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ANTIMICROBIAL SCREENING OF THE DIFFERENT PARTS OF *ACACIA NILOTICA* AND *COMETES ABYSSINICA*

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ABSTRACT

Two, Eritrean endemic, medicinal plants that are used to treat diseases associated with microbial infection were collected and identified. Leaf, stem, and seed parts of *acacia nilotica* as well as leaf and root parts of *cometes abyssinica* extracts were obtained using water and methanol. Eight crude extracts were screened for antimicrobial activities against *staphylococcus aureus* (NCTC-12981, ATCC-25923), *escherichia coli* (NCTC-12241, ATCC-25929) and *candida albicans* (NCTF-3255, ATCC-2091) using well streak method. The results from this study demonstrated that all parts of the *acacia nilotica* inhibited the growth of *staphylococcus aureus*, *escherichia coli* except water extract of stem and seed on *candida albicans*. The methanol extract of the leaf part of *cometes abyssinica* showed inhibition against all organisms. From all the different parts screened, the seed extracts of *acacia nilotica* and the leaf part of *cometes abyssinica* showed maximum inhibition. And among the solvents used, the methanol extracts showed better activities.

KEYWORDS

Antimicrobial Screening, Eritrean Medicinal Plants and Crude Extracts.

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INTRODUCTION

Human kind has been exposed to infection by microorganisms since before the dawn of recorded history[1]. In treating such infections, mainly bacterial, human beings have identified the use of different herbs since ancient times[2]. The knowledge on plant use is the result of many years of man's interaction and selection on the most desirable, the most vigorous and the most successful plants present in the immediate environment at a given time[3-5].

The world's poorest countries are most in need of inexpensive, effective treatments for diseases. World Health Organization (WHO) estimates that one-third of the global population still lacks regular access to essential drugs, and that in the poorest parts of Africa and Asia, this figure rises to over 50%[6]. According to the WHO report, more than 80% of the people in Africa depend on traditional medicine[7]. Besides, the origins of more than 50% of modern clinical drugs are natural products[8].

In Eritrea the use of herbs to treat different types of ailment is a wide spread practice[9]. However, little has been done to study the antimicrobial activity of Eritrean flora. In the present investigation, two endemic medicinal plants were selected, namely, *acacia nilotica* (Figure No.1) and *cometes abyssinica* (Figure No.2). The genus Acacia is the second largest in the family Leguminosae, with about 1350 species. It is distributed throughout tropical and warm temperate areas of the world, with the largest concentration of species in Australia (957 species), The Americas (185 species), Africa (144 species), and Asia (89 species)[10]. In Eritrea it occurs in woodland and scrub in the lower Gash river plains around Alighder (Numero-Ashera), 700-1400m. Out of these, *acacia nilotica* is one of the species that has been effectively utilized in folk medicine for the treatment of tuberculosis, leprosy, smallpox, dysentery, cough, ophthalmic, toothache, astringent, antispasmodic, and aphrodisiac by rural population. *Acacia nilotica* leaves are protein rich and highly digestible[11,12]. *cometes abyssinica* is classified under the phylum Tracheophyta and is mostly found distributed in open rocky slopes; near sea level to 2300-2700 m. It is found distributed in Egypt, south Palestine, Arabia, Ethiopia, Sudan and Somalia. Its habitat is mostly stony deserts. It has perennial life expectancy. Broad shrub to 45cm high, sometimes flowering in first season as erect annual; all parts minutely erect puberulent[13]. The objective of the study was to identify the antimicrobial effect of each plant part and ratify the traditional use of the plants in Eritrean traditional medicine.

MATERIALS AND METHOD

This research was an experimental study. Two plant species were used for this study and both hot and cold extraction methods were used. Cold extraction was used to extract leaf, stem and seed parts of *acacia nilotica* while hot extraction was used to leaf and root parts of *cometes abyssinica*. The organisms used for the screening were *staphylococcus aureus* (NCTC-12981, ATCC-25923), *escherichia coli* (NCTC-12241, ATCC-25922) and *candida albicans* (NCTC-3255, ATCC-2091).

The two plants were collected from different administrative regions. *Acacia nilotica* was collected from Keren, Anseba region while *cometes abyssinica* was collected from Mendefera, Debub region. Identification of the plants was done according to Flora of Ethiopia and Eritrea[14,15].

Each part of the collected plants were separated and thoroughly washed using running water and dried at room temperature away from sun light to avoid sun damage. After drying, each part was size reduced, using mortar and pestle, and sieved to get the appropriate size. The plant *acacia nilotica's* leaf, stem and seed parts were soaked in water and methanol. These were left soaked in a shaker for 48hrs after which they were then filtered using filter paper in Buchner's funnel associated with a vacuum pump and then concentrated using rotavapour. Unlike *acacia nilotica,* plant parts of *cometes abyssinica* were subjected to hot extraction using water for the root part and methanol for the leaf part. The extracted mixture was then subjected to filtration using Buchner's funnel and evaporated using rotavapour. Finally, the concentrated extracts were tested for their antimicrobial activity.

Antimicrobial sensitivity test and control

The method used for evaluation of antimicrobial activity is AST in which sterile Mueller Hinton agar for bacteria and Sabouraud media for fungi were inoculated with test organisms *staphylococcus aureus* (NCTC-12981, ATCC-25923), *escherichia coli* (NCTC-12241, ATCC25929) and *candida albicans* (NCTF-3255, ATCC-2091). 6mm Well streak method was used for the crude extracts of both *acacia nilotica* and *cometes abyssinica* plant

parts. The presence of inhibition was regarded as the presence of antimicrobial activity.

For the antimicrobial sensitivity test, well streak method was used for the sensitivity test for both the plants, in which holes were made using a sterile cork borer in a quality controlled media and the organisms were then inoculated. Water and Methanol dissolved extracts of each of the plant parts were delivered using a sterile pipette to each well and zone of inhibition was measured after 24-48 hours of incubation for each plant extract. The inhibition zone produced was measured in terms of millimeter.

Prior to each procedure, all the material used for the antimicrobial test was sterilized properly and media, specifically, were autoclaved at 121^{o}C for 30 minutes. As a positive control, standard drugs, amoxicillin and fluconazole, were used against the selected stock organisms and methanol was used against the selected stock organisms as a negative control.

RESULTS AND DISCUSSION

A total of eight extracts representing two plant species were screened for their antimicrobial activity. Results for the antimicrobial activity of the plants and standard antimicrobials, amoxicillin and fluconazole are summarized in Table No.1 and Figure No.3 and 4. All extracts of this study, except the root part of *cometes abyssinica*, showed antibacterial activity against *staphylococcus aureus* and *escherichia coli*. Leaf extracts (of both water and methanol) from *acacia nilotica* had inhibitory activity against all test organisms. Among the solvents used, methanol extracts of all plant parts of the two plants showed antifungal and antibacterial activities against all test pathogens. Maximum zone of inhibition is observed with seed extracts of *acacia nilotica.* The water extracts of *acacia nilotica's* seed showed inhibition zone of 17mm and 23mm against *escherichia coli* in concentrations of 50 and 100 mg/ml respectively and 44mm and 47mm against *Staphylococcus aureus*in concentrations of 50 and 100 mg/ml respectively but no effect against *candida albicans*. In addition, methanol extracts of the seed part of *acacia nilotica* showed inhibition zone of 29mm and 30mm against *escherichia coli* in concentrations of 50 and 100 mg/ml respectively, 39mm and 48mm against *staphylococcus aureus* in concentrations of 50 and 100 mg/ml respectively and 14mm and 15mm against *candida albicans* in concentrations of 50 and 100 mg/ml respectively. Extracts from *cometes abyssinica* had antimicrobial activities relatively at higher concentrations than extracts of a*cacia nilotica*. Methanol extracts of the leaf part of *cometes abyssinica* were tested in 300 and 400 mg/ml concentrations against *staphylococcus aureus*, *escherichia coli* and *candida albicans*. The zone of inhibition detected for the methanol leaf extracts were 23mm and 28mm for *escherichia coli*, 21mm and 29mm s*taphylococcus aureus* and 32mm and 45mm *candida albicans* in concentrations of 300 and 400 mg/ml respectively.

The results from this study demonstrated that the plants have antimicrobial activity against *staphylococcus aureus, escherichia coli,* and *candida albicans*. *Staphylococcus aureus is* the causative agent of most skin infection and septicemia. *Escherichia coli* is the causative agent of kidney damage (causing toxins), septicemia and diarrhea[16].

Acacia nilotica has been reported to be effective against a variety of diseases including diabetes and skin disease. The fresh plant parts of *acacia nilotica* are considered as astringent, demulcent, aphrodisiac, anthelmintic, antimicrobial, antidiarrheal, with good nutritional value in Indian traditional medicine system[17,18]. Although it has got this many uses it isn't that much known in our country. Some people in Eritrea use the seeds of *acacia nilotica* in treatment of common cold while others use it in the treatment of diarrhea and diabetes without knowing the constituents of the plant. The leaves and stem are not that much used although they have got high antimicrobial activity. In a previous study done in Nigeria, aqueous extract of *acacia nilotica* failed to show any antimicrobial

activity against same strains of *staphylococcus aureus and escherichia coli*[19]. The activity of *cometes abyssinica* parts were compared, the root had almost no effect against all organisms while the leaf had effect against all the organisms signifying that the leaf might have the highest content of active constituents which need further investigation on its chemical constituents.

When the plants were compared with the control drugs, Amoxicillin and Fluconazole, they didn't show more effect. They had slightly less effect than Amoxicillin (used at concentration of 100mg/ml) with zone of inhibition of 40mm on *escherichia coli* especially the methanol seed extract of *acacia nilotica* but they had far less effect on *candida albicans* which had zone of inhibition of 30mm with Fluconazole (used at concentration of 100mg/ml).

Table No.1: Antimicrobial activity the selected plants

S.No	Plant/Standard Antimicrobial Agent	Plant Part	Solvent	Concentration (mg/ml)	Zone of inhibition (mm)		
					SA	***EC***	***CA***
1	*Acacia nilotica*	Leaf	W	50	41	16	14
				100	43	17	15
			M	50	31	19	16
				100	35	20	18
		Stem	W	50	39	13	-
				100	44	18	-
			M	50	32	18	14
				100	34	19	15
		Seed	W	50	44	17	-
				100	47	23	-
			M	50	39	29	14
				100	48	30	15
2	*Cometes abyssinica*	leaf	M	300	21	23	32
				400	29	28	45
		root	W	200	-	-	-
				300	12	-	-
3	Amoxicillin			100	NA	40	NA
4	Fluconazole			100	NA	NA	30

SA: staphylococcus aureus, EC: escherichia coli, CA: candida albicans, W: water, M: methanol, -: no activity, mm: millimeters, NA: Not Applicable.

Figure No.1: *Acacia Nilotica*

Figure No.2: *Cometes Abyssinica*

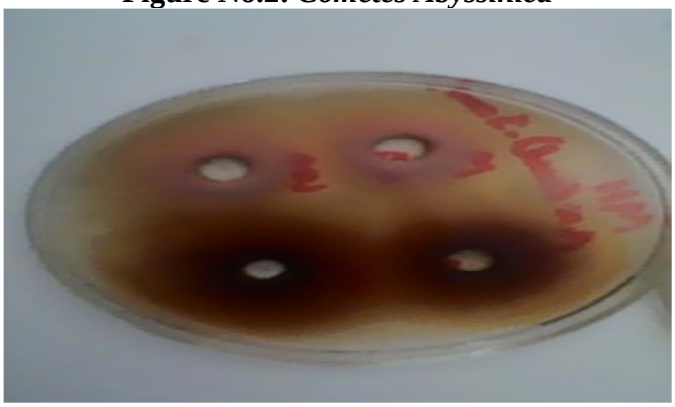

Figure No.3: Zone of inhibition of *A.Nilotica*

Figure No.4: Zone of inhibition of *C.Abyssinica*

CONCLUSION

In conclusion, the study suggests that the plants investigated have useful antimicrobial activity. The use of the plants in Eritrean traditional medicine for disease associated with bacterial/fungal infection could be justified by their antimicrobial activity. However, further phytochemical studies are required to establish the types of compounds responsible for the antimicrobial effects of these medicinal plants.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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