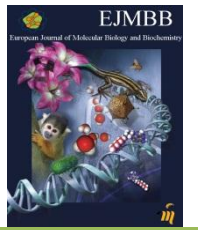
e - ISSN - 2348 - 2206
Print ISSN - 2348 - 2192

European Journal of Molecular Biology and Biochemistry

Journal homepage: www.mcmed.us/journal/ejmbb



ANTIMICROBIAL SCREENING OF WOOD EXTRACTS OF *COMBRETUM HARTMANNIANUM, ACACIA SEYAL* AND *TERMINALIA BROWNIE*

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Article Info
Received 23/05/2014
Revised 10/06/2014
Accepted 15/06/2014

Key words:-
Combretum hartmannianum Acacia seyal, Terminalia browni, Antibacterial.

ABSTRACT

The effect of the aqueous, ethyl acetate, chloroform and petroleum ether extracts from the woody part of *Combretum hartmannianum, Acacia seyal* and *Terminalia brownie* were tested for antibacterial activity against pathogenic bacteria such as *Salmonella, E. coli* and *Staphylococcus aureus*. The ethyl acetate extract gave the highest zone of inhibition against Salmonella (18 mm). All other extracts showed moderate zones of inhibition (14 - 17 mm) against all the bacteriatested. The present study has proved the usefulness of *Combretum hartmannianum, Acacia seyal* and *Terminalia brownie* plants for medicinal purposes.

INTRODUCTION

Today, natural products derived from plants are being tested for the presence of new drugs with new modes of pharmacological action. A special feature of higher plants is their capacity to produce a large number of secondary metabolites [1]. Recent studies are involved in the identification and isolation of new therapeutic compounds of medicinal importance of higher plants for specific diseases [2, 3]. Combretum and Terminalia are a genus of large trees of the flowering plant family Combretaceae. They are native to Eritrea, Ethiopia, Kenya, Somalia, Sudan, Tanzania, and Uganda [4]. The bark of *Terminalia brownie* has been used in traditional medicinefor thetreatment of cough and bronchitis in Sudan, the methanolic extract of this plantbark and leaves was found to be effective for rabbits infected with low doses of *Pasteurella multocida* strain B2 [5]. *Acacia seyal* Genus is Acacia Mill.–acacia Family Fabaceae–Pea family Species *Acacia seyal* Delile–talh. Is native to Egypt, Eritrea, Ethiopia, Ghana, Iran, Israel, Kenya, Malawi, Mali, Mozambique, Namibia, Niger, Nigeria, Saudi Arabia, Senegal, Sudan, Syrian Arab Republic, Tanzania, Uganda, Yemen, Republic of, Zambia, Zimbabwe [6]. The methanolic extract of *Combretum hartmannianum* leaves was markedly effective in inhibiting the oxidation of linoleic acid and subsequent bleaching of ß-carotene, this extract inhibited the formation of peroxides in sunflower oil and showed the highest antioxidative activity compared with 20 mg BHA [7].

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MATERIALS AND METHODS

Collection of plant materials

Materials from the woody part of three plant species were harvested in March 2010 from Western Kordofan state, Sudan. They were carefully washed, oven-dried for 1 h at 50°C and put in the shade in an aerated place till complete drying, then were ground into a fine powder.

Preparation of plant extracts

25 g of fine powder from the woody part of three plant species was added to a Soxhlet apparatus along with a solvent methanol for extraction of chemicals. The liquid extract was evaporated to dryness by vacuum distillation and stored at 4°C for further analysis [3]. Percentage yield was calculated from the dry extract powder after that the obtained methanol extract was fractionated sequentially by organic solvents as ethyl acetate, chloroform and petroleum ether.

Aqueous extraction

Water extraction was prepared by adding 100 ml hot sterilized distilled water into 25 g of the woody part of three plant species, mixed well until they became cold, and then were filtered using muslin clothes, the filtrates were evaporated and concentrated to dryness using the water bath at 100 ºC.

Methods

Antimicrobial activity

Test microorganisms

The extracts of *Combretum hartmannianum*, *Acacia seyal* and *Terminalia brownie* were tested for antimicrobial activity; the method used was cup-plate agar diffusion method [8]. All bacterial strains mainly *Salmonella* (ATCC 25921), *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus (ATCC 25923)* were obtained from the Institute for medicinal and aromatic plants, National Center for Research, Khartoum, Sudan.

Antimicrobial activity screening tests

Testing cup-plate agar diffusion method

0.2ml of the standardized bacterial stock suspension were mixed with 20 ml of sterile nutrient agar and poured into a sterile petridish, the agar left at room temperature to dry. 4 cups (10 mm in diameter) were cut using a cork borer and agar discs were removed. Cups were filled with 0.1ml of the different extracts in aspirate petri dish (aqueous, ethyl acetate, chloroform and petroleum ether extract), three replicates for each extract for each testing organisms (*Salmonella*, *Escherichia coli* and *Staphylococcus aureus*). Concentration used from each extract is 2.5mg of the extract in 1ml of the solvent and 5mg of extract in 1ml of solvent, the extracts left to diffuse for two hours, and then the plates incubated in the upright position at 37°C for 18 hours. After incubation the diameter of the inhibition zone was measured the mean value was taken positive control for each solvent was carried out to know the activity of the different solvents.

Statistical analysis

The mean values were expressed as the Mean±Standard Deviation (SD) and were analyzed using one-way ANOVA (Tukey's studentized range) using the program SPSS 19.0 for Windows. Differences were considered significant at $p<0.05$.

RESULTS

The antimicrobial activity of extracts of three medicinal plant species were assessed. Table 1, displays that the ethyl acetate extract of *Combretum hartmannianum* showed high significant ($p<0.05$) antimicrobial activity with inhibition zone of 18 mm against *Salmonella* and 15 mm against *Escherichia coli* while the other three extracts showed no antibacterial activity**.** *Terminalia brownie* aqueous, ethyl acetate, chloroform and petroleum ether extracts showed good antimicrobial activity against *Salmonella* with inhibition zone of 11, 17, 13 and 10 mm respectively. Ethyl acetate extract showed good antimicrobial activity against *Staphylococcus aureu* and *Escherichia coli* with inhibition zone of 18 and 15 mm, respectively. All extracts (except petroleum ether) of *Acacia seyal* showed high significant ($p<0.05$) antimicrobial activity for the first three bacteria species with ethyl acetate as the strongest one. Extract concentration affect on extract antimicrobial activity (Table 1). The ethyl acetate extract of *Combretum hartmannianum*, *Terminalia brownie* and *Acacia seyal* revealed the highest significant ($p<0.05$) antimicrobial activity with inhibition zone more than 17 mm. Although *Combretum hartmannianum* and *Terminalia brownie* were belonged to the same genus but their extracts exhibited different antimicrobial activity aqueous and chloroform extracts of *Acacia seyal* antibacterial activity (Table 1). But aqueous and chloroform extracts of *Combretum hartmannianum, Terminalia brownie* showed no antibacterial activity**.** On the other hand, all petroleum ether extracts of *Combretum hartmannianum, Terminalia brownie* and *Acacia seyal* showed no antibacterial activity (Table 1). Ethyl acetate extract had an antimicrobial activity against all tests *(E.c and Sa.a*) microorganisms except *Sa.a* (Table 1). The bacterium *Sa.a* was the least sensitive microorganism to plant extracts investigated in the current study. Interestingly, it was noticed that the ethyl acetate extract had antibacterial effective against all bacteria used in this study except *Sa.a* (Table 1). Moreover, it was found that aqueous, chloroform and petroleum ether extracts *of Combretum hartmannianum*, *Terminalia* and *Acacia seyal* show no antibacterial activity against all bacteria used in this study. In general, it was observed that both *E. coli* and *Sa.a* were sensitive to the same plant extracts (Table 1); they are sensitive to ethyl acetate extract. From Table 1 it's clear that if we compare the extract depending on the source (the plant) *Terminalia*

was the richest species since it showed activity by the four extracts, followed by *Acacia seyal* and the *Combretum hartmannianum* was the latest. Extracts of *Combretum hartmannianum* aqueous, chloroform and petroleum ether, extracts of *Terminalia brownie*, aqueous, chloroform and petroleum ether as well as extracts of *Acacia seyal* aqueous, chloroform and petroleum ether, they are not sensitive to all bacteria used in this study. From Table 1 increasing plant extract concentration from 2.5mg/1ml to 5mg/1ml has little increase in inhibition effect of the same extract.

Table 1. Inhibition zone in mm by different extracts of *Combretum hartmannianum*, *Acacia seyal* and *Terminalia brownie*.

Extracts		Concentration	Sa. a	E.c	S.a	Control (solvent of extracts)
			Inhibition zones (mm) of Bacteria			
Combretum hartmannianum	Aqueous	2.5mg/1ml	-	-	-	-
	Ethyl acetate		18	15	-	-
	Chloroform		-	-	-	-
	Petroleum ether		-	-	-	-
Terminalia brownie	Aqueous		11	-	-	-
	Ethyl acetate		17	18	15	-
	Chloroform		13	-	-	-
	Petroleum ether		10	-	-	-
Acacia seyal	Aqueous		9	-	11	-
	Ethyl acetate		17	15	12	-
	Chloroform		9	10	11	-
	Petroleum ether		-	-	-	-
Combretum hartmannianum	Aqueous	5mg/1ml	-	-	13	-
	Ethyl acetate		18	15	-	-
	Chloroform		-	-	-	-
	Petroleum ether		-	-	-	-
Terminalia brownie	Aqueous		13	-	-	-
	Ethyl acetate		17	18	15	
	Chloroform		13	-	-	-
	Petroleum ether		12	-	-	-
Acacia seyal	Aqueous		11	-	14	-
	Ethyl acetate		17	18	15	-
	Chloroform		14	12	-	-
	Petroleum ether		-	-	-	-

-=no inhibition, S.a = Staphylococcus aureu, E.c = Escherichia coli, Sa.a = Salmonella. Control = organic solvent (petroleum ether, chloroform and ethyl acetate), MIZD mm : >18mm: Sensitive, MIZD mm: 14-18mm: Intermediate, MIZD mm :< 14mm: Resistant.

DISCUSSION

The antimicrobial activity of 3medicinal plants has been evaluated *in vitro* against 3 bacterial species. They are frequently incriminated in human infections. Most of the tested plant extracts showed some level ofantimicrobial activity. The obtained results showed that, ethyl acetate extract of three medicinal plant species, namely: *Combretum hartmannianum*, *Terminalia brownie* and *Acacia seyal* has revealed a wide antibacterial against most test bacterial strains. These findings are consistent with those obtained in some previous studies [9, 10]. Also, the obtained results indicate that chloroform stands as the second most effective solvent after ethyl acetate. Similar findings were reported previously [11].

Extraction of antimicrobial substances from medicinal plants compared to water and petroleum ether solvents. Nevertheless, the ethyl acetate extraction method was found the most successful to extract antifungal metabolites from the plants used.

Extraction of antimicrobial metabolites from the selected plant species by petroleum ether and water was observed to be the least effective methods (Table 1). However, petroleum ether extraction method was found to be useful for the extraction of antimicrobial metabolites from *Terminalia brownie*. On the other hand, water extracts of *Acacia seyal* showed antibacterial activities. However, all solvent extracts of *Combretum hartmannianum* showed no antimicrobial activity except ethyl acetate extract.

The results of the current investigation clearly indicate that the antibacterial vary with the species of the plants. Therefore, the antimicrobial activity of plant extracts depends on the species of plant, the type of solvent and the type of testing microorganism.

CONCLUSION

The results obtained confirm the folkloric anticipation of the antimicrobial effectiveness and the therapeutic applications of the examined plants.

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