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SYNERGETIC EFFECT OF THE LEAF EXTRACTS OF *FICUS CAPENSIS* (LINN) AND *SORGHUM BICOLOR* (LINN) MOENCH AGAINST SOME HUMAN BACTERIAL PATHOGENS

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ABSTRACT
The cold extraction method was used to obtain the methanol extract of the leaf of *Ficus capensis* and *Sorghum bicolor*. The extracts were analyzed *in-vitro* for antibacterial activities against some clinical pathogenic bacteria namely: *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Staphylococcus aureus, Serratia marcescens, Pseudomonas aeruginosa, Micrococcus roseus* and *Bacillus cereus.* The leaf extract of *Ficus capensis* had inhibitory effect on all the test organisms except *Pseudomonas aeruginosa,*while the extract of *Sorghum bicolor* had no inhibitory effect on all the test organisms. However, the synergistic bioassay of *Ficus capensis* and *Sorghum bicolor* leaf extracts revealed that the growth of the tested organisms was inhibited at a concentration of 25.0 mg/ml with the highest zone of inhibition observed for *Proteus mirabilis* and *Staphylococcus aureus* (15.0 mm), while *Bacillus cereus* showed least inhibition. The result of the phytochemical screening tests revealed that the extracts contain saponin, tannins, alkaloids and cardiac glycoside. The number of organisms decreased with increased time of exposure to the extract showing the rate of killing of the extracts. Minimum inhibitory concentration of the leaf extract ranged from 3.12 to 12.5 mg/ml. The result of the antibiotic sensitivity test compared well with the commercial antibiotics used. It can be concluded that these plants can be used to discover natural products that may serve as lead for the development of new pharmaceuticals addressing the major therapeutic needs.
Key words: *In-vitro,* antibacterial, extracts, zone of inhibition, phytochemical screening and rate of killing.

INTRODUCTION

All human societies have medical beliefs that provide explanations for birth, death and disease. Throughout history, illness has been attributed to witch craft, demons, adverse astral influence or the will of gods (Crabben, 2011). One of the preventive and curative means for the illness include the use of medicinal plants which areplant defined by world health organization (W.H.O., 2003) consultative group as any plant in which one or more of its organs contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs.

Medicinal plants are the backbone of traditional medicine as majority of the world's population uses plant products as the primary source of medicine (Nunn, 2002). Many of the herbs and spices used by human to season food yield useful medicinal compounds and many familiar medications of the twentieth century were developed from ancient healing traditions that treated health problems with specific plants (Fabricant and Farnsworth, 2001). Today science has isolated the medicinal properties of a large number of botanicals, and their healing components have been extracted, analyzed and synthesized for use in pharmaceuticals preparations (Charles and Ramani, 2011).

Herbal medicine is the oldest form of health care known to mankind. Herbs had been used by all cultures throughout history (Solecki *et al.*, 1975). It was an integral part of the development of modern civilization. The plants provided food, clothing, shelter and medicine. Much of the medicinal use of plants seems to have

been developed through observations of wild animal and by trial and error. As time went on, each tribe added the medicinal power of herbs in their area to its knowledgebase. They methodically collected information on herbs and developed well-defined herbal pharmacopeias. Indeed, well into the 20th century much of the pharmacopeia of scientific medicine as derived from herbal lore of native peoples (O'Neill, 1993). Many drugs commonly used today are of herbal origin. Undisputedly, the history of herbs is inextricably intertwined with that of modern medicine. Many, drugs listed as convectional medications were originally derived from plants (Barney, 1996). Salicylic acid, a precursor of aspirin, was originally derived from white willow bark and meadowsweet plant. Cinchona bark is the source of malaria-fighting quinine. Vincristine, used to treat certain types of cancer, comes from periwinkle. The opium poppy yields morphine, codeine, and paregoric, a treatment for diarrhea laudanum, a tincture of the opium poppy, was the favoured tranquilizer in Victorian times. Once scientific methods were developed to extract and synthesize the active ingredient in plants, pharmaceutical laboratories took over from providers of medicinal herbs as the producers of the modern day drugs. The use of herbs, which for most of history had been mainstream medical practice, began to be considered unscientific, or at least unconventional, and fell into relative obscurity. The World health organization estimates that 4 billion people, 80% of the world population, presently use herbal medicine for some aspect of primary health care. Herbal medicine is a major component in all indigenous traditional medicine and a common element in ayurvedic, homeopathic, naturopathic, traditional oriental and Native American Indian medicine.

Rather than using a whole plant, pharmacologists identify, isolate, extract and synthesize individual components thus capturing the active properties. In addition to active ingredients, plants contain minerals, vitamins, volatile oils, glycosides, alkaloids, bioflavonoids and other substances that are important in supporting a particular herb's medicinal properties (Kordali *et al.,* 2003).

Ficus capensis (Linn) is an evergreen tree that belongs to the family Monoaceae. It is widely distributed in Africa and referred to as the African mustard tree or the fig tree. The plants have been used extensively in traditional medicine for the treatment of a variety of diseases. The leaves, stem, roots and fruits are used in traditional medicine for the treatment of dysentery, odema, leprosy, epilepsy, rickets, infants (Joshua, 2006) and in treatment of astringent, infertility, increase lactation, respiratory disorders and gonorrhea (Sofowora, 1982). However, *Sorghum bicolor* (Linn) Moench is an important summer season crop that belongs to the family Poaceae grown for fodder and grain purpose. The leaves, whole plant and grains are used in traditional medicine as blood tonic and in the treatment of Malaria and Fever (Sofowora, 1982).

This work is intended to assay for the phytochemical properties and synergetic effect of the leaf extracts of *F. capensis* and *S. bicolor* on some pathogenic bacteria with a view to verifying their traditional use as medicinal plants and to examine whether their action on selected bacteria are cidal or static.

MATERIALS AND METHODS

Collection and preparation of leaf samples

The plant leaves of *Sorghum bicolor* (Linn) Moench were purchased on January 13, 2010 from Kings Market in Akure, Ondo State, Nigeria while leaves of *Ficus capensis* (Linn) were collected from the Forest and Wild life Reserve of the Federal University of Technology, Akure, Nigeria, where they were found growing naturally. They were identified by Professor Akinyele at the Department of Crop, Soil and Pest Management of the Federal University of Technology, Akure, Nigeria. Voucher specimens were submitted at the Departmental herbarium.

Collection and identification of test organisms

The test organisms were collected from, Ondo State Hospital, Akure, Nigeria. The bacterial isolates used for this research work included: *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Staphylococcus aureus, Serratia marcescens, Pseudomonas aeruginosa, Micrococcus roseus* and *Bacillus cereus.* The identities of the bacterial isolates were confirmed according to the method of Olutiola *et al.* (2000).

Standardization of the test organisms

A loop-ful of test organism was inoculated into nutrient broth and incubated for 24 h. Exactly 0.2 ml from the 24 h culture of the

organisms was dispensed into 20 ml sterile nutrient broth and incubated for 3 to 5h to standardize the culture to 0.5 McFarland standards (10^6cfu/ml) before use according to the method of Oyeleke *et al.* (2008).

Extraction of the plant samples

The leaves were air dried for four weeks and crushed into powder. Exactly 600 g of the ground leaves of *F. capensis*, *S. bicolor* and mixture of 60% *F. capensis* and 40% *S. bicolor* was soaked in absolute methanol for 72 h, after which it was first sieved with a muslin cloth, and then filtered using No 1 Whatman filter paper. The filtrate was collected in a beaker and dried *invacuo* using rotary evaporator (Resona, Germany).

Phytochemical screening

The pytochemical screening was done according to the method described by Trease and Evans (1996).

Determination of antibacterial activities of leaf extracts of *Ficus capensis* and *Sorghum bicolor*

The antibacterial activity of the plant extracts was assayed using agar well diffusion method as described by Olutiola *et al.* (2000). The concentration of the extract used was 25 mg/ml (2.5 g in 100 ml of 30 % Dimethyl Suphoxide). The plates were incubated at 37°C for 24 h. Clear zones around the bored holes are indicative of the inhibition of the organisms by the extract.

Determination of minimum inhibitory concentration of leaf extracts of *Ficus capensis* and *Sorghum bicolor*

Four concentrations (12.5, 6.25, 3.12 and 1.56 mg/ml) of the methanol extract of 60% *F. capensis* and 40% *S. bicolor* leaf were assayed using the method of Doughari *et al.*(2007). The plates were incubated for 24 h at 37°C. Concentration of the crude extract below in which there was no inhibition was recorded as the minimum inhibitory concentration (MIC).

Antibiotics sensitivity test

The disc diffusion method described by (Khan *et al.*,2002) was used to determine the sensitivity of the test organisms to commercial antibiotics.

Determination of the rate of killing of organisms by the extract

A 5 ml of 25 mg/ml of the methanol extract and 5 ml of the standard culture was added together in a sterile test tube. The solution was allowed to stand for 24 h, for interaction between the organism and the extract. At intervals of one hour, 1 ml of the mixture was pour plated using nutrient agar. The microbial load was determined, after incubation at 37°C for 24 h (Ogundare and Akinyemi, 2011).

Table 1: Antibacterial activity of the methanol extracts of *Ficus capensis, Sorghum bicolor* and combination of 60% *Ficus capensis* and 40% *Sorghum bicolor*

Organisms	Zone of inhibition (mm) at the concentration of 25.0 mg/ml		
	FCE	SBE	SFE
Escherichia coli	5.0	Nil	13.0
Klebsiella pneumoniae	1.5	Nil	9.0
Proteus mirabilis	6.0	Nil	15.0
Staphylococcus aureus	4.5	Nil	15.0
Serratia marcescens	3.8	Nil	13.0
Pseudomonas aeruginosa	Nil	Nil	Nil
Micrococcus roseus	4.0	Nil	12.0
Bacillus cereus	1.0	Nil	7.0

Key: FCE- *Ficuscapensis* Extract, SBE-*Sorghum bicolor* Extract and SFE- 60% *Ficus capensis* and 40% *Sorghum bicolor*

Table 2: Phytochemical groups in methanol leaf extract of *Ficus capensis* and *Sorghum bicolor*

Phytochemical groups	*Ficus capensis*	*Sorghum bicolor*
Saponin	+ve	-ve
Tanins	+ve	+ve
Phleobatanin	-ve	-ve
Alkaloids	+ve	-ve
Anthraquinone	-ve	+ve
Cardiac glycoside		
Legals Test	+ve	-ve
Salkowski Test	+ve	+ve
Keller Killian Test	+ve	+ve
Liebermans Test	+ve	-ve

Table 3: MIC of the methanol extract of the combination of 60% *Ficus capensis* and 40% *Sorghum bicolor*

Organism	Concentration of Extract (mg/ml)
E. coli	3.12 mg/ml
K. pnuemoniae	6.25 mg/ml
Proteus mirabilis	3.12 mg/ml
S. aureus	3.12 mg/ml
Serratia marcescens	6.25 mg/ml
Pseudomonas aeruginosa	-
Bacillus cereus	12.5 mg/ml
Micrococcus roseus	6.25 mg/ml

Table 4: Antibiotics sensitivity test on bacterial isolates

	Zones of Inhibition (mm)									
Gram positive organisms	AMX	OFL	STR	CHL	CEF	GEN	PEF	COT	CPX	ERY
S. aureus	-	18.0	-	-	-	-	-	-	30.0	-
Bacillus cereus	-	17.0	7.5	7.5	-	8.0	-	-	-	-
Micrococcus roseus	-	7.0	6.0	15.0	-	5.0	7.0	-	16.0	7.0
Gram negative organisms	AUG	CRO	NIT	GEN	COT	OFL	AMX	CPX	TET	PFX
K. pnuemoniae	-	-	-	7.0	8.0	-	-	17.0	-	-
E. coli	-	-	-	-	-	-	12.0	-	18.0	-
Pseudomonas aeruginosa	10.0	-	-	10.0	9.0	-	-	-	-	-
Serratia marcescens	7.0	9.0	-	6.0	7.0	7.5	-	-	-	8.0
Proteus mirabilis	-	18.0	13.0	-	20.0	-	-	-	10.0	23.0

Key: AMX-Amoxycillin; AUG- Augmentin; CRO- Ceftriazone; OFL- Oflaxacin; PEF- Pefloxacin; CPX-Ciproflaxin; GEN-Gentamycin; PEN-Penicillin; STR-Streptomycin; TET-Tetracycline; AMP-Ampicillin; ERY- Erythromycin; CHL-Chloramphenicol; NAL-Nalidixic acid; NIT-Nitrofurantoin;; COT-Cotrimazole.

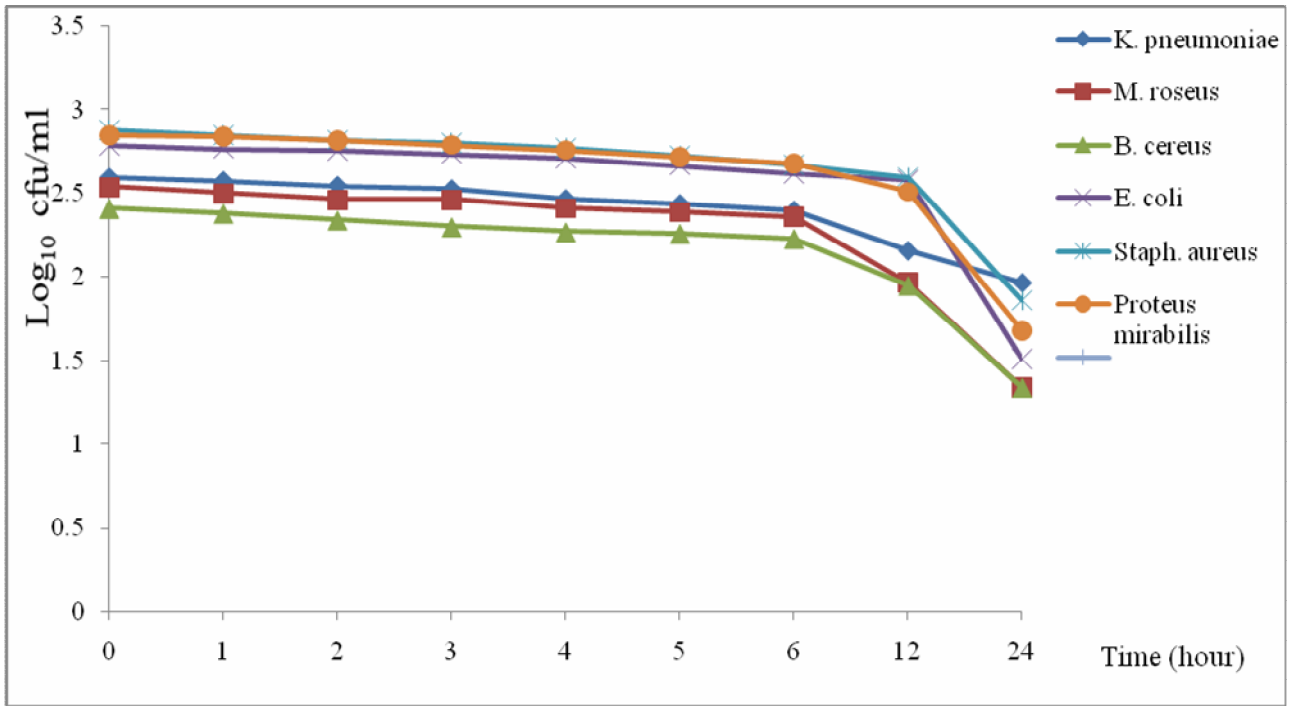

Figure 1: Rate of killing of some bacterial isolate by the synergy

RESULTS AND DISUSSION

Ficus capensis leaves are extensively used in the treatment of dysentery, odema, leprosy, epilepsy, rickets, emollient, astringent, infertility, respiratory disorders, gonorrhea and to increase lactation while *Sorghum bicolor* leaves are used to treat malaria, fever and used as blood tonic (Odugbemi, 2006). Result from this work shows the use of *F. capensis* as a more effective antimicrobial agent in combination with *Sorghum bicolor*. Table 1 shows the antibacterial activity of the methanol extract of *F. capensis* and *S. bicolor* with the combination of 60% *F. capensis* and 40% *S. bicolor*. The methanol extracts of *F. capensis* inhibited the entire test organism except *Pseudomonas aeruginosa*. The highest zone of inhibition of the synergy was recorded with *Proteus mirabilis* and *Staphylococcus aureus* with inhibition value of 15.0 mm each, hence its traditional use in the treatment of infections caused by the test organisms. This was followed by *Escherichia coli* and *Serratia marcescens* with zone of inhibition values of 13.0mm. In the case of the methanol extract of *S. bicolor* against the test organisms, there was no inhibition, which means none of the test organisms was susceptible to the methanol extract of *Sorghum bicolor*.

However, the synergistic effect of *F. capensis* and *S. bicolor* inhibited the growth of both gram positive and gram negative organism used for this research. This is indicative that the methanol extracts of the leaves showed broad spectrum activity. The high susceptibility of these organisms is a clear indication of the effect of the leaf extract as a good treatment of bacterial infections caused by *E. coli*, *P. mirabilis* and *S. aureus* confirming the traditional use of the synergy in the treatment of dysentery, intestinal and respiratory disorders and as blood tonic. *Pseudomonas aeruginosa* was not susceptible to the extract used separately and in combination. However, increase in concentration of the extracts and purification of the extract, may give high zone of inhibition on the test organism than the crude extracts.

Antimicrobial activities in plants have been reported to be as a result of phytochemicals present in the plants and that these phyto-constituents are biologically active and therefore aid antimicrobial activities (Igbinosa *et al.*,2009). Table 2 shows the result of the phytochemical test indicating the presence of various bioactive

components in the extract which are most likely responsible for the broad spectrum inhibitory effects of the extracts. The minimum inhibitory concentration (MIC) tests of the leaf extract on the test organism as shown on Table 3 shows that the minimum inhibitory concentration of the methanol extract of the synergy is at 3.12 mg/ml. The low MIC values show that the synergy has a strong antibacterial effect on the test organisms. The result of the commercial antibiotics on both gram positive and gram negative bacteria compares well with that of the crude extract used in the study (Table 4). However, the low zone of inhibition recorded with some of the test organisms, to test antibiotics may be due to ready availability of commercial antibiotics from diverse sources such as pharmacies, patent medicine stores and road side stalls which make any control of the use of antibiotics ineffective because of lack of legislation to enforce their usage (Ganguly *et al.*, 2001).This may also be as a result of drug misuse, drug abuse and self-medication which have led to high level of microbial resistance (Martino *et al.*, 2002). The high inhibition values recorded by the antibiotics than the plant extracts may be due to the purity level of commercial antibiotics, as reported by Doughari *et al.*, (2007) that antibiotics are in a refined state while plant extract are still in crude state. The effectiveness of the antibiotics may also be due to their molecular size that aids their solubility in diluents (Mailard, 2002).The result of the rate of killing of the organism by the synergy (Figures 1) shows that the numbers of organisms present at each hour declined till the 24th hour. The effect of the extract on the test organisms can be considered to be of a broad spectrum activity because both gram positive and gram negative bacteria were inhibited.It is believed that if the extract is further purified, stronger inhibitory results will be achieved as this research was conducted on crude extract and was reported by Lenta *et al.* (2007) that crude extract are liable to contamination and deterioration which reduces their antibacterial activities. The structure of the active phytochemical components can also be determined and an assayed, for its toxicological analysis will assess its safety and level of tolerance in human body.

The availability and accessibility to plants' part makes the use of *F. capensis* and *S. bicolor* a cost effective alternative medicine in the treatment of infectious caused by the test organisms. Herbal medicine has proven to be the basis of modern medicine, so therefore plants should be exploited scientifically to its fullest in other to answer the lingering human health problems and to reserve them from going into extinction.

This work has been able to show that extract produced from the dry leaves of *F.capensis* and *S. bicolor* has antibacterial activity against the test bacteria used. In other to obtain greater antibacterial activity, the leaf extracts can be further purified and the active component (s) in the fraction identified.

REFERENCES

Barney, D. P. (1996).Clinical Applications of Herbal Medicine.4th Edition. Woodland Publishing, p. 45.

Charles A. and Alex Ramani V. (2011).Phytochemical screening and antimicrobial resistance of *Alseodaphne semecarpifolianees*.Journal of Chemical and Pharmaceutical Research.3 (5): 205-211

Doughari, J., Pukuma, M. and De, N. (2007).Antibacterial effects of *Balanites aegyptiaca* L. Drel.and *Moringa oleifera* Lam. on *Salmonella typhi*, African Journal of Biotechnology 6 (19): 2212-2215

Fabricant, D. S and Farnsworth N. R. (2001)."The value of plants used in traditional medicine for drug discovery". Environmental Health Perspective 109 (1): 69–75

Ganguly, R., Mishra, P. and Sharma, A. (2001). Sensitivity of *Salmonella typhi* isolated from patients in Roorkee to antibiotics and two Unani drugs. Indian Journal of Microbiology 41: 211 – 213.

Igbinosa, O. O., Igbinosa, E. O. and Aiyegoro, O. A. (2009). Antimicrobial activity and phytochemical screening of stem bark extracts from *Jatropha curcas* (Linn), African Journal of Pharmacy and Pharmacology 3(2):058-062.

Jan van der Crabben, (2011). Ancient History Encyclopedia.

Joshua, K .(2006). Conservation of Indigenous Medicinal Botanicals in Ekiti State.Nigeria Research Paper.

Khan, M. R., Kihara, M. and Omotosho A. D. (2002).Antibacterial and antifungal activities of *Barrington asiatica.*Fitoterapia 5: 255-260.

Kordali, S., Cakir, A. and Drum, M. E (2003).Antifungal activity of the leave of three *Pistacies* from Turkey.Fitoterapia74:164-167.

Lenta, B., Wniger, B., Antheaume, C., Noungoue, D., Ngroueta, S., Assob, C., Senecheau, V., Fokou, A., Devkota, P., Tsamo, E. and Sewald, N. (2007).Anthraquinones from the stem bark of *Strereospernum* Zenkeri with antimicrobial activity. Phytochemistry 11(68): 1595-1599.

Mailard, J. Y., (2002). Bacterial target sites for biocide action. Journal of Applied Microbiology 9:16-27.

Martino, P. D. and Gagniere, H., Berry, H. and Bret, L. (2002). Antibiotics resistance and virulence properties of *P. aeruginosa* strains from mechanical ventilated patients with pneumonia in intensive care units; comparison with imipenem – resistant extrarespiratory tract isolates from uninfected patients. Journal of Microbes and Infecion 4: 613 – 620.

Nunn, John F. (2002). Ancient Egyptian Medicine.University of Oklahoma Press.

O'Neill, M.J., and Levis, J.A., (1993). In Human medicinal agents from Plants.(Editors Kinghorn, A.D., and Balandrin, M.), ACS, Symposium series, 534: 48-55.

Odugbemi, T (2006*).* Outline of Medicinal Plants in Nigeria.1st Edition, University of Lagos Press, Nigeria, p. 77.

Ogundare, A. O and Akinyemi, A. I. (2011). Phytochemical and antibacterial properties of *Combretum mucronatum* (Schumach) leaf extract. African Journal of Microbiology Research 5(18), 2632-2637

Olutiola, P. O., Famurewa, O. and Sontag, H. G.(2000).An Introduction to General Microbiology: A Practical Approach.2nd Edition.HeidebergerVerlagsanstait and DruckereiGmb. Heldelberg Publisher. Pp. 35-66.

Oyeleke, S. B., Dauda, B. E. N. and Boye, O. A. (2008).Antibacterial activity of *Ficus capensis.* African Journal of Biotechnology 7 (10): 1414-1417

Sofowora, A. (1982). Medicinal Plants and Traditional Medicines in Africa. 6th Edition.Chicester John Willey and Sons, New York. p. 150.

Solecki, R., and Shanidar, I.V., (1975**).** A Neanderthal flower burial in northern Iraq. Science 190: 880 - 881.

Trease, G. E. and Evans W. C. (1996). Pharmacognosy.15th Edition, London J & A Churchhill Ltd. London. pp.234-492.

World Health Organization, (2003). Fact sheet no. 134, revised May, 2003 - Traditional Medicine