Global Research Journal of Agricultural and Biological Sciences Vol.3(4) pp.370 –372 October 2012.
Available online http://www.globalresearchjournals.org/?a=journal&id=grjabs

Case Study.

ANTIBACTERIAL AND ANTIFUNGAL EFFECT OF THE ETHANOL LEAF EXTRACT OF NEWBOULDIA LAEVIS

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Accepted 28th September 2012.

The Ethanol leaf extract of *Newbouldia Laevis* was screened for antibacterial effect against three Gram negative bacteria *(Pseudomonas aeruginosa, Klebsiella pneumonia and Proteus mirabilis)* two gram positive *(Staphylococcus albus and Bacillus subtilis)* and also for antifungal activity against five fungi *(Candida stellatoidea, Aspergillus niger, Aspegillus terrens, Penicillium oxalicum and Candida albicans)*. Inhibitory activity of the plant extract was higher in the fungi than in the bacteria. Among the bacteria, the inhibitory effect of leaf extract was higher on the gram negative than the gram positive bacteria. Streptomycin had inhibitory activity on all the tested organisms and the activity was higher than that of *Newbouldia Laevis* on some of the organisms.

Key words: Antibacterial, Antigungal, Ethanol extract, *Newbouldia laevis*, invitro.

INTRODUCTION

Newbouldia Laevis (Akoko in yoruba language) belongs to the family *Bignoniaceae* and it is a medium sized angiosperm which grows to a height of about 7-8 (up to 15) meters; more usually it is a shrub of 2-3 metres (Arbonnier, 2004). (Kuete *et al*.,2007) isolated ten compounds from the root bark of this plant, (Oliver-Bever, 1956) studied the leaves and bark of Congolese *N. leavis* and reported the absence of flavonoids, saponins, quinones, terpenes or steroids while (Vollekova *et al*.,2001) reported the presence of alkaloids in this plant. The phytochemical screening of the crude methanol leaf extract of *N. laevis* by(Usman and Osuji,2007) in Nigeria revealed the presence of Flavonoids, tannins, terpenes, steroidal and cardiac glycosides, alkaloids and saponins were found to be absent. The young leaves are crushed in little pieces in water and the extract is put into the eye to cure eye inflammation and redness it is also administered to stop vaginal bleeding in threatened abortion (Kargbo, 1982). The root and leaves are known to be used for the treatment of round worms, elephantiasis, dysentery, malaria stomachic, migraines and convulsions (Lewis and Manony, 1977; Akunyili, 2000). The leaf, stem and fruits have been used for Febrifuge and wound healing (Iwu, 2000).

MATERIALS AND METHODS

Plant Collection: The leaves of *Newbouldia laevis* were collected from Ago-Iwoye, Ogun-State, Nigeria. The herbarium specimen was identified at El Kaf herbarium Olabisi Onabanjo University, Ago-Iwoye. Ogun-State. The leaves were air dried and pulverized into fine powder. 500g of the powdered form was extracted with 80% Ethanol using soxhelet extraction. The extract was concentrated under reduced pressure to yield a dark green mass. The crude extract was stored in a refrigerator until required.

Organisms: *Staphylococcus albus, Pseudomonas aeruginosa, Bacillus subtilis, Klebbiella pneumonia and Proteus mirabilis*, were obtained from Ogun State University Teaching hospital while the fungi *Candida stellatoide, Candida albicans, Aspergillus terrens, Aspergillus niger and Penicullum oxalicum* were obtained from yam soil.

Bacterial inoculation and incubation with extracts

Nutrient agar and nutrient broth (oxoid) were prepared according to the manufacturers' recommendations and agar-well diffusion method was used for the inoculation of the bacteria. Plates containing 30 ml of sterile nutrient agar each were inoculated with standardized innocula (1.5 x 108 cells/ml) (Olafimihan and Fawole, 2003) using sterile Pasteur pipette. Wells of 6 mm diameter were made at the centre of each plate and 0.15 ml of the various concentrations of the extract was dispensed into each well.

The extracts were allowed to diffuse into the medium for 1hr at room temperature. This was then incubated for 24 h at 37^{o}C after which the zones of growth inhibition were measured and recorded in millimeter. The control sample was set up in a similar manner except that the extract was replaced with sterile distilled water.

Antifungal Assay

Antifungal effect of the extracts was tested using the agar dilution method described by (Collins and Lyne, 1970). Varying concentrations of the extracts were prepared and incorporated into Potato dextrose agar. The plates were incubated at 25°c for 48 hours and inhibition of growth was noted.

RESULT

Table 1 showed the antibacterial and antifungal effect of the ethanol leaf extract of *Newbouldia laevis* on 5 bacterial and 5 fungi. The table also showed the effect of an antibiotics and antifungal drug on these organisms; while water served as control. The result revealed that the ethanol leaf extract of *N. laevis* had higher inhibitory effect on the 5 fungi than the bacteria. Inhibitory activity was highest on *A. Niger* and the least effect on *C. albicans.* The inhibitory activity of *N. laevis* was higher on the Gram negative bacteria than the gram positive bacteria. Inhibitory activity was the highest on *P. mirabilis* and the lowest on *S. albus*. Streptomycin was more effective in inhibiting the growth of all the bacteria and most active on *P. aeruginosa* and the least on *B. subtilis*. Water as control had no inhibitory activity on all the organisms tested. Ketoconazole had a high inhibitory activity on *A. terrens* while it was not active on others.

Table 1 :Antibacterial and Antifungal Effect of the Ethanol leaf extract of *N. laevis* on some microorganisms.

Microbes/ Extract.	*S. Albus*	*P. aeruginosa*	*B. Subtilis*	*K. Pneumonia*	*P. Mirabilis*	*A. terrens*	*A. niger*	*P. oxallicum*	*C. Stellatoidae*	*C. albicans*
Newbouldia Laevis	12 □ 0 +	20 □ 0 +	18 □ 0.4 +	26 □ 1 ++	21 □ 0.7 +	27 □ 0.7 ++	36□0.7 +++	20 □ 0.4 +	21 □ 0.7 +	17 □ 0.6 +
Streptomycin	20 ++	25 ++	37 +++	15 +	18 +	-	-	-	-	-
Ketoconazole.	-	-	-	-	-	22 +	-	-	-	-

Key
10 – 23 mm = low inhibition (+)
24 – 35 mm = moderate inhibition (++)
36– above = high inhibition (+++)
(-)=No Activity.

DISCUSSION

The antibacterial and antifungal effect of the ethanol leaf extract of *N. laevis* revealed that the broadest activity of the extract was against the gram negative bacteria studied *(K. pneumonia, R. mirabilis and P. aeruginosa)* and this is in agreement with the findings of (Usman and Osuji, 2007). The extract exhibited considerable inhibitory effect on *K. pneumonia* which is known to play a significant role in hospital acquired urinary tract and wound infections. It is also an opportunistic pathogen for patients with chronic pulmonary diseases, nasal mucosa atrophy and rhinoscleroma. It was also active on *S. albus* known to cause parasitic skin diseases.

The high antibacterial inhibitory effect of this extract could be related to the presence of the plant secondary metabolites as reported by different authors (Oliver-Bever,1956 ;Vollekova *et al*.,2001; Usman and Osuji, 2007).

The antifungal inhibitory effect of the extract was the least on *C. albicans* and this uphold the fact that there's no report that the plant is used to treat ringworm which is caused by *C. albicans*.

N. laevis had the highest inhibitory activity against *A. niger* which causes aspergillosis in man, otomycosis (fungal ear infection), which can cause pain, temporary hearing loss and, in severe cases, damage to the ear canal and tympanic membrane.

Streptomycin had inhibitory activity on all the bacteria used in this study and the activity was higher than that of *N. laevies* extract. Protein synthesis site of the organisms were attacked by streptomycin (Joyce-Elaine, 2006).

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