2012 International Conference on Environment, Chemistry and Biology
IPCBEE vol.49 (2012) © (2012) IACSIT Press, Singapore
DOI: 10.7763/IPCBEE. 2012. V49. 38

Phytochemical and Antimicrobial Studies on the Extracts from Leaves of *Cajanus Cajan* and *Eucalyptus Globulus*

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Abstract. Resistance of microorganisms to antibiotics is a serious global problem. The aim of this study was to investigate the antimicrobial activity of *Cajanus cajan* and *Eucalyptus globules* leaf extracts and to relate these to their constituents. Phytochemical tests, thin-layer chromatography and antimicrobial activities using cup-plate and dilution methods were carried out. Phytochemical tests revealed the presence of reducing sugars, glycosides, saponins, tannins, resins and acidic compounds in both samples. Terpenoids was found in *E. globules*. Thin-layer chromatography revealed four and three bands for *C. cajan* and *E. globules* respectively. Preliminary antimicrobial screening for *C. cajan* and *E. globulus* showed inhibitory activity against *Staphylococcus aureus (9mm and 14mm)* and *Bacillus subtilis(7mm am 12mm)* and very slight inhibitory action on *Pseudomonas aeruginosa* and *Trichophyton rubrum* while others were resistant to the action of the extracts. Zones of inhibition ranged between 1mm and 14 mm. Minimum inhibitory concentrations for both plants on the organisms used were between 100mg/ml and 50mg/ml. The research has shown that these plants possess some therapeutic potential.

Keywords: Phytochemical, *Cajanus cajan*, *Eucalypyus globules*, antimicrobials

1. Introduction

Resistance of some microorganisms to antibiotics is a serious global problem (Westh *et al*, 2004) thus the urgent need to identify traditional plants with new antimicrobial compounds, diverse chemical structures and novel mechanisms of action for new and re-emerging infections diseases (Rojas *et al*, 2003). Plants have been found useful to man not only as food or as sources of raw materials for industrial purposes, but also as sources of medicaments (Azoro, 2004). Unlike the orthodox medicines, plants apart from their efficacy have little or no side effects. For example, in the treatment of hypertension, herbs are used to lower blood pressure, clean the arteries, slow and regulate the heart beating rate in order to improve the circulation of blood and to relax the mind (Mann *et. al*., 1983). This is unlike the fundamental conventional drugs that dilate the arteries or the veins until they reach their maximum elastic point which may suddenly burst and give rise to vascular accident, causing stroke or death (Ogundare, 2011). Medicinal plant is any plant which in one or more of its organs, contains substance(s) that can be used for therapeutic purpose or which are precursors for the synthesis of useful drugs (Sofowora, 1999). Some plants reportedly used for the traditional treatment of ailments like stomach disorder, cold, fever etc such as *Ocimum gratissimum, Zingiber officinale, Vernonia amygdalina*, Mistletoe etc have been scientifically screened and shown to possess antimicrobial activity.

Cajanus Cajan an annual or biennial shrub is of the family fabaceae and is commonly called pigeon pea or field pea. This plant has been found to be anti-viral (measles, catarrh and hepatitis), antibacterial

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antisickling, and regulates blood pressure. It can also be applied in the treatment of the psychosomatic disorder called Abutilon (Sofowora, 1979). The antisickling effect of this plant may be due to the prevention of sodium metabisulifite from inducing sickling of red blood cells by aqueous alcohol extracts and cajaminose (constituent). Or due to the effectiveness of seed extracts (main antisickling fraction is phenylalanine) of the plant to restore normal morphology of erythrocytes from blood samples of patients affected by sickle-cell anaemia (Iwu *et.al.,* 1993). *Cajanus* Cajan in addition to other plant leaves such as *Rauwolfia vomitoria, Indiogofera macrophylla,* and whole herb of *Olyra latifolia* is used as a remedy for small pox (Iwu, 1993).

Eucalyptus globules characterized by the possession of white peeling bark, belong to the family Myrtaceae. The plant is commonly called Eucalyptus leaf, blue-gum tree or Eucalyptus. Pharmacological studies revealed that the essential oil posses antimicrobial (including antitubercular) activity. Medically, the leaves are employed as a remedy for cold and they also serve as ingredients in the pot herb mused as stem therapy for malaria. The plant is listed for catarrh and as a rube-facient in Martindale

Extrapharmacopeia (Iwu, 1993). The leaves exude fragrance when crushed and as such can be used for flavouring. Excessive consumption of eucalyptus is toxic and may have side effects such as nausea, vomiting, dizziness, feeling of suffocation and muscular weakness (Iwu, 1993).

The study was carried out to determine the phytochemical content and inhibitory effect of the methanol extract of the test plants on common bacteria and fungi especially those with known antibiotic resistance, such as; *S. aureus, E. coli* and *Pseudomonas aeruginosa*.

2. Materials and Methods

The leaves of *Eucalyptus globules* and *Cajanus cajan* were collected from University of Nigeria, Nsukka Enugu state and Nnewi, Anambra state respectively. The plants were authenticated at the Department of Botany, UNN.

2.1. Extraction of Samples

The leaves were dried at suitable temperature, ground using lab mortar and electric blender. Some quantity of the powder was used for phytochemical analysis while fifty grams of each of the plant was soaked in 400ml of the methanol for 48hrs. This was filtered with clean muslin cloth and then Whatman No 1 filter paper and the filtrate evaporated to dryness under an electric fan.

2.2. Thin-layer Chromatography

Thin –layer chromatography was done (using silica gel C dissolved in distilled water and a thickness 0.5mm) to separate the different constituents present in the plant extracts. The chamber was saturated with chloroform (10): petroleum ether (7): diethyl ether (3).

2.3. Preliminary Screening tests on Plant Extracts for Antimicrobial Activities

Preliminary screening of the plant extract was carried out using cup-plate method done according to the method of Collins and Lyne (1979) and incubated at 37^0c for 24hrs (bacteria) and room temperature for 4 days.

- **For bacteria:** Five Petri-dishes containing 15ml NA media were poured and allowed to set. Ig each of the extracts (*Cajanus cajan* and *Eucalyptus globules* was dissolved in 1ml of 50% methanol (Tortoriello et al, 1995) and then shaken. Each of the five plates was streaked with one of the following test organisms (*Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa* and *Salmonella Kitambo* using a sterile swab for each.
  In each of the plates, 3 bores were carefully made using 0.4cm borer and then labeled A to C for *Cajanus cajan, Eucalyptus globules* and control (50% methanol) respectively.
  The extracts and control were added aseptically into the appropriate bore and then incubated for 24 hrs at 37^oC and zone of inhibition measured at the end of incubation period.
- **For fungi:** Same method used for bacteria was employed but in this case, chloramphenicol was added to the medium (SDA). Fungi used were *Basidiobolus species, Trichophytor rubrum* and *T. mentagrophyte* labeled 1, 2 and 3 respectively and grown at room temperature (28^0C) for 4days and inhibition zones measured.

2.4. Determination of the Minimum Inhibitory Concentration (MIC)

This was found for the two extracts using serial dilution method. For the investigation of the antibacterial and antifungal activities, the different concentrations (200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml and 12,5mg/ml) were prepared using 50% methanol as diluent. One sterile test tube contained just the 50% methanol and served as control. Five different NA plates were streaked with the five different test bacteria and 3 SDA plates were streaked with the 3 different fungi. One Petri dish was used for each test organism and 5 holes were made using cork borer in each of the plates. The holes were carefully labeled to represent the appropriate concentrations of 200ml/ml, 12.5mg/ml. 0.5ml of each dilution was added in each hole as labeled and these were all incubated at 37^0c for 24 hrs bacterial and room temperature for four days for fungi. At the end of the incubation period, inhibition zones formed on the medium were measured in mm.

2.5. Phytochemical Screening

The crude extracts were subjected to preliminary Phytochemical screening (using method described by Harbone, 1984 and modified by Trease and Evans, 1989) to determine the presence of secondary bioactive metabolites such as saponins, alkaloids, glycosides, acidic compounds etc.

3. Results and Discussion

Leaf extracts used in this study inhibited Gram positive bacteria and *Trichophyton rubrum* (slightly). It's an indication that they are of narrow spectrum activity. *Eucalyptus globulus* extract exhibited highest antimicrobial activity.

The *E. globules* extract showed inhibitory activity on *B. subtilis*, *S. aureus* while *C. cajan* showed inhibition on same organisms too. Slight zones of inhibition were observed with *P. aeruginosa* and *Trichophyton rubrum.* There was no zone of inhibition observed with *E. coli*, *S. kitambo, Basidiobolus species* and *T. mentagrophyte.* Table 1 shows the results of phytochemical analysis. The result of preliminary screening test when degree of activities was measured as inhibition zones is shown in table 3a and 3b while MIC results are given in table 4a and 4b.

Table 4 showed that MIC values of the extracts on the microorganisms used were between 100 and 50mg/ml

Table 1 : Result of Phytochemical analysis

Phytochemical	*Cajanus Cajan*	*Eucalyptus globulus*
Reducing sugars	+	+
Glycosides	+	+
Cardiac glycosides	-	-
Anthracene glycosides	-	-
Cyanogenetic glycosides	-	-
Tannins	+	+
Saponins	+	+
Flavonoids	-	-
Steroidal aglycon	-	-
Terpenoids	-	+
Oils	-	+
Alkaloids	-	-
Carbohydrates (CHOs)	+	+
Protein	-	-
Resins	+	+
Acidic compounds	+	+

Table 2: Results of TLC separation

	Cajanus cajan			*Eucalyptus globulus*		
Substance Spots	Colour	Distance Moved (cm)	RF	Colour	Distance moved (cm)	RF
1	Brownish	3.8	0.22	Greenish	2.7	0.15
2	Greenish	15.5	0.91	light brown	15.0	0.83
	Light brown	16.2	0.95	Brownish	17.5	0.83
	Light brown	16.5	0.97			

Solvent front for *Cajanus Cajan* is 18cm

Solvent front for *Eucalyptus globulus* 18cm

Table 3a: Results of antibacterial screening test

Organisms	Zones of inhibition (in mm)	
	Cajanus Cajan	*Eucalyptus globulus*
Pseudomonas aeruginosa	1	_
Bacillus subtilis	7	12
Escherichia coli	_	_
Salmonella Kitambo	_	_
Staphylococcus aureus	9	14

Table 3b: Results of antifungal screening test

Organisms	*Cajanus cajan*	*Eucalyptus globulus*
Basidiobolus species	_	_
Trichophyton rubrum	2	1
Trichophyton mentagrophtye	_	_

Table 4a: Results of Minimum inhibitory concentration of Cajanus cajan on test organisms

Concentration(mg/ml)	Sa(mm)	Bs(mm)	Pas (mm)	Tr (mm)
200	9	7	1	2
100	3	2	_	_
50	1	1	_	_
25	_	_	_	_
12.5	_	_	_	_

Table 4b: Results of Minimum inhibitory concentration of Eucalyptus globules on test organisms

Concentration(mg/ml)	Sa(mm)	Bs(mm)	Pa (mm)	Tr (mm)
200	14	12	1	1
100	9	4	_	_
50	2	1	_	_
25	_	_	_	_
12.5	_	_	_	_

Key: - No inhibition Sa: *Staphycoloccus aureus, Bs – Bacillus subtilis, Pa- Pseudomonas aeruginosa, Tr- Trichophyton rubrum*

The results obtained showed reasonable inhibitory effect by the extracts on S*taphylococcus aureus* and *Bacillus subtilis* with very slight (insignificant) inhibition on *Pseudomonas aeruginosa.* There was also very slight inhibition on *Trichophyton rubrum.* None of these extracts was found to be bacteriocidal or fungicidal.

Phytochemical analysis revealed in addition to other substances, the presence of tannins and saponins in both plants. The presence of these two phytochemicals may be responsible for the antimicrobial effects exhibited by these plants on *Staph. aureus* and *B. subtilis* since these substances are known to have antimicrobial effects. Tannins, for example are known to be made up of phenolic compounds and phenols, and phenolic compounds have been used extensively as disinfectants. Action of tannin may be due to protein denaturation and is found to be non-specific (Carter, 1986). Tannins possess astringent and homeostatic properties and are therefore widely used as topical application on sprains, bruises, and superficial wounds and infections.

The non-bacteriocidal or fungicical effects of these plant extracts may be attributed to the low level of saponins contained in these plants or the possible masking of the toxic action of saponnins by other constituents. It can also be due to the absence of alkaloids in the plants. Alkaloids are known to cause death of susceptible organisms by their ability to bind to the DNA of the organisms thereby affecting replication and subsequent synthesis (Evans, 1992).

The absence of flavonoids in *Eucalyptus globulus* as opposed to previous reports is of interest. This may be due to differences in geographical location of the plant which is known to influence the composition of a plant greatly (Evans, 1992). *Eucalyptus globulus* was also found to contain oil and terpenoids. Terpenoids from some plants has been found to show anti HIV activity on different targets like reverse transcriptase, integrase and protease (Cowan, 1999).

Some of the organisms used in this study are implicated in nosocomial infections, hence the antimicrobial activity of these extracts to such organisms may be vital in the clinical management of nosocomial infections.

4. Conclusion and Recommendation

The phytochemical analysis carried out on these plants revealed that the test plants possess bioactive substances that have been proven to be antimicrobial by research for instance tannins are known to be made up of phenolic compounds, and phenols and phenolic compounds have been used extensively as disinfectants. Of interest in this work is the absence of flavonoids in *E. globules* as opposed to previous reports. This however may be due to differences in geographical location of the plants which is known to influence the constituents of a plant greatly (Evans, 1992). The significant differences in the antimicrobial activities of the plant extracts can be attributed to the differences in the phytochemical constituents of these plants as revealed by phytochemical analysis. *E. globules* in addition to all constituents present in *C. cajan* also possesses terpenoids and oil.

The solubility of the extracts in the solvent used may have contributed to the results obtained (Oloke and Kolawole, 1988). This research has shown considerable scope for the improvement of the therapeutic potentials of these plants.

Research to identify the individual phytochemical responsible for the antimicrobial activity seen is recommended. By so doing, the use of these extracts for medical and pharmacognostic purposes will be made possible and a better place given them in the pharmaceutical world for man's use in clinical medicine.

5. Acknowledgements

The authors are grateful to the Botany Department of University of Nigeria, Nsukka who authenticated the plants used for this research and to Kevin Onwuekwe and Mary Onwuekwe for there contributions.

6. References

[1] H. Westh, C. Zinn, V. Rodahl *et al*. An International multicenter study of antimicrobial consumption and resistance in *Staphylococcus aureus* isolates from 15 hospitals in 14 countries. *Microb. Drug Resist.*2004, 10:169- 176.

[2] R. Rojas , B. Bustamante, Bauerj *et al*. Antimicrobial activity of selected Peruvian medicinal plants. *J. Ethnopharmacol*. 2003, 88:199-204.

[3] C. Azoro. Towards effective integration of herbal medicine practice. The Nigerian Journal of Research and production. 2004, 3: 132-137

[4] J. Mann, R. Davidson, J. Hobbs, D. Barthorpe. Natural products (their Chemistry and Biological Significance). 1st edition, Longman group, UK .1998, pp. 339-389.

[5] A. Ogundare. Antibacterial properties of the leaf extracts of *Veronia amygdalina*, *Ocimum gratissimum*, Corchorous *olitorius* and *Manihot palmate*. *Nig. Journal of Microbiology*. 2011, 25: 2269-2279

[6] A. Sofowora. Medicinal Plants and traditional medicine in Africa. 3rd Edition. Spectrum books Limited, Ibadan. 1999, pp 172-188.

[7] A. Sofowora. African Medicinal plants: Proceedings of a Conference. University of Ife press, lle-Ife. 1979, p. 24.

[8] M. Iwu. Handboook of African Medicinal plants. CRC press, Florida. 1993, pp. 135 – 136.

[9] M. Iwu, A. Duncan, C. Okunji. New antimicrobials of plant origin. In perspectives on new crops and new uses. Janick, J. (Ed.) Alexandria, V.A. ASHS Press. 1999, pp. 457-462.

[10] K. Collins, P. Lyne. Microbiological methods, 4th Edition Butterworths, London. 1979, pp 234-249.

[11] J. Tortoriello, M. Meckes.- Fisher, M. Villareal, B. Berlin, E. Berlin. Phytomedicine. *International Journal of Phytotheraphy and Phytopharmacology.* 1995, 2 (1): 57-66.

[12] J. Harbone. Phytochemical methods, 1st Edition Chapman Halls, London. 1984, pp 20-22.

[13] G. Trease, W. Evans. Pharmacognosy. Braillene Tindall can, 13th ed. Macmillian Publishers 1989.

[14] S. Carter (Ed). Cooper and Gunn's Tutorial Pharmacy. 6th Edition G.B.S Publishers and Distributors, India. 1986, pp 343-344.

[15] W. Evans. Trease and Evans Pharmacognosy: Baillene and Tindall, Eastborne, London. 1992, pp. 243-351.

[16] M. Cowan. Plant products as antimicrobial agents. *Clinical Microbiology Review*. 1999, 12: 564-582

[17] J. Oloke, D. Kolawole. The antibacterial band antifungal activities of certain components of *Aframomum melleguetea* fruits. Fitoterapia. 1998, 59 (5), 384-388.