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Synergistic Effect of *Acacia senegalensis* and *Kigelia africana* on Vaginal *Candida albicans*

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Abstract

Background: Plant extracts with medicinal values have been used to treat many diseases that can be bacterial, fungal or parasitic among many others. Plants with medicinal value produce certain chemical element known as phytochemicals that have antimicrobial activity. These plants extracts are considered potential source of new antimicrobial agents, serving as alternatives to conventional drugs that the microorganisms have develop resistance to *Candida albicans* is yeast that is mainly found in the mucosal cavity of the vagina and the intestinal tract as a normal microbiota but it can cause systematic infection in immunocompromised individuals.

Objective: This study was aimed at determining the synergistic effect of *Acacia senegalensis* and *Kigelia africana* leaves extracts against *Candida albicans* isolated from the vagina.

Methods: *Candida* spp were isolated from the vagina and *Candida albicans* isolates were obtained after identification using Germ tube test. The synergistic effect of the plants was evaluated using agar well diffusion method and the minimum inhibitory concentration was determined using double fold dilution method.

Results: The results displayed *A. senegalensis* as the most active against *Candida albicans,* having the highest zone of inhibition 13 mm and 7 mm for ethanolic and aqueous extracts respectively at 200 mg/ml concentration and 10 mm for ethanolic extract of *A. senegalensis* at 100 mg/ml concentration, followed by synergy of ethanolic extract of *A. senegalensis* and *K. africana* which has the zone of inhibition of 7 mm for aqueous and ethanolic at 200 mg/ml.

Conclusion: The results suggest that individual plant extract has better activity on *Candida albicans* than a combination of the extracts hence *K. aficana* and *A. senegalensis* shows antagonistic effect rather than a synergistic activity on the *Candida albicans* isolate.

Keywords: Synergistic; *Kigelia africana*; *Acacia senegalensis*; *Candida albicans*; Antifungal; ethanolic and aqueous leaf extracts

Introduction

Fungi are eukaryotic organism that includes unicellular (like yeast) or very complex multicellular (like filamentous) microorganism, don't have chlorophyll (non-photosynthetic-heterotrophic) [1-6].

They have rigid cell walls which contain chitin-chitosan, and they have relationships with animals, some of them symbiotic, commensals or parasite in/on animals which cause infection (like *Candida albicans*), *Candida* is a type of yeast that is the reason for a number of undesirable symptoms, this yeast may be only those gut flora, an assembly of microorganisms that live-in mouth also digestive system [7].

The point that *C. albicans* populace begins getting crazy for control and its resistance to synthetic antifungal drugs has contributed to the search and use of natural based plants extracts to kill the microorganism [8].

Kigelia africana (syn. *Kigelia pinnata*, *Kigelia aethiopica*) is commonly referred to as sausage or cucumber tree because of its huge sausage or cucumber-like fruit. It belongs to the Bignoniaceae family and occurs throughout tropical Africa. It grows particularly well in wetter areas, spreading across the wet savannah and riverine areas [9].

Parts of the plant are used for treating a wide range of ailments traditionally based mainly on cultural practices [9]. The fruit is used to treat skin ailments like fungal infections, boils, psoriasis and eczema. Dysentery, ringworm, tapeworm, post-partum haemorrhage, malaria, diabetes and pneumonia are also treated with the fruit. The fruit is also applied for the treatment of solar keratosis and malignant melanoma [10].

The bark is used to treat venereal diseases while the root is applied to treat ulcer [9]. *Acacia senegalensis* also called *Sengalia senegal* is a small thorny deciduous tree from the genus *Senegalia*, which is known by several common names, including Gum acacia, Gum Arabic tree, Sudan gum and Sudan gum Arabic.

In parts of India, it is known as Kher or Khor [11]. It is native to semi-desert regions of Sub-Saharan Africa. It has been reportedly used for its astringent properties, to treat bleeding, bronchitis, cough, diarrhea, dysentery, catarrh, gonorrhea, leprosy, typhoid fever and upper respiratory tract infections [12].

Methods and Materials

Isolation of *Candida albicans*

High Vaginal swab specimens were collected by a gynecologist at antenatal clinic of Bauchi specialist hospital by rolling sterile cotton tipped swabs in the female vagina.

The samples were transported to Abubakar Tafawa Balewa University microbiology laboratory for processing. *Candida albicans* was isolated by aseptically streaking the swab stick containing the sample onto Potato Dextrose agar (PDA) containing dextrose that stimulate fungal growth and incubated at 37°C for 24 hours [13].

Plant sample collection: The leaves of *A. senegalensis* (dakwara) and *K. africana* (nonon giwa) were collected at Bauchi Local Government Area, Bauchi State of Nigeria and confirmed at Bauchi State Agricultural Development Programme (BSADP). The leaves with no flowers and no sign of damage were selected.

Preparation of plant extract: The leaves from the plants were washed with clean de-ionized water, spread on a clean sack and dried at room temperature. The dried leaves were grounded into coarse powder using a mortar and pestle [14,15].

The extraction was done by preparing a plant material to solvent ratio of 1:10 w/v that is 25 g of the prepared powder was weighed and soaked in 250 ml of water and another 25 g of the powder was weighed and soaked in ethanol [16]. The mixtures were kept at room temperature for 72 hours under continuous shaking. The crude extracts were filtered through glass funnel and the filtrates of the extracts were dried at 60°C in a water bath. The dried extracts was weighed, labeled and stored in a refrigerator [17].

Characterization and identification of the test organism: Characterization was made by initial examination of the colonies on the plate. Physiological examination for growth of pasty, creamy to white and smooth, glabrous colonies [18] was carried out which are the morphological growth characteristics of *Candida albicans* on Potato Dextrose Agar.

Biochemical test such as Germ tube test was used for presumptive identification of *C. albicans* by emulsifying a colony of the yeast in 1 ml of human serum in a small test tube and incubated at 37°C for 2-4 hours. *Candida albicans* presence was indicated by the presence of a short hyphal (filamentous) extension arising laterally from a yeast cell, with no constriction at the point of origin.

Preparation of stock and standard solution of aqueous and ethanolic extract of *A. senegalensis* and *K. africana*: 0.2 g each of *A. senegalensis* and *K. Africana* extracts was dissolved in 1 ml of 4% Dimethyl Sulphoxide (DMSO) to form a stock solution of 200 mg/ml. It was then serially diluted to 100 mg/ml, 50 mg/ml, 25 mg/ml and 12.5 mg/ml concentration [19].

Evaluation of synergistic activity of plant extracts: Agar well diffusion method was used to test the antimicrobial activity of the plant extract on the *Candida albicans* isolate as described by Cheesebrough [20]. The method was described by Kirby and demonstrated by Cheesebrough [21]. The organism was inoculated onto the surface of Mueller Hilton agar using sterile cotton swab dipped into test tubes containing the standard inoculum.

Excess inoculum was removed by pressing and rotating the swab firmly against the inside wall of the petri dish. The plates were allowed to dry for few minutes and a sterile cork borer of 6 mm diameter was used to bore wells on plate. 1 ml of each of the different concentration of the plant extracts was pipette inside the well. The plates were allowed to stay for 30 minutes before incubation. All the plates were incubated overnight at 37°C for 24 hours. After overnight incubation, the plates were examined for zone of inhibition [22].

The zone of inhibition is the area surrounding the well and there is no growth of the inoculated organisms. The diameter of the zone of inhibition is measured in millimeter using a transparent ruler and recorded.

The actual zone of inhibition is obtained by subtracting the diameter of the well which is 6 mm from the diameter of the zone of inhibition. The synergistic effects of the plant extracts were determined by combining the plants extracts before pipetting into the well bored in the agar and incubated at 37°C for 24 hours. The zone of inhibition was measured after incubation and recorded [20].

Determination of Minimum Inhibitory Concentration (MIC): The MIC was determined by serially dilution of plant extracts of various concentrations of 200 mg/ml, 100 mg/ml, 50 mg/ml, 25 mg/ml and 12.5 mg/ml respectively. Equal volume of each extract and nutrient broth were mixed in the test tubes. Specifically 0.1 ml of standardized inoculums was added to each test tube.

The tubes were incubated at 37°C for 18-24 hours. Two control tubes were maintained for each test batch. This included antibiotic control (containing extract and growth media without inoculum) and organism control (tube containing the growth medium, saline and the inoculum). The lowest concentration (highest dilution) of the extract that produced no visible bacterial growth (no turbidity) when compared with the control were regarded as MIC [23].

Results

The results are shown in Tables 1 and 2.

(Zone of inhibition mm)							
Conc [mg/ml]	*A. senegalensis*		*K. africana*		Synergistic effect		Water
	EE	AE	EE	AE	EAK	AAK	Control
12.5	0	0	0	0	1	0	0
25	0	0	0	0	1	0	0
50	3	2	0	0	2	1	0
100	10	3	2	1	4	2	0
200	13	7	5	3	7	5	0

Key: EE; Ethanolic Extract, AE; Aqueous Extract, EAK; Ethanolic extract of *A. senegalensis* and *K. africana*, AAK; Aqueous extract of *A. senegalensis* and *K. Africana*, 0=No zone of inhibition.

Table 1: Synergistic effect of ethanolic and aqueous extract of *K. Africana* and *A. senegalensis* on *Candida albicans* showing diameter of zones of inhibition obtained at different concentrations.

Conc (mg/ml)	12.5	25	50	100	200
Extracts	+	+	+	-	-
EA	+	+	+	+	-

AA	+	+	+	+	+
EK	+	+	+	+	+
AK	+	+	+	+	+
EAK	+	+	+	+	-
AAK	+	+	+	+	+
OC	+	+	+	+	+
AC	-	-	-	-	-

Key: EA; Ethanolic extract of *A. senegalensis*, AA; Aqueous extract of *A. senegalensis*, EK; Ethanolic extract of *K. africana*, AK; Aqueous extract of *K. Africana*, EAK; Synergy of ethanolic extract of *A. senegalensis* and *K. africana*, AAK; Synergy of aqueous extract of *A. senegalensis* and *K. Africana*, AC; Antibiotics control, OC; Organism control. -: No growth, +: Growth seen.

Table 2: Determination of Minimum Inhibitory Concentration (MIC) of *A. senegalensis*, *K. africana* and their synergistic effect at various concentrations.

Discussion

Antimicrobial and synergistic effect of *K. Africana* and *A. senegalensis* and *A. senegalensis* on *Candida albicans*

Antimicrobial and synergistic effect of ethanolic and aqueous extract of *K. Africana* and *A. senegalensis* on *Candida albicans* showing diameter of zones of inhibition obtained at different concentrations.

The effectiveness of *A. senegalensis* and *K. africana* and their synergistic effects was confirmed by agar well diffusion method and growth of inhibition zones were measured in millimetre (mm), the result indicated that *A. senegalenis* was the most active against *Candida albicans*, having the highest zone of inhibition 13 mm and 7 mm for ethanolic and aqueous extracts respectively at 200 mg/ml concentration and 10 mm for ethanolic extract of *A. senegalenis* at 100 mg/ml concentration, followed by synergy of ethanolic extract of *A. senegalensis* and *K. africana* which has the zone of inhibition of 7 mm for aqueous and ethanolic at 200 mg/ml.

Determination of minimum inhibitory concentration

The minimum inhibitory concentration (MIC) was determined using broth dilution method, In *K. africana*, growth was seen in all concentrations including the highest concentration, which 200 mg/ml for the aqueous and ethanolic extract, and that of *A. senegalensis* was determined at 200 mg/ml and 100 mg/ml for aqueous and ethanolic extract respectively, and the MIC of the synergistic effect of ethanolic extract of *A. senegalensis* and *K. africana* was determined at 200 mg/ml, while for the aqueous extract growth was seen in all concentrations.

As shown by the lower MIC of *A. senegalensis*, it may have better antibacterial activity than the synergy of *A. senegalensis* and *K. africana* and *K. africana* extract against *Candida albicans*.

Conclusion

The results also shows that individual plant extract has better activity on *Candida albicans* than a combination of the extracts hence *K. africana* and *A. senegalensis* shows antagonistic effect rather than a synergistic activity on the *Candida albicans* isolate Hence, extracts of *A. senegalensis* may be used as the alternative source for treating several infectious disease caused by *Candida albicans* [24,25].

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