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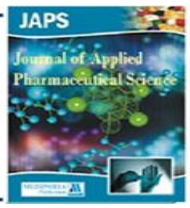

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Evaluation of the antimicrobial activities of crude extract of *Cryptolepis sanguinolenta* and *Crateva adansonii* leaves and their interactions

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ABSTRACT

Activities of crude extract of *Cryptolepis Sanguinolen* and *Crateva Adansonii* leaves and their interactions were evaluated.Crude methanol extracts of *Crateva adansonii* and *Cryptolepis sanguinolenta* leaves were obtained by cold maceration. Antimicrobial activities of the extracts were carried out against six bacteria ie *Pseudomonas aeruginosa, Escherichia coli, Salmonella typhii, Staphylococcus aureus, Klebsiella pneumoniae, Bacillus subtilis and* two fungi which includes *Aspergillus niger* and *Candida albicans* using agar dilution method MICs of methanol extract of *Crateva adansonii* against the six bacteria are *Pseudomonas aeruginosa* -12.5 mg/ml, *Escherichia coli* - 6.25 mg ml, *Salmonella typhi* - 12.5 mg/ml, *Staphylococcus aureus* - 2.5 mg/ml, *Klebsiella pneumonia* - 6.25 mg/ml, *Bacillus subtilis* - 12.5 mg/ml, fungi *Aspergillus niger* - 12.5 mg/ml, *Candida albicans* - 12.5 mg/ml. While the MICs of methanol extract of *Cryptolepis sanguinolenta* against the six bacteria. *Pseudomonas aeruginosa* - 12.5 mg/ml, *Escherichia coli* - 6.25 mg ml, *Salmonella typhii* - 12.5 mg/ml**,** *Staphylococcus aureus* - 12.5 mg/ml**,** *Klebsiella pneumoni* - 12.5 mg/ml. *Bacillus subtilis* - 6.25 mg/ml and for fungi *Aspergillus niger* - 12.5 mg/ml, *Candida albicans* - 6.25 mg/ml. Combined activity of the two plant extracts against *Escherichia coil, klebsiella pneumoniae, Bacillus subtilis* and *Candida albicans* was carried out at the ratios of 1:1, 2:1 and 1:2 of extracts of *Crateva adansonii* and *Cryptolepis sanguinolenta* respectively. Combination 1:2 and 2:1 were found to be effective and able to inhibit *Escherichia coli and Candida albicans.* The result of this work shows that the extracts of *Cryptolepis sanguinolenta* and *Cranteva Adansonii* have both antifungal and antibacterial effects and their combination is effective in some bacterial and fungal infection most especially *Candida albicans* infection.

Keywords: Crude extracts, *Cryptolepis sanginolenta, Crateva adansonii,* combination interactions, antibacteria, antifungal

INTRODUCTION

Traditional medicines have been used for about five thousand years for curing, suppressing and prevention of diseases of human (Malo, 2001). Scientific research into medicinal plants has led to the development of many valuable drugs through isolation, identification, purification, characterization and standardization of active components of these plants (Trease and Evans, 2002). Nature has made available plants which can be used for the cure of various diseases that continually plague mankind (Boye and Ampofo 1990). among this plants are *Crateva adansonii* and *Cryptolepis sanguinolenta*. *Cryptolepis sanguinolenta* (Yellow dye plant**)** is a thin stemmed twining and scrambling shrub. The leaves are petiolate, glabrous, elliptic or oblong – elliptic up to 7 cm long and 3 cm wide (Wright *et al.*, 1996). Its common name among the various tribe of Ghana include nibima (among the Twi speaking people), kadze (among the Ewe), and

gangamau (among Hausa). The major alkaloid, Cryptolepine, was isolated from *Cryptolepis sanguinolenta* in Nigeria and later in Ghana by Dwuma- Badu and his co-workers (Boye and Ampofo 1990), (Lori and Shelland, 2001).

Crateva adansonii is a small handsome tree, often stunted as a result of leaves being cut for food. The plant is also called the sacred garlic pear and temple plant (NPGS/GRIN: www.arsgrin.gov.2008). The plant's common name in Hausa is ungududu, in Youruba is eegun- orun, in Igbo is amakarode (Peter, 1997). The plant is called spider tree because the showy flowers bear long, spidery stamens. The plant belongs to the family Capparidaceae (Ryan and Ray, 2004).

Indications for combination of antimicrobial agent include achieving a broad antimicrobial spectrum for empirical therapy (Walker *et al.*, 2004); treatment of poly microbial infection involving organisms not susceptible to the same drugs (Madigan and Martinko, 2006) ; reducing the risk or advent of antimicrobial resistance; and reducing the risk of adverse drug reactions by minimizing exposure to potentially toxic antimicrobiall (Lansing *et al*., 2002). Combination therapy often is used when dealing with infection caused by both aerobic and anaerobic microorganisms (Evans *et al.*, 2007). Antimicrobial Interactions can be antagonistic, indifference, additive or synergistic (Carter, S.J. (2000), (Fotadal *et al.*, 2005). Antimicrobial combinations of the extracts of *Crateva adansonii* and *Cryptolepis sanguinolenta* was carried out using pour plate diffussion method (Sampson *et al.*, 2001).

MATERIALS AND METHODS

Test organism

Clinical isolates used *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella Pneumoniae*, *Pseudomonas aeruginosa, Bacillus subtilis, Salmonella typhi. Candida albicans* , *Aspergillus niger* . The bacteria and fungi were both collected from the Department of Pharmaceutical Microbiology, University of Nigeria Nsukka. The fungi were preserved at room temperature on Sabouraud Dextrose**.** Agar (SDA) slant while the bacteria were preserved in refrigerator
temperature (4^oC) on nutrient agar (NA) slant.

Nutrient media

Nutrient agar (FLUKA limited) for bacteria were used for inoculation on plates, slants and tubes. Sabouraud Dextrose agar (FLUKA Limited) for fungi were used for inoculation of plates, slants and tubes as well.

Antibiotics

Gentamicin, Chloramphenicol, Nystatin.

Solvents

Methanol (Merck,Germany), Dimethyl sulphoxide (DMSO) (May & baker, England), Distilled Water (Laboratory grade).

Equipment

Test tubes, petri -dishes, pipette, measuring cylinder, flat bottom flask, burnsen burner, autoclave, refrigerator, cotton wool, Weighing balance, foil, wire loop, masking tape and

Collection of plant materials

The plants of *Crateva adansonii* and *Cryptolepis sanguinolenta* were collected from Orba in Udenu Local Government Area of Enugu State and identified by Mr. Alfred Ozioko, retired botanist, University of Nigeria, Nsukka.

Extraction of plant materials

200 mg each of ground leaves of *Crateva adansonii* and *Cryptolepis sanguinolenta* were extracted by cold merceration in 500 ml of methanol overnight; the content was filtered to get the extract. The extracts were poured into well labeled metal plates and left to dry completelyfor 24 hrs.

Innoculum preparation

The micro-organisms used were collected from the Department of Pharmaceutics and Pharmaceutical Microbiology University of Nigeria, Nsukka. A single colony of microbial growth in agar plate was sub-cultured into 5 ml of sterile nutrient broth in a test tube and
incubated at 37^oC for 24hrs, while fungal suspensions were left at room temperature for 48 hrs.

Preliminary sensitivity test

Antibacterial activities

50 mg of each of the extracts was dissolved with 1 ml of DMSO and 1 ml of each extracts was introduced in already prepared molten nutrient agar in a bottle, mixed and asceptically poured into Petri dish and allowed to gel. The plates were now ready for inoculation of the organisms. Each of the plate was divided into six according to the number of the organisms used. The organisms were asceptically inoculated and incubated for 24 hrs at 37^o C.

Antifungal activities

50 mg of each of the extracts was dissolved with 1 ml of DMSO and a 1 ml volume of each extracts were pipetted and was poured in a sterile Petri dish. Each of the extract was poured in already prepared molten SDA in a bottle, mixed thoroughly and poured in petri dish and allowed to gel. The plates were now ready for inoculation of the organisms. Each of the plate was divided into 2 according to the number of the organisms used. The organisms were asceptically inoculated and incubated for 4 days (96 hrs) at 28^0C

Determination of MIC for bacteria by agar dilution method

50 mg of each of the extracts was dissolved with 1 ml of DMSO and 1 ml volume of each of the extract was pipette and poured in petri dishes. Two folds serial dilution was done. Four sterile tubes for each of the extracts containing 1 ml of sterile water

were placed on test tube rack. One ml of the extract was pipetted into the first tube and shaken vigorously after which the same quantity was transferred into subsequent tubes up to the 4th tube where one ml was taken and discarded. Each of the serial dilutions of the extracts were poured asceptically in different bottle containing molten nutrient agar, shaken thoroughly and poured in well labeled petri dishes and allowed to solidify. The plates containing different concentrations (25 mg/ml, 12 mg/ml, 6.25 mg/ml, 3.125 mg/ml) of each of the extracts were divided into six according to the number of organisms used. The organisms were inoculated asceptically and incubated at 37 ^{o}C for 24 hrs.

Determination of MIC for fungi by agar dilution method

50 mg of each of the extracts was dissolved with 1 ml of DMSO and 1 ml volume of each of the extract was pipette and poured in petri dishes. Two folds serial dilution was done. Four sterile tubes for each of the extracts containing 1 ml of sterile water were placed on test tube rack. One ml of the extract was pipetted into the first tube and shaken vigorously after which the same quantity was transferred into subsequent tubes up to the 4th tube where one ml was taken and discarded.

Each of the serial dilutions of the extracts were poured asceptically in different bottle containing SDA, shaken thoroughly and poured in well labeled petri dishes and allowed to solidify.

The plates containing different concentrations (25 mg/ml, 12.5 mg/ml, 6.25 mg/ml, 3.125 mg/ml) of each of the extracts were divided into two according to the number of organisms used. The organisms were inoculated asceptically and incubated at 28^{0} C for 4 days.

Interaction Studies

Combined activity of the two extracts against the selected bacteria and fungi by pour plate method

MICs of the separate solutions of the two extracts (Leaves of *Cryptolepis sanguinoleta* and leaves of *Crateva adansonii)* were determined. Using the MICs the extracts (Leaves of *Cryptolepis sanguinolenta* and leaves of *Crateva adansonii*) were mixed in the following ratios 1:1, 2:1 and 1:2 respectively. The ratios of the extracts were asceptically poured in petri dishes containing prepared molten nutrient agar and mixed thoroughly and allowed to gel. Each of the plate was divided into three according to the number of the selected organisms. The organisms were asceptically inoculated and incubated at 37 ^{o}C

RESULTS AND DISCUSSIONS

Preliminary sensitivity test result

The preliminary sensitivity test result, shows that the methanol extract of leaves of *Crateva adansonii* has activity against *Bacillus subtilis, E. coli*, *Klebsiella pneumonia* and *Aspergillus niger* while the methanol extract of leaves of *Cryptolepis sanguinolenta* showed activity against *Bacillus subtilis, Staph. aureus and Aspergillus niger* (Table-1).

Table 1: Susceptibility of the organisms to the extracts of *C.adansonii* and *C. sanguinolenta.*

ORGANISMS	*Crateva adansonii*	*Cryptolepis sanguinolenta*
Bacillus Subtilis	-	-
Staphylococcus aureus	+	-
Escherichia coli	-	+
Pseudomonas aeruginosa	+	+++
Klebsiella Pneumoniae	-	+
Salmonella typhi	+++	+++
Candida albicans	+++	+
Aspergillus niger	-	-

Key: - = Complete inhibition, + = Slight growth, +++ = Heavy growth.

Table 2: The MIC of methanol extract of *Crateva adansonii* against the six bacteria used.

ORGANISMS	25 mg/ml	12.5 mglml	6.25 mglml	3.125 mglml
Pseudomonas aeruginosa	-	-	+	+
Escherichia coli	-	-	-	+
Salmonella typhi	-	-	+	+
Staphylococcus aureus	-	-	+	+.
Klebsiella pneumoniae	-	-	-	+
Bacillus subtilis	-	-	+	+

Key: + = Growth, - = Inhibition.

MICs of methanol extract of *Crateva adansonii* against the six bacteria.

Pseudomonas aeruginosa	-	12.5 mg/ml
Escherichia coli	-	6.25 mg ml
Salmonella typhi	-	12.5 mg/ml
Staphylococcus aureus	-	12.5 mg/ml
Klebsiella pneumonia	-	6.25 mg/ml
Bacillus subtilis	-	12.5 mg/ml

The result of the methanolic extract of leaves of *Crateva adansonii* against *Pseudomonas aeruginosa*, *E. coli, Salmonella typhi, Staph. Aureus, Klebsiella pneumonia* and *Bacillus subtilis* at different concentrations showed activity against all the bacteria at the concentration of 25 mg/mI and 12.5 mg/mI while at the concentration of 6.25 mg/ml, only *E.coli* and *Klebsiella pneumonia* were inhibited. At the concentration of 3.125 mg/ml, all tested bacteria were not sensitive (Table-2).

Table 3 : MIC of methanolic extract of *C. adansonii against* two fungi.

ORGANISMS	25 mg/ml	12.5 mglml	6.25 mglml	3.125 mglml
Aspergillus niger	-	-	+	+
Candida albicans	-	-	+	+

Key: + Growth, - = Inhibition.

MICs of methanolic extract of *C. adansonii against* two fungi.

Aspergillus niger	-	12.5 mg/ml
Candida albicans	-	12.5 mg/ml

The result of the methanolic extract of leaves of Crateva adansonii against *Aspergillus niger* and *Candida albicans* at different concentrations showed that the two organisms were

sensitive at 25 mg/mI and 12.5mg/mI concentrations but resistant at 6.25 mg/mI and 3.125 mg/ml (Table 3).

Table 4: MIC of methanolic extract of *C. sanguinolenta* against the six bacteria used.

ORGANISMS	25 mg/ml	12.5 mglml	6.25 mglml	3.125mglml
Pseudomonas aeruginosa	-	-	+	+
Escherichia coli	-	-	-	+
Salmonella typhi	-	-	+	+
Staphylococcus aureus	-	-	+	+
Klebsiella pneumonia	-	-	+	+
Bacillus subtilis	-	-	-	+

Key: + = Growth, - = Inhibition

MICs of methanol extract of *Cryptolepis sanguinolenta* against the six bacteria.

Pseudomonas aeruginosa	-	12.5 mg/ml
Escherichia coli	-	6.25 mg ml
Salmonella typhi	-	12.5 mg/ml
Staphylococcus aureus	-	12.5 mg/ml
Klebsiella pneumonia	-	12.5 mg/ml
Bacillus subtilis	-	6.25 mg/ml

The result of the methanolic extract of leaves of *Cryptolepis sanguinolenta* against *Pseudomonas aeruginosa*, *E. coli*, *Salmonella typhi*, *Staph aureus*, *Klebsiella pneumonia and Bacillus subtilis* at different concentrations showed activity against all the bacteria at the concentrations of 25 mg/mI and 12.5 mg/ml while at the concentration 6.25 mg/mI, only *E.coli* and *s* showed activity. At the concentration of 3.125 mg/ml all bacteria were resistant (Table 4).

Table 5: MIC of methanolic extract of *C. sanguinolenta* against *A. niger* and *C.albicans*.

	25 mg/ml	12.5 mglml	6.25 mglml	3.125 mglml
Aspergillus niger	-	-	+	+
Candida albicans	-	-	-	+

Key: + = Growth, - = Inhibition.

MIC of methanolic extract of *C. sanguinolenta* against *A. Niger* and *C.albicans*

Aspergillus niger	-	12.5 mg/ml
Candida albicans	-	6.25 mg/ml

The result of the methanolic extract of leaves of *Cryptolepis sanguinolenta* against *Aspergillus niger* and *Candida albicans* at different concentrations showed that the two organisms were sensitive at 25 mg/mI and 12.5 mg/mI concentrations. At 6.25 mg/mI, only *Candida albicans* was sensitive and both were resistant at 3.125 mg/ml concentration (Table 5).

COMBINED ACTIVITY OF THE TWO EXTRACTS AGAINST SELECTED ORGANISM

Table 6, is the result of the combined activity of the two extracts (leaves of *Crateva adansonii* and leaves of *Cryptolepis sanguinolenta* against *E. coli, Klebsiella pneumonia* and *Bacillus subtilis* at the ratio of 1.1 showed that all organisms tested were resistant (Okore, 2005).

Table 6: Sensitivity of some of the bacteria to combined extract at equal concentrations.

Concentration (6.25 mg/mI : 6.25 mg/ml)	Organisms	Result
Ratio A : B 1 : 1	*Escherichia coli*	+
	Klebsiella Pneumoniae	+
	Bacillus subtilis	+

Key: Plant A –*Crateva adansonii*,Plant B – *Cryptolepis sanguinolenta*, + = Growth.

Table 7: Sensitivity of some to combacteriabined extract at 1:2 combination.

Concentration (6.25mg/mI : 12.5mg/ml) Ratio A : B 1 : 2	Organisms	Result
	Escherichia coli	-
	Klebsiella Pneumoniae	+
	Bacillus subtilis	+

Key: Plant A - *Crateva adansoii*, Plat B - *Cryptolepis sanguinolenta* + = Growth, - = inhibition.

The result of the combined activity of the two extract (leaves of *Crateva adansonii* and leaves and root of *Cryptolepis sanguinolenta* against selected bacteria ie *E. coli Klebsiella pneumonia* and *Bacillus subtilis* at the ratio of 1:2 showed that out of three organisms tested, only *E.coli* was sensitive (Table 7).

Table 8: Sensitivity of some bacteria to combined extract at 2:1 combination.

Concentration (12.5mg/ml : 6.25mg/ml) A : B 2 : 1	Ratio	Organisms	Result
		Escherichia coli	-
		Klebsiella Pneumoniae	+
		Bacillus subtilis	+

Key: Plant A - *Crateva adansonii* extract. Plant B - *Crypotolepis sanguinolenta* extract. + = Growth, - = Inhibition.

Table 8, which is the result of the combined activity of the two extract (leaves of *Crateva adansonii* and leaves and root of *Cryptolepis saniguinolenta* against selected bacteria ie. *E. coli Klebsiella pneumonia* and *Bacillus subtilis* at the ratio of 2.1 showed that, out of the three organisms tested, only *E. coli* was sensitive.

Table 9: Sensitivity of *C. albicans* against combined extract at equal concentration.

Concentration (6.25mg/ml : 6.25mg/ml) A: B 1:1	Ratio	Organism	Result
		Candida albicans	+

Key: Plant A –*Crateva adansonii*, Plant B –*Cryptolepis sanguinolenta*, += Growth, - = Inhibition.

The result of the combined activity of the two extract (leaves of *Crateva adansonii* and leaves of *Cryptolepis sanguinolenta*) against *Candida albicans* at the ratio of 1:1 showed that the organisms was resistant.

Table 10: Sensitivity of *C.albicans* to combined extract at 1:2 combination.

Concentration		Organism	Result
(6.25mg/ml : 12.5mg/ml)	Ratio A: B 1: 2	*Candida albicans*	-

Key: Plant A –*Crateva adansonii*, Plant B –*Cryptolepis sanguinolenta*, + = Growth - = Inhibition.

The result of the combined activity of the two extract (leaves of *Crateva adansonii* and leaves *Cryptolepis sanguinolenta*) against *Candida albicans* at the ratio of 1:2 showed that the organisms was sensitive (Costanzo *et al*.,2005).

Table 11: Sensitivity of *C.albicans* to combined extract at 2:1 combination.

Concentration	**Organism**	**Result**
(12.5mg/ml : 6.25mg/ml)	*Candida albicans*	-
Ratio A: B 2:1		

Key: Plant A –*Crateva adansonii*, Plant B –*Cryptolepis sanguinolenta*, + = Growth = Inhibition

The result of the combined activity of the two extracts (leaves of *Crateva adansonii* and leaves of *Cryptolepis sanguinolenta)* against *Candida albicans* at the ratio of 2:1 showed that the *Candida albicans* were sensitive to the combination of the two plants.

CONCLUSION

The results show that the extracts of the leaves of the two plants have bacteriocidal and fungicidal activities individually. The interaction studies, shows that at the combination ratios 2:1 and 1:2 of the extracts were either additive or synergistic inhibiting the bacteria and the fungi at these conbination ratios, while at combination ratio 1:1 the interaction was antagonistic as there were no inhibition of both bacteria and fungi. It is better for the extract of *Cryptolepis sanguinolenta* and *Crateva adansonii* to be used alone in the treatment of bacteria, instead of the combination using higher concentrations. The information gathered in this work in terms of antimicrobial activities serve as nucleus for further investigation and that the combination interactions can be considered in the treatment of *Candida albicans.*

RECOMMENDATION

This extracts of *Cryptolepis sanguinolenta* and C*rateva adansonii* used in this work has been critically examined and has been found to be bacteriocidal and fungicidal. The extract of *Cryptolepis sanguinolenta* has been shown to be a good anti-candida, it is thereby recommended in the treatment of *Candida albicans*.

APPRECIATION

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CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest in execution of this research work.

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