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Chemical composition and antibacterial activity of *Xeromphis nilotica* bark extracts

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Abstract

This present study was carried out in Khartoum state-Sudan, during March 2019. The dried bark of *Xeromphis nilotica* was extracted successively with (Ethyl acetate, Ethanol) and (Methanol, Water). The phytochemical screening carried out on four different extracts of *Xeromphis nilotica* bark and it contain amount of secondary metabolites such as (Alkaloids, Carbohydrates, Tannins, Phenols, Sterols & Triterpenes, Saponins, Flavonoids, Amino acids and Proteins) were studied. The antibacterial activity of extracts were evaluated against four standard bacteria (Gram positive; *Bacillus subtilis*, *Staphylococcus aureus*) and (Gram negative; *Escherichia coli*, *Pseudomonas aeruginosa*) and the results showed high inhibition zone (21mm) in methanol extract against *Escherichia coli* and low inhibition zone in aqueous extract against all bacterial strains tested.

Keywords: Folk medicine; Elsalihen area; *Xeromphis nilotica*; Shagart-Elmarfaein

1. Introduction

Xeromphis nilotica belong to Rubiaceae family, is the medicine plant that distributed in tropical and subtropical regions [1], the genus *Xeromphis* is represented in Sudan by one species, namely *Xeromphis nilotica*(Stapf) Keay [2], which is widespread in Central and East Africa as well as in Cameroon and Nigeria [3]. Locally it is known as Shagart-Elmarfaein [4]. It grows as a medium height shrub (usually less than 3 m) with grey globose drupes, stiff spines, and deciduous leaves clustered below the spines [5], it is use in different traditional medicines systems of Sudan for antispasmodic, anti-dysenteric, anti-inflammatory, immunomodulatory and anti-fertility properties [6-10]. Many species of plants grow abundantly in the Sudan and other African countries and are used by the village populations for treatment of various disorders [11] [12], and are the main medicinal source to treat infectious diseases [13], in many rural area, the medicinal plants had been an important methods in the treatment of diseases. The medicinal plants contain a number of secondary metabolites compounds such as alkaloids, flavonoids, tannins, saponins, amino acids, proteins, carbohydrates, Phenol, Sterols & Triterpenes [12], the traditional medicinal plants are increase in both developing and industrialized countries. [14] reported that both literate and illiterate people still use local plants as drugs in many conditions [15].

The aim of the present study was to determine the phytochemical screening and antibacterial activity of *Xeromphis nilotica* bark.

2. Materials and methods

All the chemicals and reagents used in this study were of analytical grade such as chloroform, distilled water, ethanol, methanol, acetic anhydride, sulphuric acid, gelatine salt, ferric chloride, reagents (Wagner, Hager, and Dragendorffs), aluminum chloride and potassium hydroxide.

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2.1. Plant material, collection and identification

Xiromphis nilotica bark was collected from Elsaliheen area, locality of algoze, South kordofan State- Sudan and identified in herbarium of natural research Centre and compared with herbarium of Faculty of Science University of Khartoum

2.2. Preparation of Crude Extracts

50g of the dried plant was weighted and extracted successively with (ethyl acetate, ethanol) and (methanol aqueous) by shaker apparatus for four hours at room temperature, each extract was filtrated through Whatman No 1 filter paper, followed by concentrated under vacuum room. The crude extracts were then kept at -20 °C in sterile universal bottles.

2.3. Phytochemical screening of different crude extracts

General phytochemical screening for the active constituents was carried out for extracts using the methods of [16].

2.4. Preparation of media

28g of powdered nutrient agar was weighted, dispersed in 1 liter of distilled water and allowed to soak for 10 minutes, swirl to mix then sterilized by autoclaving for 15 minutes at 121°C, cooled to 47c, mixed well then poured into Petri-dishes [17].

2.5. Testing of organisms:

The two (gram positive and negative bacteria were tested in table (2). The bacterial strains tests used for screening were *Bacillus subtiles* (NCTC 8236 Gram positive bacteria). *Staphylococcus aureus* (ATCC 25923 Gram positive bacteria). *Escherichia coli* (ATCC 25922 Gram negative bacteria) and *Pseudomonas aeruginosa* (ATCC 27853 Gram negative bacteria).

2.6. Testing for antibacterial Activity

The cup-plate agar diffusion method [17], was adopted with some minor modifications to assess the antibacterial of the prepared extracts. One ml of the standardized bacterial stock suspension 10⁸–10⁹ C.F.U/ ml were thoroughly mixed with 100 ml of molten sterile nutrient agar which was maintained at 45 °C. 20 ml aliquots of the inoculated nutrient agar were distributed into sterile Petri-dishes. The agars was left to set and in each of these plates 4 cups (10 mm in diameter) were cut using a sterile cork borer (No. 4) and agar discs were removed. Alternate cups were filled with 0.1 ml sample of the extracts using automatic micro-liter pipette, and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37 °C for 18 hours. Two replicates were carried out for the extracts against each of the tested organisms. After incubation the diameters of the resultant growth inhibition zones were measured.

3. Results and discussion

Table 1 Results of phytochemical screening of *Xiromphis nilotica* bark

Secondary metabolites	Reagent	Successive method of extraction			
		Aqueous	Methanol	Ethyl acetate	Ethanol
Alkaloids	dragendroffs	+	+	+++	+++
	Winger	+	+	+++	+++
	Hager	+	+	+	+
Flavonoids	KOH	-	++	++	+++
	NH ₄ OH	-	++	++	+++
	ALCL ₃	-	++	+++	+++
	Mg	-	++	+++	+++
Saponins	Foam test	+++	+++	-	+++
Phenols	Ferric chloride	++	+++	-	+++

Sterols & Triterpenes	Liebermann's	+	++	++	+++
	Salkowski	+	++	++	+++
Tannins	Ferric chloride test	++	+++	+	+++
	Gelatin test	++	+++	+	+++
Carbohydrates	Molisch,H ₂ SO ₄	+++	+++	+	+++
Proteins	Biuret reagent	-	-	+	++
Amino acids	ninhydrin	-	-	+	+

Key: Very high=(++++), High= (+++), Moderate= (++) , Trace amount= (+) and absent= (-).

Phytochemical screening for major constituents was undertaken using standard qualitative methods of [16]. The plant extracts were screened for the presence of (Alkaloids, Carbohydrates, Tannins, Phenols, Sterols &Triterpenes, Saponins, Flavonoids and Amino acids).

Table 2 Results of antibacterial activity of *Xeromphis nilotica* bark

Extracts	Concentrations	Zone of inhibition in diameters (mm)			
		<i>Escherichia coli</i>	<i>pseudomonas aeruginosa</i>	<i>staphylococcus aureus</i>	<i>Bacillus subtilis</i>
Ethyl acetate	100	17	17	16	16
	50	16	15	14	15
	25	15	14	13	14
	12.5	14	13	12	13
Ethanol	100	18	19	18	19
	50	17	17	16	18
	25	16	15	15	16
	12.5	15	14	14	15
Methanol	100	21	20	20	19
	50	18	19	19	18
	25	17	18	18	17
	12.5	15	16	14	15
Aqueous	100	15	15	14	15
	50	14	13	13	14
	25	13	13	12	13
	12.5	12	12	11	12

Key:Concentrations of extracts (100, 50, 25, 12.5mg/ml) .Zone of inhibition in (mm), - no inhibition, <9mm inactive, 9-12mm partially active, 13-18mm active, >18mm very active.The methanol and ethanol extracts showed high inhibition zone between (21-18)against four tested microorganisms (*E.c*, *S.a*, *B.s*, and *P.s*),

The Aqueous extract was recorded low inhibition zone between (15-11) comparing with other extracts.

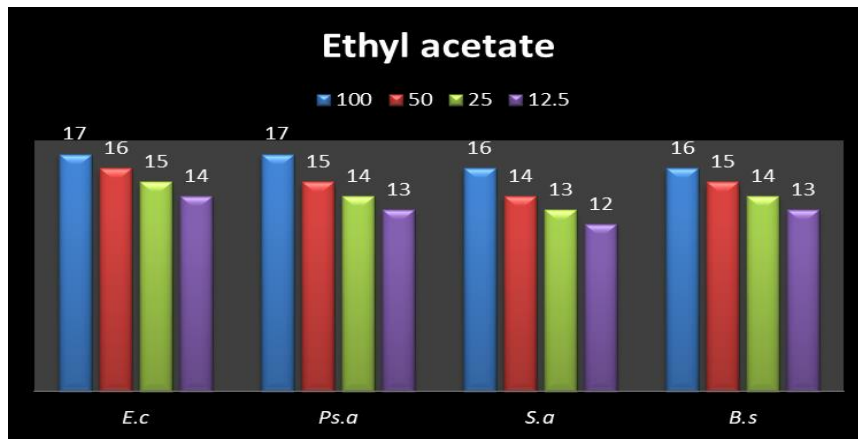


Figure 1 Antibacterial activity of ethyl acetate extract of *Xeromphis nilotica* bark at Concentrations (100, 50, 25 and 12.5). Each bar represent zone of inhibition in diameters (mm)

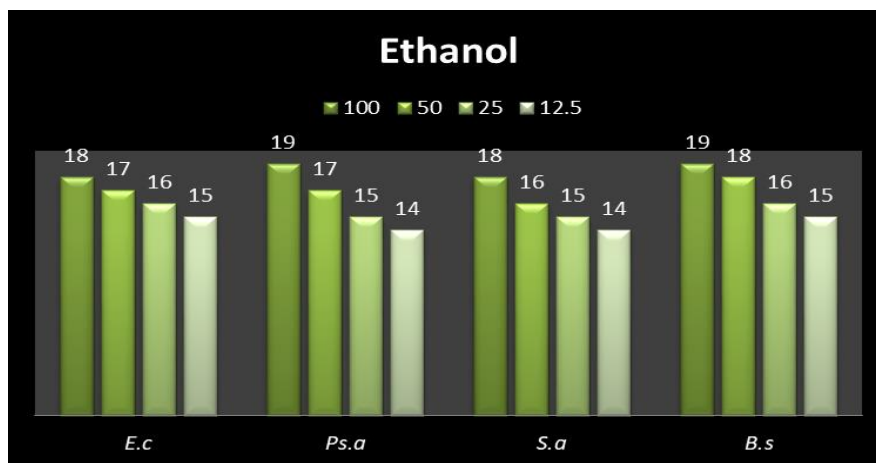


Figure 2 Antibacterial activity of ethanol extract of *Xeromphis nilotica* bark at Concentrations (100, 50, 25, and 12.5). Each bar represent zone of inhibition in diameters (mm)

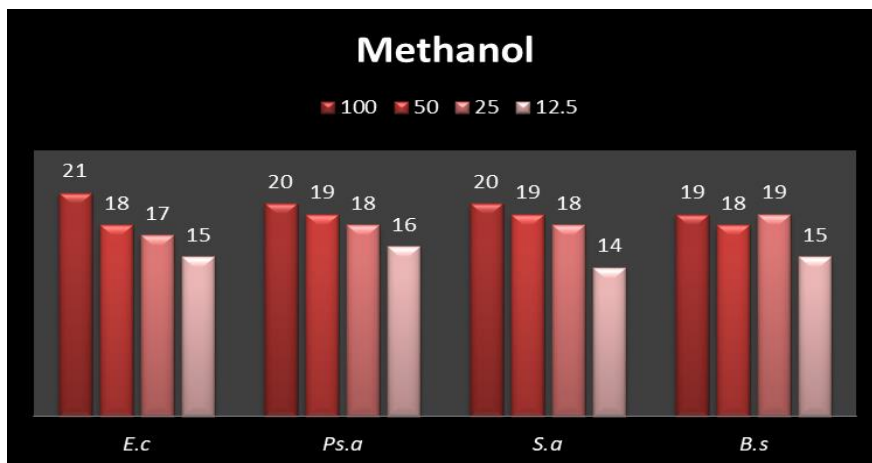


Figure 3 Antibacterial activity of methanol extract of *Xeromphis nilotica* bark at Concentrations (100, 50, 25 and 12.5). Each bar represent zone of inhibition in diameters (mm)

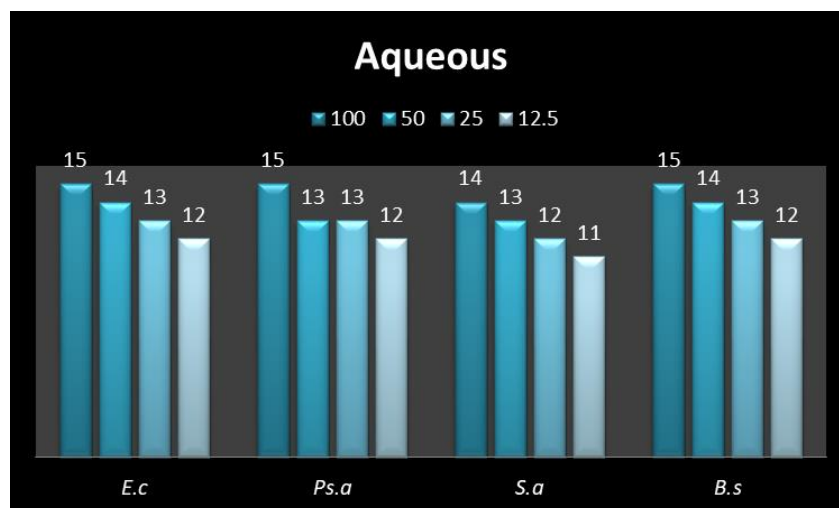


Figure 4 Antibacterial activity of aqueous extract of *Xeromphis nilotica* bark at Concentrations (100, 50, 25, and 12.5). Each bar represent zone of inhibition in diameters (mm)

4. Discussion

Phytochemical analysis provided significant ideas for the development of new herbicides and drugs against deadly diseases. Natural products of wild plants still play a central role in the biological activities and healthcare system of large proportions of the world's population [18, 19]. The total of four extracts of *Xeromphis nilotica* bark (Methanol, Ethanol, Ethyl acetate and aqueous) were used to evaluate the antibacterial profile and phytochemical screening of the plant. The *Xeromphis nilotica* bark found to be good source of (Alkaloids, Flavonoids, Carbohydrates, Tannins, Phenols, Sterols & Triterpenes, Saponins and Amino acids). The antibacterial assay shows that all of the extracts were found to be active against the tested pathogens, but the aqueous extract of the plant shows minimum activity against *staphylococcus aureus* at concentration 12.5 when compared with other extracts. The presence of different phytochemicals in the plant is the possible answer for its active antibacterial profile.

5. Conclusion

It was concluded from the present study that the crude extracts of *Xeromphis nilotica* bark has significant inhibitory effect against both gram positive and gram negative bacterial culture. Among different extracts methanolic extract was very effective. The extraction bark of *Xeromphis nilotica* used in this study showed the significant phytochemicals and the bark contained high amounts of secondary metabolites. In general the bark extracts has better chemical characteristics.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

Authors have no any conflict of interest.

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