ANTIBACTERIAL ACTIVITY OF *NEWBOULDIA LAEVIS* AQUEOUS LEAF EXTRACT AGAINST SELECTED WOUND PATHOGENS

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ABSTRACT

The ethanolic leaves extract of Newbouldia laevis was subjected to preliminary phytochemical screening and in-vitro antibacterial test. The extract revealed the presence of alkaloid, cardiac glycosides, steroids and saponins. The antibacterial activity of the plant extract was assayed by agar diffusion techniques, the test organisms were Stapylococcus aureus and Klebsiella pneumonia, all were clinical isolates. The extract did not inhibited the growth of the test organisms at concentration between 250mg/ml to 3.125mg/ml. this study therefore did not justify the traditional use of the plant as remedy for wound healing.

Keywords: In-vitro antibacterial activity, Phytochemicals *Newbouldia laevis*,

INTRODUCTION

Antibioctics were considered to be miracle drugs when they first became available half a century ago, but their popularity rapidly led to overuse. Over the last decade, it has become clear that antibioctics are losing their effectiveness as pathogens evolve resistance against them, a problem compounded by the fact that new drugs only rarely reach market. Moreover, bacteria can acquire drug resistance in a multitude of ways, so getting around the resistance problem is not a straight forward matter. To address these issues, pharmaceutical companies have recently revived efforts to developed new antibiotics, due to the appearance of antibiotics resistant strain such as methicillin resistant *Stapylococcus aureus* (MRSA).

Natural products are both a fundamental source of new chemical diversity and an integral component of today s pharmaceutical compendium. however many currently available antifungal and antibacterial agents have undesirable toxicity, and the wide spread use of these drugs has led to rapid developments of drugs resistance strain, which are leading cause of failure in both clinical and agricultural applications.

According to the world health organization (WHO), a medicinal plants is any plant in which one or more of its component part contain a substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs(1). for years, medicine had depend exclusively on leaves, flowers and barks of the plants, only recently that synthetic drugs came into use and many instances, these carbon copies of chemicals identified in plants (2) traditionally the used of plants preparation as sources of drugs are based on the experience of superstition passed from generation to generation(3).

Recently research has focused on natural product alternatives for disease control in developing countries. The majority of rural dwellers do not have access to modern health care, so they depend on medicinal plants to prevent or eliminate diseases.

Medicinal plants are cheaper, more accessible to most of the population in the world. Thus the plant around us can be investigated for the purpose of identifying those that may be potent against infectious organism and hence useful in treating ailments caused by microorganisms. Many of this of these plants contains large variety of chemical substances referred to as secondary metabolites such as flavonoids, tannins among others and these have significant biological effects on humans.

Therefore this study aims at determining the antibacterial properties and assay for the in-vitro effects of aqueous extract of *Newbouldia laevis* leaves.

MATERIAL AND METHODS

Source and identification of plant materials

The sample was collected from Botanical Garden Department of Biological Science ABU Zaria, the plant materials (fresh) were identified at Herbarium of the Department of Biological Science ABU Zaria on the 16th July 2012.

Extraction

The leaves of *Newbouldia laevis* were dried shed and pulverized into fine powder using a pestle and mortar, 110g of the dried leaves powder was extracted with 1000ml of distilled water in a soxhlex exractor for 72hours to obtain the aqueous extract. The extract was then concentrated to dryness on water bath and weight (4).

Preparation of Different Concentration of the Extracts

A stock solution of the extract was prepared by dissolving 2g of the extract in 8ml of distilled water to obtained a concentration of 0.25g/ml 2.5x10-1g/ml, serial dilution was then carried out in 4 test tube to obtain concentration of 0.125g/m l, 0.0625g/ml and 0.03125g/ml respectively by transferring 4ml of the stock in to 3ml of distilled water and 4ml from there to the next until the final concentration (5)

Phytochemical Screening

The crude extract was subjected to phytochemical screening using standard methods (6) to show the bioactive components present in the leaves of the plant.

Test organisms

The test organisms used in this study were clinical isolates of *Staphlococcus aureas and Klebsiella pneumonia* obtained from the University Health Services (sickbay).

Antibacterial Testing of the Extract

Sensitivity testing of the plant extract was determining using agar diffusion method as described by trobi *et al* (1994), Russell and fur (1997) with little modifications, the bacterial isolate were first grown in nutrient agar slant for 18-24hours before used. The isolates were later subculture on Mueller Hinton agar (oxoid) four bottles of sterile nutrient agar were poured into sterile Petri dishes and allowed to set the solidified sterile media were flooded with 0.5ml of the standardized inoculums of the test bacteria, and drained off. A sterile (flamed) cork borer (12mm) was used to bore the concentrations 0.25g/ml-0.03125g/ml of the aqueous extract were carefully inoculated to fill the bore holes. 30min of pre diffusion time was allowed after which the plates incubated at 370c for 18hours. The procedure was done in duplicate for each test organism. Positive and negative control plates were also prepared and incubated along side (7).

Results

Phytochemical analysis of ethanolic extract revealed the presence of carbohydrate (+), saponin (+), alkaloid (+), glycosides (+), flavanoid (-), tannins (-), triterpene (-), unsaturated steroid (-), and cardic steroid are absense as shown in table 1

The in-vitro antimicrobial screening presented in table 2 shows the susceptibility test against gram positive and gram negative organisms. The extract did not exhibit any considerable level zone of inhibition against the test organism.

Table 1: Phytochemical Screening test for the plant extracts

Test	Compound	Inference
Molisch test	Carbohydrates	+
Keller-kilian	Cardiac Glycosides	+
Frothing test	Saponins	+
Ferric chloride test	Tanins	-
Shinoda test	Flavonoids	-
Mayer s test	Alkaloids	+
Salkowski test	Steroid	-
Bucchard test	Triterpene	-

Table 2: *In-vitro* antibacterial activity of Aqueous *Newbouldia laevis* extract on selected clinical isolates

Test organisms	0.25g/ml	0.125g/ml	0.0625g/ml	0.03125g/ml	PC
S aureus	NA	NA	NA	NA	40
K pneumonia	NA	NA	NA	NA	36

KEY:

NA= NO ACTIVITY

PC= POSITIVE CONTROL-CIPROFLOXACIN

Discussion

The extract did not showed any remarkable activity against the gram positive and gram negative bacteria, the gram negative bacteria are generally regarded more difficult to inhibited due tom the presence of a thick murcin layer that tend to prevent the entry of inhibitors (8). However the extract of *Newbouldia leavis* do not inhibited gram positive bacteria which have a record of developing resistance to all most all synthetic b-lactam antibiotics drugs such as MRSA

The resistance form by this microorganism to the aqueous extract may also due to the absence of the potent secondary metabolites on the leaves which may be acting synergistically with one another. The overall observe non antibacterial activities of the aqueous extract could be traced to the absences of secondary metabolites like tannins, flavonoid and steroid etc, which are responsible to microbial infection and have been found to be antimicrobial substances against wide array of microorganism in-vitro.

A number of other factors may also lead to the negative result observed in this work, as noted by Sofowara (9) practices used in traditional medicine include the collection of certain plants only at certain season using cold extraction, instead of hot extraction for some herbs. using fallen dead leaves of certain plant rather than fresh ones etc have rationalized as been due to seasonal, diurnal, or age variation, inactive constituents of plant and the thermo ability of the active ingredients of certain plants, thus the disparity between observation in this work and Usman s work could be due to such variations inactive ingredients due to seasonal diurnal or age.

In this work matured fresh leaves of *Newbuldia laevis* was collected and dried before the soxhlex extraction procedure was carried out. The continuous extraction process for 72hrs, followed by evaporation to dryness could have lead to inactivation of the potent active ingredients.

Therefore this work prove that the aqueous extract of *Newbouldia laevis* had no observable activities against Staphylococcus aureus and *Klebsiella pnemoniae* invitro, the plant extract might still have some beneficial antimicrobial activity in-vivo. Thus, the negative invitro results recorded in this work do not neceesary contradict the positive in vivo activity claimed by Usman *et al* (2007).

Conclusion

The aqueous extract of *Newbouldia laevis* did not show any observable antimicrobial activity in-vitro against the test organisms. The resistance by the organism to the aqueous extract may be due to absence of potent secondary metabolites on the leaves which act synergic ally.

References

- Junaid SA, Olabode AO, Onwuliri FC, Okworiu AEJ, Ajina SE (2006). The antimicrobial properties of *Ocimum grastissimum* Extracts on selected Bacterial Gastrointestinal Isolates. *Afri. J. Biotechnol*. 5(22):2315-2321
- Sofowora, A. (1993). Medicinal plants and traditional medicine in Africa spectrum books Ltd,Ibadan Nigeria pp191-289.
- Sofowora A, (1993). Screening plants for bioactive agents. in Medical plants and Traditional Medicines in Africa. 2nd ed. Spectrum books ltd, sunshine house, Ibadan Nigeria, pp. 134-156
- Trease, G.E and Evans, W.C (2002). Pharmacognosy. 15th Ed. Saunders publishers, London. Pp 42-44, 221-229, 246-249, 304-306, 331-332, 391-393.
- Ogbulie, T.N., Ogueke, C.C. and Okorondu, S.I. (2004). Antibacterial properties of A.cardifolia, M. Nig. J. Microbial. 18:249-255
- Sofowora A, (1993). Screening plants for bioactive agents in Medical plants and Traditional Medicines in Africa. 2nd ed. Spectrum books Ltd, sunshine house, Ibadan Nigeria, pp. 134-156
- Cheesebrought M (2000) District Laboratory Practice in Tropical Countries. part2. Cambridge University Press; Pp 63-70
- Martin G (1995) Ethanobotany: A Method Manual. london: Chapman and hall p 80
- Usman H, and J.C Osuji.,2007, Pytochemical and invitro antibacterial assay of the leaf extracts of Newbouldia laevis. *Afr.jo. TraCAM*,4:476-480
- Usman, H, Haruna AK Akpula,IN Ilyas M.,Ahmadu, AA and Musa, Y.M (2005). Pytochemical and Antibacterial Screenings of the leaf Extract of *Celtis integrifolia* Lam. *J. Trop. Biosci*.5(2):pp 72-76.