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Original Research Article

Phytochemical investigation and Antimicrobial activity of Sudanese *Faidherbia albida* and *Khaya senegalensis* bark extracts and their combination against wound infection pathogens

Mojahid Mohammed Khalid[1], Einas Gamal Mohieldin[2], Afraa Mubarak Alhaj[3] and Ayat Ahmed Alrasheid[1]*

[1]Pharmacognosy Department, Faculty of Pharmacy, University of Medical Sciences and Technology, Khartoum, Sudan.

[2]Pharmacology Department, Faculty of Pharmacy, University of Medical Sciences and Technology, Khartoum, Sudan.

[3]Microbiology Department, Faculty of Medical Laboratory Sciences, University of Medical Sciences and Technology, Khartoum, Sudan.

***Corresponding Author's E-mail: ayatwarag@yahoo.com**

Abstract

Antimicrobial resistance has become an important public health problem in Sudan. Some bacteria resistant to developed antibiotics. This study was conducted on the ethanolic bark extracts of Sudanese *Faidherbia albida* and *Khaya senegalensis* and their combination to carry out phytochemical screening and to evaluate the antimicrobial activity with the determination of the minimum inhibitory concentration. Qualitative phytochemical screening of ethanolic extract of the bark of *F. albida* indicated the presence of sterols, tannins and alkaloids, while the ethanolic extract of the bark of *K. senegalensis* contain alkaloids, triterpenes, tannins, saponins, cardiac glycosides, and reducing sugars. The total tannins content of the ethanolic bark extracts was determined using double beam Spectrophotometer. The result was found to be 9.099 ppm for *F. albida*, 9.63 ppm for *K. Senegalensis* and 10.983 ppm for the combination of both extracts (1:1). Antimicrobial activity and minimum inhibitory concentration for the both samples was determined using disc diffusion method. It was found that there is higher activity against the two Gram positive bacteria and *Proteus spp* Gram negative bacteria. than *Klebsiella spp*. Generally *K. senegalensis* showed slightly higher antibacterial activity than *F.albida*, but it showed low antifungal activity. Further researches are needed for the identification and isolation of the compounds responsible for the antimicrobial activity.

Keyword: *F. albida*, *K. senegalensis*, wound, antimicrobial activity, tannin

INTRODUCTION

Herbs are defined in several ways, depending on the context in which the word is used. In botanical nomenclature, the word refers to non-woody seed-producing plants that die to the ground at the end of the growing season. In the culinary arts, it refers to vegetable products used to add flavor or aroma to food. But in the field of medicine, the term has a different, yet specific meaning, where it is most accurately defined as crude drugs of vegetable origin utilized for the treatment of disease states, often of a chronic nature, or to attain or to maintain a condition of improved health (Awang., 2009).

Sudan folklore-medicine represents a unique blend of indigenous cultures with Egyptian, Indian, Arabian, East and West African culture; because Sudan has a wide diversity of climate which is responsible for its varied vegetation and very rich flora. Sudan is rich in medicinal

plants which need to be researched upon to extract the active ingredients for treatment of widespread diseases in rural areas (Khalid *et al*., 2012). *F. albida* belongs to family Fabaceae. It can attain a very large size; heights of over 30 m and a diameter of 1.5 m. The lifespan is generally about 70 to 90 years. The main area of natural distribution of *F. albida* is Africa, right across the African continent from Senegal and Gambia to the Red Sea (Egypt, Sudan, Ethiopia, Somalia, and Kenya)(Wood ., 1992). In Sudan, *F. albida* is most common on silty loams with sufficient subsoil moisture, e.g., along rivers. It is found in most parts of Sudan but its best development is in the western part of the country, particularly in the Jebel Marra area (Goda ., 1992). In folkloric medicine, it is used for fevers by the Masai people of Kenya as well as for diarrhea in Tanganyika. A liniment, made by steeping the bark, is used for bathing and massage in pneumonia. (Salawu *et al*., 2010) The bark's infusion is used for difficult delivery, and is used as a febrifuge. In northern Nigeria, especially among the cattle rearing nomads, a decoction of the stem bark is taken orally for the management of the sleeping sickness and malaria, also used in cleansing fresh wounds, in a manner similar to that of potassium permanganate, and for colds, haemorrhage, leprosy and ophthalmic in West Africa (Irvine, 1961). *Khaya senegalensis* belongs to family Meliaceae, it's a deciduous tree, 15-20 m tall, with diameter up to 1.5 m. The bark is dark grey, and the slash is dark pink with red latex (Adesogan and Taylor,1968). Natural distribution of *K. senegalensis* extends from Mauritania and Senegal east to northern Uganda, within the rainfall range 650–1300mm and even up to 1800 mm (Nikiema and Pasternak 2008). *Khaya senegalensis*is traditionally used in the treatment of malaria, jaundice, edema, diarrhoea, dysentery, anaemia, wound infections and headache (Aliyu., 2006).

Wound infection

In the last two decades, increased numbers of antibiotic-resistant bacteria have been isolated from wounds, and although they do not always cause infection in the host, their carriage can be a source of infection for other vulnerable groups of patients. If these organisms do cause wound infection in the patient, then the treatment is more difficult as the availability of antibiotics diminishes (Edwards-Jones, 2016). Wounds subject to infection can be surgical, traumatic, or physiologic. The latter include the endometrial surface, after separation of the placenta, and the umbilical stump in the neonate. Traumatic wounds comprise such diverse damage as deep cuts, compound fractures, frostbite necrosis, and thermal burns. Sources of infection include: the patient's own normal flora; material from infected individuals or carriers that may reach the wound on fomites, hands, or through the air; and pathogens from the environment that can contaminate the wound through soil, clothing, and other foreign material (Ryan and Ray,2010). The study was done on the medicinal plants used traditionally in the treatment of diseases caused by microorganisms; to discover alternative ways to overcome the infections caused by antibiotics resistant bacteria; since the medicinal plants may have compounds that archives bactericidal activity by new mechanism of action that can help discovering new bactericidal active compounds. Natural compounds, unlike the synthetic ones, show high margin of safety with minimal side effects, they are cheaper and more available.

MATERIALS AND METHODS

Materials

Plant materials

The bark samples of *F.albida* was collected from near River Nile in Khartoum, Sudan. *K. senegalensis* was collected directly from the field in western Sudan. Then they were identified and authenticated by the Research Centre Institute.

Culture Media

Mueller-Hinton agar

Thirty eight grams of Mueller Hinton agar powder were weighed, dissolved in 1 liter of distilled water and allowed to soak for 10 minutes. The medium was placed in water bath to dissolve, swirled to mix and sterilized by autoclaving for 15 minutes at 121°C. It was then cooled to 47°C, mixed well then poured into sterile Petridishes.

Sabaroud dextrose agar

Sixty two grams of the powdered Sabaroud dextrose agar, was weighed, dispersed in 1 L water and allowed to soak for 10 minutes, swirled to mix then sterilized by autoclaving for 15 minutes at 121°C.It was cooled to 47°C, mixed well and then poured in to sterile Petri dishes.

Methods

Plant preparation and Extraction

The stem barks were removed from the plants, washed and air-dried for 5-days at room temperature in the shade. The stem barks were pounded using the mortar and pestle to get fine small powder particles. About 50 grams

of powdered material of the bark of each *F. albida* and *K. senegalensis* were extracted using ethanol 96% at room temperature for three days. Extracts were first filtered then the filtrate was put to dryness and percentage yield was calculated.

Qualitative phytochemical screening tests

Phytochemical screening has been carried out in order to detect the different classes of chemical constituents in the different extracts of each plant using standard procedure (Trease and Evans., 1989).

Quantitative determination of total tannins content

The tannins content was determined using $FeCl_3$and gelatin test. 1 ml of each extract of *F. albida* and of *K. senegalensis* (1mg/ml) and their combination (1:1) was transferred to test tubes, 1ml of 1% $K_3Fe(CN)_6$ and 1 ml of 1% $FeCl_3$ was added, and the volume was made up to 10 ml with distilled water. 1 ml of each extract (1mg/ml) was transferred into a test tube to make combination of the extracts. After 5 minutes incubation, absorbance was measured at 510 nm in a double beam Spectrophotometer. Tannic acid was used as standard calibration curve (Shivakumar *et al*., 2012).

Antimicrobial activity and minimum inhibitory concentration (MIC)

The culture media was sterilized by autoclave, and then poured in the Petri dishes and bacteria were transferred into a clean tube filled with normal saline. A swap was emerged in the tube and moved slowly on the sterilized culture media. The paper discs were impregnated with the plants extracts, and combination solution of both extracts (1:1) in concentrations of 6.25, 12.5, 25, 50 and 100 mg/ml. DMSO was used as a negative control, while Ciprofloxacin (5 mcg) was used as a positive control. Antibacterial discs were dispensed onto the surface of the inoculated agar plates and Petri plates were incubated for 24 h at 37°C. Diameters of clear zone of inhibition produced around the discs were measured and recorded (Kil *et al.,* 2009).

RESULTS AND DISCUSSION

Phytochemistry

The *K. senegalensis* bark extract was found to have extractive yield (16.98%) and *F.albida* bark extract (1.86%).

Qualitative phytochemical screening

Phytochemical screening of ethanolic extract of the bark of *F. albida* showed the presence of sterols, tannins and alkaloids, while the flavonoids were not detected.
The ethanolic extract of the bark of *K. senegalensis* showed that it contains alkaloids, triterpenes, tannins, saponins, glycosides, and reducing sugars. While the flavonoids, sterols and anthraquinones were not detected. Results are presented in Table 1.

Quantitative analysis of total tannin content

Tannin containing plants have been used traditionally as styptics and internally for the protection of inflamed surfaces of skin, mouth, and throat. Theoretically, the effect is directly proportional to the total tannins content. The total tannins content of (1mg\ml) *F. albida* extract was found to be 9.099 ppm, and9.63 ppm for *K. senegalensis*, while for the combination of both samples (1:1) it was found to be 10.983 ppm. The results showed that the amount of tannins present in the combination of both samples is larger than the amount of tannins presents in either of the samples individually.

Antimicrobial activity and Minimum Inhibitory Concentration (MIC)

Antibacterial activity of the ethanolic extract of *F.albida* and of *K. senegalensis* and their combinations was tested against two isolated Gram positive, (*S. aureus* and *E. faecalis)* and two isolated Gram negative bacteria (*Klebsiella spp.* and *Proteus spp*.)and the antifungal activity was tested against *C. albican* using disc diffusion method. Results are presented in Table 2. Both extracts showed activity against these microorganisms with some variation in activity. It was found that there is higher activity against both Gram positive bacteria and *Proteus spp*. Gram negative bacteria than *Klebsiella spp.* and C*. albican*. Generally *K. senegalensis* showed higher antibacterial activity than *F. albida*, while *F. albida* showed higher antifungal activity. The ethanolic extract of *F.albida* with concentration 100mg/ml showed the highest activity against*S. aureus* (19mm) and *Proteus spp*. (17mm) followed by *E. faecalis* and *Klebsiella spp.* (13mm), with the lowest zone of inhibition observed against *C. albican* (11mm).The ethanolic extract of *K. senegalensis* 100mg/ml showed higher activity against *S. aureus* (22mm) and *Proteus spp*. (20mm) followed by *E. faecalis* and *Klebsiella spp.* (13 and14 mm) respectively. The activity against *C. albican* was lower compared with that obtained by *F.albida* extract (10mm). Antimicrobial activity of ethanolic extract (96%) of the bark of *K. senegalensis* with concentration(100mg/ml) was higher against *S. aureus* than the activity in the previous study

Table 1. Phytochemical screening of bark extracts of *F. albida* and *K. senegalensis*

Test	Specific test	Results	
		F. albida	***K. senegalensis***
Alkaloids	Wagner's test	+ve	+ve
	Dragendroff's test	-ve	+ve
	Mayer's test	-ve	+ve
Flavonoids	lead acetate test	-ve	-ve
Sterols	Salkowski's test	+ve	-ve
	Lieberman's test	+ve	-ve
Triterpenes	Salkowski's test	-ve	+ve
	Lieberman's test	-ve	+ve
Tannin	$FeCl_3$ test	+ve	+ve
	Gelatin's test	+ve	+ve
Saponins	Foam's test	-ve	+ve
Glycosides	Keller kiliani's test	+ve	+ve
	Kedd's test	-ve	+ve
Reducing sugars	Fehling's test	-ve	+ve
Anthraquinone	Ammonia test	-ve	-ve

Table 2. Antibacterial activity of plants extracts and Standard drug

Sample	Concentration	Diameter of Inhibition Zone (mm)			
		Gram positive bacteria		Gram negative bacteria	
		S. aureus	***E. faecalis***	***Klebsiella spp.***	***Proteus spp.***
F.albida	100 mg/ml	19	13	13	17
	50 mg/ml	16	9	8	15
	25 mg/ml	14	7	-	-
	12.5 mg/ml	11	-	-	-
	6.25 mg/ml	6	-	-	-
Khaya senegalensis	100 mg/ml	22	13	14	20
	50 mg/ml	18	9	8	14
	25 mg/ml	15	7	-	10
	12.5 mg/ml	12	-	-	-
	6.25 mg/ml	7	-	-	-
Combination	100 mg/ml	24	15	15	20
	50 mg/ml	20	12	9	15
	25 mg/ml	16	8	-	12
	12.5 mg/ml	12	7	-	9
	6.25 mg/ml	10	-	-	-
Ciprofloxacin	5 mcg	50	10	13	15

Table 3. Antifungal activity of plants extracts against *Candida albicans*

Sample	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml
F.albida	11	7	**6**	-	**-**
K.senegalensis	10	7	-	-	**-**
Combination (1:1)	13	8	**6**	-	**-**

Table 4. Minimum inhibitory concentration (MIC) of *F.albida* and *K.senegalensis* and their combination

Microorganism	MIC (mg/ml)*		
	F.albida	***K.senegalensis***	**Combination (1:1)**
Staphylococcus aureus	6.25	6.25	6.25
Enterococcus faecalis	25	25	12.5
Klebsiella spp.	50	50	50
Proteus spp.	50	25	12.5
Candida albican.	25	50	25

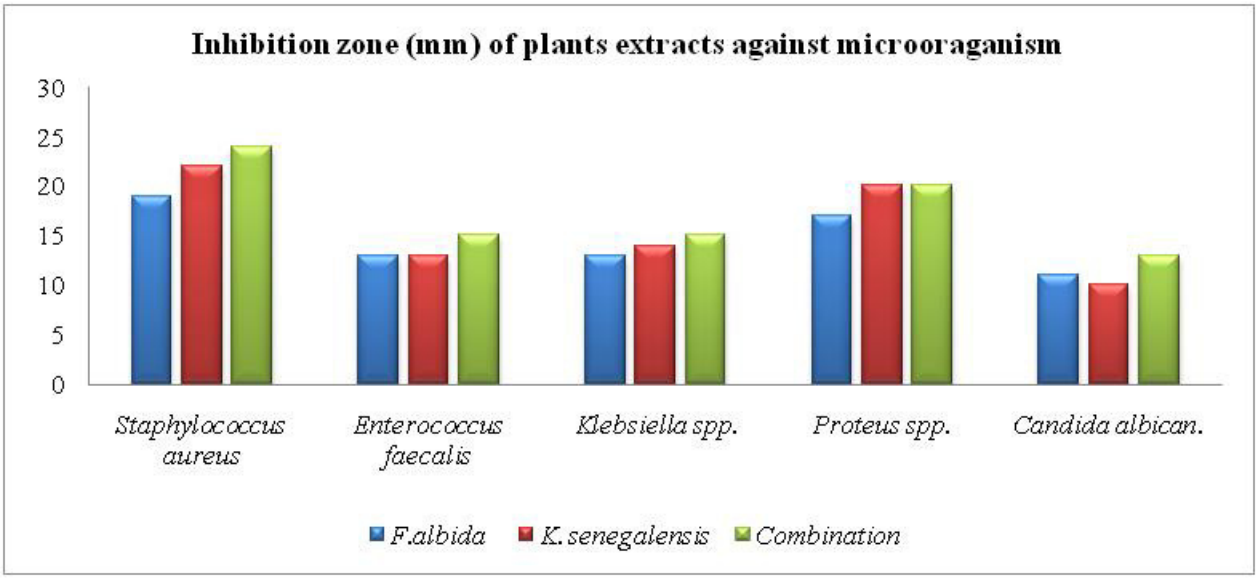

Figure 1. Inhibition zone of *F. albida*, *K. senegalensis* and their Combination

conducted by Kuta*et al.* (2015) who used ethanolic extract (50%) of the stem bark of *K. senegalensis*. This may be due to the differences of solvent concentration used in the extraction process or to some environmental differences related to the plants, or may be due to destruction of metabolites during the steam evaporation process used in the mentioned study. The combination of both *F.albida* and *K. senegalensis* (1:1) showed additive effect in the activity against these pathogens. The highest activity was recorded against *S. aureus* (24mm) followed by *Proteus spp*. (20mm), *E. faecalis* and *Klebsiella spp.* (15mm) while *C. albican* gave inhibition zone of 13 mm. Both extracts and their combination showed an extra ordinary activity against *Proteus spp*., *E. faecalis* and *Klebsiella spp.* that is higher than ciprofloxacin standard drugs. The activity may be due to effective compounds which detected in both extracts i.e. alkaloids, tannins or glycosides as seen in phytochemical screening. Although the pathogens isolated from patients could have been resistant species (like *E. faecalis, Proteus spp.,* and *Klebsiella spp.*that showed resistance to ciprofloxacin according to EUCAST.

CONCLUSION

Generally, the antimicrobial activity of combination of both extracts of *F.albida* and *K. senegalensis* was found to be better than each extract separately. Both plants extracts and the combination could be a source of important bioactive secondary metabolites and further investigations are important for the identification of active principles to development new drugs against various diseases.

Conflict of Interests

Authors declare no conflict of interests.

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