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Vol. 8(24), pp. 2376-2380, 11 June, 2014
DOI: 10.5897/AJMR2013.6360
Article Number: 806DEBE45351
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Antimicrobial potential of *Rothmannia longiflora* Salisb and *Canna indica* Linn extracts against selected strains of fungi and bacteria

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Received 15 September, 2013; Accepted 5 February, 2014

This study was conducted to investigate the antimicrobial activities of the methanolic extract of *Rothmannia longiflora* and *Canna indica* leaves against 10 pathogenic bacterial and fungal strains. The methanolic extract from the two plants were screened for their minimum inhibitory concentration (MIC) against the microbial growth of *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Salmonella typhi, Klebsiella pneumonia, Candida albicans, Aspergillus niger, Phjapus stolometer* and *Penicillium notatum.* The results show that inhibition of microbial growth decreased with decreasing concentrations of the plant extracts. While concentrations of 200 and 100 mg/ml completely inhibited microbial growth, lower concentrations (50 and 25 mg/ml) showed partial inhibition, extracts at lower concentrations than these had no effects on microbial growth of microorganisms tested. Comparatively, *R. longiflora* gave better results than *C. indica*. This study confirmed the effectiveness of methanolic extracts of the two plants as inhibitory effect to microbial growth of several pathogenic microorganisms and testified to the basis of the ethnomedicinal uses of these two plants against several microbial infections.

Key words: Antimicrobial potential, *Rothmannia longiflora*, *Canna indica*, bacteria, fungi, Nigeria, methanolic extract.

INTRODUCTION

Plant-derived substances have recently become of great interest owing to their versatile applications. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical inter-mediates and chemical entities for synthetic drugs. The increase of microbial infections have increased dramatically in the past 20 years because of the increase in the number of people whose immune systems are compromised by AIDS, aging, organ transplant and cancer therapy.

Accordingly, increase in the rates of morbidity and mortality because of microbial infections have been regarded as a major problem (Tatli and Akdemir, 2005). Worse still, there are global problems of multiple antibiotics resistance as well as emergence of new and resurrection of previously eradicated diseases. Most of the current antimicrobial drugs simply reduce the level of growth of bacteria or fungi, and some of them are very toxic to the kidney, the hematopoietic and central nervous

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system (Tatli and Akdemir, 2005). Furthermore, antimicrobial resistance among enteric pathogens is becoming a matter of serious concern (El-Mahmoud et al., 2008) and poses a great threat to global human health. Further, new microbial strains are being continuously discovered, which are refractory to the current arsenal of drugs (Erturk et al., 2006). This is because antimicrobial resistance leads to therapeutic failures of empirical therapy (Parekh and Chanda, 2007).

As a result, it has become necessary to fight against emerging and re-emerging infectious diseases with a view to discover and invent new agents of greater therapeutic profile to mitigate frequent outbreaks of diseases which has posed a new threat to global health security (Mohanta et al., 2007). The continuous evolution of bacterial resistance to currently available antibiotics has necessitated the search for novel and effective antimicrobial compounds (Fagbemi et al., 2009), to which these micro-organisms are yet to develop resistance (El-Mahmoud et al., 2008).

With the rising problems of side effects and limited efficacy of antibiotic drugs (Gupta et al., 1998), there is an urgent need for the development of alternative antimicrobial substances and researchers are nowadays turning to natural products from plants (Nitta et al., 2002), as their main source of bioactive compounds with antimicrobial properties, to complement the existing synthetic antimicrobial drugs that are gradually becoming less potent against pathogenic microorganisms (Lawal et al., 2012).

The rate of interest in plant derived drugs has shoot up due to the fact that herbal medicine is safer and less costly than the synthetic drugs which possess serious side effects. It is therefore necessary to screen plants for promising biological activity (Lawal et al., 2010).

Rothmannia longiflora Salisb. is a shrub belonging to the plant family of Rubiaceae, having trumpet-like flower with fruits that are longitudinal in shape. It has been reported to have antimalarial effects and also used in the treatment of measles and to give tribal marks (Ikpi et al., 2009). It is used as chewing stick in the treatment of filariasis, dysentery, and fever and also as an analgesic and emetic. Furthermore, the plants is considered to have febrifugal and analgesic properties, and a decoction of the leaves, twigs, bark and roots is applied internally or externally in lotions, washes and baths. In Nigeria the roots are used to treat bowel complaints (Jansen, 2005).

The plant, *Canna indica* L. is a native of the Caribbean and Tropical America; and belongs to the family Cannaceae. The introduction of this plant to Africa, especially Nigeria is unknown, but it is widely distributed throughout Nigeria, where it is known locally as “ebesalebo” in Edo, “nkwa ebotri” amongst the Efik, “bakalekale” in Hausa, “aberekamwo” in Igbo land and “iroro” amongst the Yorubas. It has been reported to be used ethnomedicinally for the treatment of malaria in South-western Nigeria, as well as a cure for diarrhoea and dysentery and in the treatment of fever, bruises and cut (Odugbemi et al., 2007). Josephine et al., 2013 reported that methanolic extracts of *C. indica* possessed anti-diarrhoeal properties.

This study was therefore conducted to investigate the antimicrobial activities of the methanolic extract of *R. longiflora* and *C. indica* leaves on some bacterial and fungal strains.

MATERIALS AND METHODS

Plant materials

Fresh leaves of *R. longiflora* and *C. indica* were collected from Forestry Research Institute of Nigeria. The plants confirmation and identification was done at Forest Herbarium, Ibadan (FHI) in Forestry Research Institute of Nigeria (FRIN). The leaves were air dried for five days and pulverised using hammer mill and kept for analysis.

Test micro-organisms

A total of 10 test microorganisms used in this study, which include bacterial and fungal strains. The bacteria species used are *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Salmonella typhi* and *Klebsiella pneumoniae*; while the fungi species include *Candida albicans, Aspergillus niger, Phjapus stolometer* and *Penicillium notatum*. The micro-organisms were collected from Departments of Pharmaceutical Microbiology and Veterinary Microbiology of the University of Ibadan and the International Institute of Tropical Agriculture (I. I. T. A), Ibadan.

Preparation of the extracts

100 g of the powered plant leaf samples were macerated with 100 ml of 90% methanol for 72 h and pooled to obtain the crude methanol extract. The extract was filtered through filter paper to remove all insoluble matter, including cellular materials.

Antimicrobial assays

4 ml of the sample was measured into the first test tube and five other test tubes contained 2 ml of methanol. From the first tube that contains 4 ml of the original sample, 2 ml was taken into the second tube to make up to 4 ml. This was done until the 6th test which was the last test tube for the extract. The 7th and 8th tubes contain positive and negative controls respectively.

Pour plate method (Bacteria)

An overnight culture of each organism was prepared before taking scoopful of the organism from the stroke and inoculated each into the sterile nutrient broth of 5 ml for 18 - 24 h at 37°C. From overnight culture 0.1 ml of each organism was taken into 9.9 ml of sterile distilled water to get (1:10) 10^{-2} of the dilution of the organism.

From the diluted organism, 0.2 ml was taken into the prepared sterile nutrient agar which was incubated at 45°C. These were then aseptically poured into sterile petri dishes and allowed to solidify for about 45 min. using a sterile cork -borer. The wells were made accordingly into the graded concentrations of the extract including

Table 1. Antibacterial and antifungal activities of methanolic leaf extract of *Rothmannia longiflora* at different concentrations

Test organism	Mean inhibition zone of *R. longiflora* at different concentration						
	200 mg/ml	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml	Gmc (10 ug/ml)
Bacteria							
Staphylococcus aureus	17	14	12	10	na	na	38
Isherichia coli	15	12	10	na	na	na	36
Bacillus subtilis	14	10	na	na	na	na	38
Pseudomonas aeruginosa	12	10	na	na	na	na	36
Salmonella typhii	19	14	12	10	na	na	34
Fungi							
Klebsiella pneumoniae	14	10	na	na	na	na	36
Candida albicansna	17	14	12	10	na	na	26
Aspergillus niger	15	12	10	na	na	na	24
Phjapus stolometer	12	10	na	na	na	na	26
Penicillum rotatum	14	12	10	na	na	na	24

Gmc: Gentamycin; NA : Not active.

the controls. The duplicates were made to ascertain the results obtained. The plates were allowed on the bench for about 2 h to allow the extract to diffuse properly into the nutrient agar that is, Pre diffusion. The plates were incubated for 18-24 h at 37°C.

Surface plate method (fungi)

A sterile Sabouraud Dextrose Agar was prepared and aseptically poured into the sterile plate in duplicate and allowed to solidify. 0.2 ml of the 10^{-2} of the organism was then spread on the surface of the agar using a sterile inoculation loop to cover all the surface of the agar. Wells were made using a sterile cork-borer of 8 mm in diameter and into each well, the graded concentration of the extract was put including the controls. All the plates were allowed to stand for 2 h to allow the extract to diffuse properly into the agar i.e. pre diffusion. The plates were incubated for 48 h at 27°C.

Minimum Inhibition Concentration (MIC) test

The method used for determining the minimum inhibitory concentrations (MICs) for the extract followed those of Clinical Laboratory Standardization Institute (CLSI, M100-S18), 2 ml of each concentration to be used was added to 18mls of the agar, and allowed to set. The diluted organisms were streaked on the agar. The plates were incubated for 24 h at 37°C and checked for growth. The MIC was taken as the lowest concentration of the extract that showed no visible growth. This was applied to the controls as well.

RESULTS AND DISCUSSION

The study was conducted to investigate the antimicrobial potentials of the methanolic extracts of *R. longflora* and *C. indica* on 10 selected pathogenic micro-organisms. The antimicrobial potentials of these 2 plants were determined based on the zones of inhibition of microbial growth of the test micro-organisms. The mean zones of inhibition of growth of the isolates are a function of relative antibacterial and anti-fungal activities of the extracts. The zone of inhibition is simply the area on the agar plate that remains free from microbial growth. The size of the zone of inhibition is usually related to the level of antimicrobial activity present in the sample or product - a larger zone of inhibition usually means that the antimicrobial is more potent (Lawal et al., 2012). Some of the plates exhibited clearly distinct zone of inhibition, while some of them did not. Tables 1 and 2 present the calculated mean zones of inhibition (mm) of methanolic leaf extracts of *R. longiflora* and *C. indica* on the bacterial and fungal strains used for this study respectively.

The results show that at the higher concentrations of 200 and 100 mg/ml, methanolic extracts of *R. longiflora* showed inhibition of all the microorganisms (Table 1). The results were similar at the same concentrations in *C. indica*, except for *P. aeruginosa* at 100 mg/ml (Table 2). Inhibition of the methanolic extracts of these plants reduced with decreasing concentrations, with no inhibition recorded at concentrations of 12.5 mg/ml and lower (Tables 1 and 2). Comparatively, *R. longiflora* showed higher inhibition to microbial growth than *C. indica*.

Resistant bacteria have become commonplace in healthcare institutions and increased mortality rate due to resistant *P. aeruginosa, S. aureus, K. pneumoniae, E. coli, Enterobacter* spp., and coagulase-negative staphylococci and enterococci has been reported (Karlosky et al., 2003; NNIS, 2004; Kang et al., 2005). With this increased incidence of antimicrobial resistance and appearance of new infectious for their antimicrobial activity and resistance modifying ability (Gibbon, 2004; Coutinho et al., 2009). While the natural products are known to play significant roles in the development of novel drugs and served as leads for the treatment and

Table 2. Antibacterial and Antifungal activities of methanolic leaf extract of *Canna indica* at different concentrations.

Test organism	Mean inhibition zone of *C. indica* methanol extract at different concentration						
	200 mg/ml	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml	Gmc (10 ug/ml)
Bacteria							
Staphylococcus aureus	13	10	na	10	na	na	36
Esherichia coli	13	10	na	na	na	na	38
Bacillus subtilis	13	10	na	na	na	na	36
Pseudomonas aeruginosa	10	na	na	na	na	na	36
Salmonella typhii	14	10	na	na	na	na	34
Fungi							
Klebsiella pneumoniae	16	13	10	na	na	na	32
Candida albicansna	15	13	10	na	na	na	26
Aspergillus niger	14	12	10	na	na	na	24
Phjapus stolometer	16	14	12	10	na	na	29
Penicillum rotatum	15	13	10	na	na	na	28

Gmc, Gentamycin; na, not active.

prevention of diseases (Belini et al., 2008), plant-derived antimicrobials provide the much needed therapeutics (Olajuyigbe and Afolayan, 2013) .

The leaves of *C. indica* showed antimicrobial activity (Abdullah et al., 2012), analgesic activity, and the rhizomes showed a good anthelmintic activity against Pheritima posthuma (Nirmal et al., 2007). The leaves haves chemical constituents like lignin, furfural and hemicelluloses, while rhizomes has 5,8- Henicosdine, Tetracosane and Tricosane (Deming and Tinoi, 2006). The water extract of rhizomes of *C. indica* has been reported to have HIV-1reverse transcriptase inhibitory activity (Woradulayapinij et al., 2005) while its essential oil shows antibacterial activity (Indrayan et al., 2011). Methanolic extract of Aerial Parts of *C. indica* shows antioxidant activity (Joshi et al., 2009). Furthermore, anthocyanins and methylated anthocyanidin glycosides were also isolated from *C. indica* flowers (Srivasta and Vankar, 2010; Srivasta and Vankar, 2010).

Previous studies have shown that *R. longiflora* is the only higher plant that possessed 4-oxonicotinamide-1-(1'-β-D-ribofuranoside), which is only found in human urine. It is an interesting compound, particularly in influencing enzymatic processes. Furthermore, Ebigwai et al., 2012 reported the larvicidal effects and use of *R. longiflora* extracts against *Simulium yahense* larvae. *S. yahense* is responsible for causing onchocerciasis.

Conclusion

This study has confirmed the effectiveness of methanolic extracts of the two plants as inhibitory to microbial growth of several pathogenic microorganisms. Furthermore, the results explain the basis of the ethnomedicinal uses of these two plants against several microbial infections. However, further studies might be required to isolate the active ingredients in the plants.

Conflict of Interests

The author(s) have not declared any conflict of interests.

ACKNOWLEDGEMENT

We appreciate the assistance given by the field overseer of Pharmacy flora Unit of Forestry Research Institute of Nigeria for the plant collection. Mr. Michael and the staff of FHI for proper identification, and also, thank Mr. Felix of Department of Pharmaceutical Microbiology, University of Ibadan.

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