the plant using the method of Odebiyi and Sofowora (1978) and Trease and Evans (1989).

***Determination of antimicrobial activities of extract***

The antimicrobial activities of n-hexane, chloroform, ethyl acetate and methanol extracts were determined by agar well diffusion method described by Omenka and Osuoha (2000) with slight modifications.

The culture plates were each seeded with test organisms and allowed to solidify and thereafter punched with a sterile cork borer (5.0mm diameter) to cut uniform wells. The open wells were filled with 0.05ml of the extract. The plates were then incubated at 37$^{0}$C for 24 hours. For the fungi, the antimicrobial test was carried out using Saboraud dextrose agar (SDA) plates incubated at 30$^{0}$C for 72 hours. The zones of inhibition were then measured and recorded and compared with positive standard controls, streptomycin (0.4mg/ml) for bacteria and nystatin (50,000 I.U/ml) for fungi.

**Minimum inhibitory concentration (MIC)**

Various concentrations of the root extracts of *Corpolobia lutea* were prepared: 6.25mg/ml, 12.5mg/ml, 25mg/ml, 50mg/ml and 100mg/ml. The culture plates were again seeded with test bacterial organisms and allowed to solidify and thereafter punched with a sterile cork borer (5.0mm diameter) to cut uniform wells. The open wells were filled with 0.05ml of the extract.The plates were then incubated at 37$^{0}$C for 24h.

The lowest concentration of the extract that showed inhibition of growth of the test organisms was read and taken as the minimum inhibitory concentration (MIC).

## RESULTS

***Phytochemical screening***

The phytochemical screening indicated the presence of the following chemical constituents: Saponins, Anthraquinones, Flavonoids, Cardiac glycosides, Terpenes and Simple sugar (table 1).

**Table 1**: Results of phytochemical screening

| Test | Inference |
|---|---|
| Saponins | + + + |
| Simple Sugar | + + + |
| Terpenes | + + + |
| Flavonoids | + + |
| Tannins | - |
| Alkaloids | - |
| Anthraquinones | + + |
| Cardiac Glycosides | + + + |

Key: + Trace, ++ Positive, +++ Strongly positive, -Absent

***Antimicrobial activities***

The results of the antimicrobial activities are as shown in table 2. The ethyl acetate extract gave the widest zone of inhibition (21.0mm) followed by chloroform when tested on *E. coli*. Methanolic and n-hexane extracts gave no activity against *E. coli*. The chloroform extract showed the widest zone of inhibition (20.0m) against *S. aureus* followed by methanolic and ethyl acetate extracts in that order. Methanol, ethyl acetate and chloroform extracts gave similar zones of inhibition when tested against *B. subtilis*.

None of the extracts had any antimicrobial activity against *P. aeruginosa* and fungal species. The n-hexane extract showed no activity against all the gram-positive and gram-negative and fungal organisms tested. The ethyl acetate and methanol extracts were more active against gram-positive organisms than against gram-negative organisms.

***Minimum inhibitory concentration***

Against *Staphylococcus aureus*, the minimum inhibitory concentration for chloroform extract was 25mg/ml; ethyl acetate, 50mg/ml and methanol, 50mg/ml. When tested against *B. subtilis*, the MIC for methanol extract was 100mg/ml; ethyl acetate, 50mg/ml and chloroform, 100mg/ml whereas against *E. coli*, the MIC for ethyl

**Table 2**: Antimicrobial activity of four extracts of *Carpolobia lutea* root

| - Bacterium gram (-)/(+) | Extract concentration (mg/ml) | Growth inhibition zone diameter (mm) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Methanol | Ethyl acetate | Chloroform | n-Hexane | Antibiotic control (streptomycin 0.4 mg/ml) | Nystatin (50,000 i.u/ml) |
| *E. Coli* - | 100 | - | 21.0 | 12.0 | - | 34.5 | |
| | 50 | - | 9.5 | - | - | | |
| | 25 | - | - | - | - | | |
| | 12.5 | - | - | - | - | | |
| *P. aeruginosa* - | 100 | - | - | - | - | 42.0 | |
| | 50 | - | - | - | - | | |
| | 25 | - | - | - | - | | |
| | 12.5 | - | - | - | - | | |
| *S. aureus* + | 100 | 17.0 | 12.0 | 20.0 | - | 40.0 | |
| | 50 | 10.0 | 9.0 | 15.0 | - | | |
| | 25 | - | - | 9.5 | - | | |
| | 12.5 | - | - | - | - | | |
| *B. subtilis* + | 100 | 12.5 | 12.5 | 12.0 | - | 54.0 | |
| | 50 | - | 9.0 | - | - | | |
| | 25 | - | - | - | - | | |
| | 12.5 | - | - | - | - | | |
| Fungus *Candida albicans* | 100 | - | - | - | - | | 25 |
| | 50 | - | - | - | - | | |
| | 25 | - | - | - | - | | |
| | 12.5 | - | - | - | - | | |
| *Tinea capitis* | 100 | - | - | - | - | | 28 |
| | 50 | - | - | - | - | | |
| | 25 | - | - | - | - | | |
| | 12.5 | - | - | - | - | | |

acetate extract was 100mg/ml, chlorofom extract, 100mg/ml.

Generally, the most potent of these extracts is chloroform extract with MIC of 25mg/ml (table 3).

## DISCUSSION

The antimicrobial potentials of substances are useful tools in the control of various infections caused by micro-organisms especially genitourinary infections. The commonest cause of infertility in women in Nigeria is infection, particularly pelvic inflammatory disease (Abudu, 1985; Otubu, 1985). The bacterial organisms found in this study to be susceptible include *E. coli*, *S. aureus* and *B. subtilis* which have been implicated in many systemic infections such as respiratory and genitourinary tract infections.

The extracts of *C. lutea* appeared more active against gram-positive than gram-negative bacteria.

The phytochemical screening of this plant showed the presence of flavonoids and saponins both of which have been shown to possess antibacterial properties (Hostettman *et al*., 1995; Oboh *et al*., 1998). Flavonoids are known for their anti-inflammatory, anti-arthritic and antibacterial properties (Trease and Evans, 1989). Therefore, the antibacterial activities of this plant may be ascribed to the presence of saponins and flavonoids.

These results support the popular ethnopharmacological use of this plant for the treatment of sterility, genitourinary infection and gingivitis. Biossay-directed fractionation of the most active extract is in progress to isolate and identify the compounds responsible for the antibacterial activity.

**Table 3**: Minimum inhibitory concentration (MIC) of four extracts of *Carpolobia lutea* root

| - Bacterium gram (-)/(+) | Extract concentration (mg/ml) | Growth inhibition zone diameter (mm) | | | |
|---|---|---|---|---|---|
| | | Methanol | Ethyl acetate | Chloroform | n-Hexane |
| *E. coli* - | 100 | - | 12.0 | 12.0 | - |
| | 50 | - | - | - | - |
| | 25 | - | - | - | - |
| | 12.5 | - | - | - | - |
| | 6.25 | - | - | -- | - |
| *P. aeruginosa* - | 100 | - | - | - | - |
| | 50 | - | - | - | - |
| | 25 | - | - | - | - |
| | 12.5 | - | - | - | - |
| | 6.25 | - | - | - | - |
| *S. aureus* + | 100 | - | - | - | - |
| | 50 | 10 | 9.0 | - | - |
| | 25 | - | - | 9.5 | - |
| | 12.5 | - | - | - | - |
| | 6.25 | - | - | - | - |
| *B. subtilis* + | 100 | 12.5 | - | 12.0 | - |
| | 50 | - | 9.0 | - | - |
| | 25 | - | - | - | - |
| | 12.5 | - | - | - | - |
| | 6.25 | - | - | - | - |

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