Antimicrobial and Resistance Modulatory Activities of Corynanthe pachyceras

Article *in* Pharmacognosy Research · January 2009

CITATIONS
11

READS
192

3 authors:

Francis Adu
Kwame Nkrumah University Of Science and Technology
42 PUBLICATIONS **231** CITATIONS
SEE PROFILE

Stephen Gbedema
Kwame Nkrumah University Of Science and Technology
47 PUBLICATIONS **336** CITATIONS
SEE PROFILE

K. Annan
Kwame Nkrumah University Of Science and Technology
66 PUBLICATIONS **615** CITATIONS
SEE PROFILE

Pharmacognosy Research [Phcog Res]
Vol 1, Issue 5, Sep-Oct, 2009 Page 280-284
(An Official Publication of Pharmacognosy Network Worldwide)
Received: May 23, 2009
Modified: Jun 10, 2009
Accepted: Aug 22, 2009

PHCOG RES.: Research Article

Antimicrobial and Resistance Modulatory Activities of *Corynanthe pachyceras*

Adu Francis[1]*, Gbedema Stephen Yao[1], Annan Kofi[2]

[1]*Department of Pharmaceutics;*
[2]*Department of Pharmacognosy, College of Health Science, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.*

*** Author for correspondence:** *Tel: +233 208168429 Email: franceadu@yahoo.com*

ABSTRACT

As part of the project to identify plant natural products which modulate bacterial multidrug resistance (MDR) the methanol extract of *Corynanthe pachyceras* (Rubiaceae) K. Schum., were tested for *in vitro* antibacterial and resistance modifying activities against different bacterial strains including resistant staphylococcus *SA1199B, RN4220* and *XU212* possessing the Tet(K), Msr(A), and Nor(A) multidrug resistance efflux mechanisms respectively. Using the Micro-well dilution method the MIC of the stem bark extract ranged between 128-512µg/ml against the selected bacteria used. At 10µg/ml, none of the different extracts displayed any antibacterial action but in combination with tetracycline, erythromycin and norfloxacin, the stem bark extract displayed a 2-fold, 4-fold and 8-fold potentiation of activities of these antibiotics against *XU212*, *SA1199B* and *RN4220* possessing the Tet(K) [tetracycline resistant], Nor(A) [norfloxacin resistant] and Msr(A) [macrolide resistant] transporters respectively.

Keywords: *Corynanthe pachyceras*, resistance modulators, antibacterial, *Staphyloccocus aureus.*

INTRODUCTION

Medicinal herbs have been known to be a good source of biodynamic compounds of therapeutic value since ancient times. In recent times there has been a global trend for revival of interest in herbal medicines (1). In Africa majority of people use plant based medicines to treat illness and ailments and the demand for medicinal plants increases as the population grows (2).

Infectious and parasitic diseases resulting from a vast array of microbes represent the main type of ailment afflicting people from poor third world nations, including Ghana. For example, HIV/AIDS, tuberculosis, malaria and leishmaniasis among others represent some of the most common viral, bacterial and protozoal diseases in the tropics, causing millions of deaths each year (3).

Different classes of antibiotics have been used to control bacterial infections. However, the usefulness of existing antimicrobial agents is rapidly fading, tipping the balance in favour of multi-drug resistant pathogens, including MRSA, and there appears to be few, if any, new classes of drugs currently in use to fight against the multi-drug resistant pathogens. Multidrug-resistance (MDR) exhibited by many bacterial species is a major problem in treating both hospital and community acquired infections. Many strains of MRSA possess efflux pumps such as the specific TetK and MsrA transporters which export or extrude certain tetracyclines and macrolides, and the multidrug resistance proteins NorA and QacA which confer resistance to a wide range of structurally unrelated antibiotics (4). The inhibition of efflux pumps or mechanism in a bacterial cell could therefore be a means of reversing resistance.

A bacterial resistance modifying agent therefore, reduces the minimum inhibitory concentration (MIC) for an antibiotic to which resistance has already occurred. This could be of great benefit in combinatory therapy, perhaps facilitating the re-introduction of antibiotics that are no longer effective due to resistance (5).

Corynanthe pachyceras (Rubiaceae) K. Schum. is a lower storey forest tree growing in tropical West Africa. Locally known as Pamprama (Fantis), the plant is used in folklore as intoxicant, local anaesthetic, aphrodisiac and a febrifuge. It is also used in the treatment of boils and chronic wounds (6).

As part of an ongoing study to screen local plants for bacterial modulatory actions, *C. pachyceras* was assessed for its actions against different strains of microorganisms including three resistant strains of *Staph. aureus*; *SA1199B, RN4220, XU212*. We here report the effect of the methanolic extract on the resistant *Staphylococcus* strains and also in combination with standard antibiotics tetracycline, erythromycin and norfloxacin respectively.

MATERIALS AND METHODS

Plant materials

Plant materials were collected in December, 2007 from the arboretum of the Bobiri forest in the Ashanti Region of Ghana and authenticated at the Department of Pharmacognosy, College of Health Science, Kwame Nkrumah University of Science and Technology, Ghana where a herbarium specimen (FP/HM/132) have been deposited.

Preparation of plant extract

Fifty five grams (55g) of the dried powdered plant part (roots, stem bark, and leaves) was packed into a cellulose thimble (28×100 mm) and soxhlet-extracted with 400ml methanol (Sigma) over 48 hours until the material was exhausted. The extract was concentrated under reduced pressure using the rotary evaporator and dried over nitrogen to give a yield of 5.29, 4.91 and 3.32g respectively for roots, stem bark, and leaves.

Test organisms

The bacteria used for the tests were obtained from the National Culture Type Collection (NCTC), UK and included both Gram positive and Gram negative bacteria. The Gram positive bacteria used were *Bacillus subtilis* (NCTC 10073), *Staphylococcus aureus* (NCTC 4163) *Streptococcus faecalis* (NCTC 775), *Micrococcus flavus* (NCTC 7743), as well as resistant strains of *Staph. aureus* SA1199B, RN4220 and XU212. Gram negative bacteria used were *Escherichia coli* (NCTC 9002) and *Pseudomonas aeruginosa* (NCTC 10662).

Antibacterial assay

Inocula of the microorganisms were prepared from the 24 h Mueller-Hinton broth (Sigma) cultures and suspensions were adjusted to 10^5CFU/ml. Minimum inhibition concentration (MIC) values of the extracts were determined based on a micro-well dilution method (7). The 96-well sterile plates were prepared by dispensing 180 µl of the inoculated broth plus a 20 µl aliquot of the plant extract made up in broth or 20 µl broth in the case of negative control in each well. Norfloxacin, erythromycin and tetracycline (Sigma) were included as positive controls. Plates were covered and incubated for 24 h at 37°C. Bacterial growth was determined after addition of 50 µl p-iodonitrotetrezolium violet (0.2 mg/ml, Sigma).

Bacterial modulation assay

The modulation assay was performed using 10µg/ml of the extract to find the effect of combining them with standard antibiotics norfloxacin, tetracycline and erythromycin on resistant strains of *Staph. aureus SA1199B* (NorA), *XU212* (tetK) and *RN4220* (MsrA) respectively following the method of Oluwatuyi *et al.* (8). 100µl of Muller-Hinton Broth (MHB) was placed in each well except wells in column 1. 200µl of antibiotic was placed in wells of column 1 and serially diluted 2-fold to give a dilution range 512-1µg/ml. To the wells in first three rows were added 100µl of the extract (made so as to bring the net concentration in each well to 10µg/ml) except those in columns 11 and 12. To the wells in rows four to six were added 100µl of reserpine (solution made so as to bring the net concentration in well to 10µg/ml). Wells in rows seven and eight were however, maintained free from both the extract and reserpine. Wells in columns 11 and 12 were used as general and sterile controls respectively. Plates were incubated at 37°C for 24 hours, after which 20µl of MTT was added to each well and incubated for further 30 minutes. Inhibition of bacterial growth was visible as a clear well and the presence of growth detected by the presence of a blue colour in the well. All experiments were performed in triplicate under aseptic conditions.

Phytochemical screening of plant extracts

The methanolic extracts were used for the phytochemical tests. The screening procedures were adapted from Wall *et al.*,(9) and Harborne (10). The test for tannins was

carried out by subjecting 0.1g of each plant extract in 2ml of distilled water, filtered and ferric chloride reagents added to the filtrate. For cardiac glycosides, Legal's test and the Killer-Kiliani test were adopted (0.1g of extract was added to 2ml acetic anhydride plus H_2SO_4). The test for alkaloids was carried out by subjecting 0.5 g aqueous extract in 5 ml 1% HCl, boiled, filtered and Mayer's reagent added. Cyanogenic glycosides were identified by subjecting 0.1g extract in 5ml sterile water and filtered. Sodium picrate paper was added to the filtrate and heated to boil. The extract was also tested for carbohydrates using resorcinol solution. The extract was subjected to frothing test for the identification of saponin. Haemolysis test was further performed on the extracts in water to remove false positive results. Fehling's solution was added to the extract and heated to detect reducing sugar. The extract was also tested for free glycoside bound anthraquinones. Extract was added to 10 ml benzene, filtered and ammonia solution added. The presence of flavonoids was determined using 1% aluminum chloride solution in methanol, concentrated HCl, magnesium turnins, and potassium hydroxide solution.

RESULTS AND DISCUSSIONS

The three different extracts from the various morphological parts showed moderate antimicrobial activity as indicated by their MICs (Table 1). The stem bark extract with MIC ranging 128-512µg/ml against all organisms tested however, was consistently more active than the others, including the resistant strains of *Staph. aureus*, even though the difference in activity was not very significant. The standard antibiotics tetracycline, erythromycin and norfloxacin used as positive controls recorded the expected very low MICs except against the specific strains to which they were resistant. Thus showing the strength of these antibiotics over the plant extracts in their capacity to deal with microbial infections.

The results of the bacterial resistance modulation assay using a sub-lethal concentration, i.e, 10µg/ml, of the plant extracts in addition to different concentrations of standard antibiotics showed that the stem bark and root extracts were able to reduce the MIC of norfloxacin from 32µg/ml to 16µg/ml (2-fold potentiation) against *SA1199B*. The two different extracts also showed good modulation actions with erythromycin and tetracycline against *RN4220* and *XU212* respectively. Reserpine, which was used as control (11) had 4-fold potentiation of tetracycline activity against *XU212* and norfloxacin activity against *SA1199B* (Table 2). However, reserpine had no effect on erythromycin against *RN4220* (MIC 256 µg/ml) whilst the stem bark extract showed a significant effect (8-fold potentiation).

Phytochemical tests on the extracts established the presence of reducing sugars, glycosides, saponins, condensed tannins and alkaloids.

However, the chemical profile of the different morphological parts appeared similar which might explain the observed antibacterial action of the plant. The study further shows that, like many other plants, the distribution of chemical principles may not be uniform, with different parts of the plant storing different metabolic intermediate or end products, or different levels of same substances. Different classes of compounds of plant origin have been found to possess bacterial resistance modulatory actions (5). These include alkaloids, tannins and saponosides, diterpenoids, triterpenoids and steroids (12–14).

Microbiological resistance mutations may be expressed in several ways including production of inactivating enzymes, changes in cell wall permeability, alterations to structural target, bypass of metabolic pathway, changes in efflux mechanisms and multiple resistance mechanisms (15). Efflux pumps occur naturally in bacterial cells and are concerned with the removal of waste products (16). However, changes in their conformation can make them remove antimicrobials.

Table 1. Antimicrobial activity of *C. pachyceras* expressed as minimum inhibitory concentration (MIC; µg/ml)

	Minimum Inhibitory Concentration (µg/ml)					
Organism	**Bark**	**Leaf**	**Root**	**TET**	**ERY**	**NOR**
B. subtilis	128	>512	512	2	2	1
Staph. aureus	128	256	256	2	1	1
Strept. faecalis	256	512	256	4	8	2
M. flavus	128	512	256	1	1	1
E. coli	128	256	128	2	4	2
Ps. aeruginosa	250	>512	512	2	4	2
SA1199B	512	>512	>512	8	8	32
RN4220	512	>512	>512	128	4	4
XU212	512	>512	>512	8	256	4

Key; **TET** – Tetracycline; **ERY** – Erythromycin; **NOR** – Norfloxacin; n=3

Table 2. Antimicrobial susceptibility of test strains in the absence and presence of 10µg/ml of extracts and reserpine

	MIC (µg/ml) of test strain expressing the indicated efflux protein		
Antimicrobial agent	**XU212 (TetK)**	**RN4220 (MsrA)**	**SA1199B (NorA)**
Tetracycline	128	NT	NT
+ bark	32		
+ leaf	64		
+ root	64		
+ reserpine	32		
Erythromycin	NT	256	NT
+ bark		32	
+ leaf		128	
+ root		32	
+ reserpine		256	
Norfloxacin	NT	NT	32
+ bark			16
+ leaf			32
+ root			16
+ reserpine			8

All MICs were determined in triplicate. NT = not tested

Gram positive organisms including *Staph. aureus* can show resistance to tetracyclines by this mechanism (17). However, it is common for organisms to exhibit resistance by using a combination of two or more of the above mechanisms. Macrolide resistance in *Streptococci* may be due to a combination of increased efflux and ribosomal modification, a scenario commonly referred to as multi-drug resistance (18).

In this study, the observed potentiation of the antimicrobial effects of the standard antibiotics used (tetracycline, erythromycin and norfloxacin) after combining them individually with 10 µg/ml of the plant extracts suggests it could modify the resistance mechanisms present. In the case of *SA1199B*, *RN4220* and *XU212* where the mechanisms of resistance have been confirmed to be by efflux (5), some components in the extracts, in low concentrations may act as substrates for the efflux proteins which the resistant bacteria overexpress thus allowing the respective antibiotics to enter the bacterial cell to cause toxicity.

CONCLUSION

The study has shown that the methanolic extracts of the stem bark, leaves and roots of *C. pachyceras* have potent antibacterial and resistant modifying actions. All have broad spectrum antibacterial activity. The stem bark showed the greatest activity against the bacteria used. From the study it can be said that *C. pachyceras* is a potential source of useful anti-infective drugs. The high yield of extract from the stem bark coupled with its high potency against the various organisms makes it the preferred morphological part to be used.

ACKNOWLEDGEMENT

We are grateful to the Ghana Government for the financial support in undertaking this project.

REFERENCES

1. Calixto J.B. Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines *Braz J Med Biol Res* **33**(2): 179–89 (2000).
2. Kofi-Tsekpo M. Institutionalization of African traditional medicine in health care systems in Africa. *Afr J Sci* **11**:i–ii (2004).
3. Broder S., Hoffman S.L. and Hotez J.P. Third world problems: The application of genomics to the diseases plaguing the developing world may have huge medical and economic benefits for those countries and might even prevent armed conflict. *European Molecular Biology Organization (EMBO) Report*, **3**(9): 806–12 (2002).
4. Koyama J. Anti-infective quinone derivatives of recent patents. *Recent Patents on Anti-infective Drug Discovery* **1**:113–25 (2006).
5. Gibbons S. Plants as a source of bacterial resistance modulators and anti-infective agents. *Phytochem Rev* **4**:63–78 (2005).
6. Jans L., Poorter L., van Rompaey R.S. A. R. and Bongers F. Gaps and Forest Zones in Tropical Moist Forest in Ivory Coast. *Biotropica* **25**: 258–269 (1993).
7. Eloff J.N. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. *Planta Med* **64**:711–3 (1998).
8. Oluwatuyi M., Kaatz G.W. and Gibbons S. Antibacterial and resistance modifying activity of *Rosemarinus officinalis*. *Phytochemistry* **65**:3249–54 (2004).
9. Wall M.E., Eddy C.R., McClenna M.L. and Klump M.E. Detection and estimation of steroid and sapogenins in plant tissue. *Anal Chem* **24**:1337–42 (1952).
10. Harborne J.B. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis, (Chapman A & Hall. London, 1973) 279.
11. Annan K., Houghton P.J., Hylands P.J. and Gibbons S. Antibacterial and Resistant Modifying activity of *Paullinia pinnata*. *J Pharm Pharmacol* **57**:S-74 (2005).
12. Brenwald N.P, Gill M.J. and Wise R. The effect of reserpine, an inhibitor of multi-drug efflux pumps on the *in vitro* susceptibilities of fluoroquinolone-resistant strains of *Streptococcus pneumoniae* to norfloxacin. *J Antimicr Chemother* **40**: 458–60 (1997).

13. Lee C.J., Galazzao J.L., Ianiro T. and Warren M.S. Novel bacterial efflux pump from microbial fermentation. In 40th Interscience Conference on *Antimicrobial Agents and Chemotherapy*, Toronto, Canada, American Society of Microbiology (2000).
14. Silvia A.M., Gavit N.E., Pere W.D. and Hayashi S.F. Identification of a tetracycline efflux resistance reverser UK-57, 562. in 39th Interscience Conference on *Antimicrobial Agents and Chemotherapy*. San Francisco, CA, American Society for Microbiology (1999).
15. Wickens H. and Wade P. Understanding antibiotic resistance. *The Pharma J* **274**:501–4 (2005).
16. Poole K. Efflux-mediated antimicrobial resistance. *J Antimicr Chemother* **56**:20–51 (2005).
17. Miyamae S., Ueda O., Yoshimura F., Tanaka J. and Nikaido H. A MATE family multidrug efflux transporter pumps fluoroquinolones in *Bacteroides thetaiotaomicron*. *Antimicrob Agents Chemother* **45**:3341–3346 (2001).
18. Di Perri G. and Bonora S. Which agents should we use for the treatment of Multi-drug resistant *Mycobacterium tuberculosis*? *J Antimicr Chemother* **54**:593–602 (2004).