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Endangered medicinal plant of aloe and its antibacterial activity

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Abstract
Aloe castellorum and *Aloe pseudorubroviolacea* of the genus Aloe belong to the family Asphodelaceae was found to be highly endangered in the Arabian Peninsula. It has rich medicinal properties and also very important to human health. It was found to be threatened species also remain only at very few localities and highly endangered in the Arabian Peninsula. Described here morphology character and antibacterial activity. The two plant extracts having significant antibacterial activity, meanwhile the methanolic extract of *Aloe castellorum* and *Aloe pseudorubroviolacea* shows higher antibacterial activity than other extracts.

Keywords: endangered medicinal plant, antibacterial activity, asphodelaceae

Introduction

The Aloe extracts are medically significant and their application have been used to treatment of gastrointestinal conditions, various ailments, antimicrobial, skin cancer, heart attacks, arthritis, leukemia, psoriasis, eczema, burns, digestive problems,high blood pressure and diabetes in worldwide (Hossain *et al*., 2013, Maharjan and Laxmipriya, 2015.,Miller and Morris, 2004.,Watt and Breyer-Brandwijk, 1962) [7, 14, 21, 6]. Infectious diseases caused through fungi and bacteria are among the major causes of illness and mortality globally. Since dried leaf exudates of *Aloe* species have been used as mainstay therapy for both humans and animals in various parts of the world. Few studies about provide evidence that *Aloe vera* secure to utilize and donot cause poisonous impacts (Sehgel *et al*., 2013). Since aloin and its metabolites exhibit antimicrobial properties, it is reasonable to conduct safety assessment of aloe-derived products for intestinal microbes, as well. Previous rodent studies have provided evidence that administration of aloe leaf or purified polyphenolic compounds causes lesions, cytotoxicity (Xia *et al*., 2007 [9]; Boudreau *et al*., 2017) [9]. Aloe has a history of traditional use by Native Americans for stomach disorders and intestinal disorders including constipation, hemorrhoids, Colitis and colon problems.

There are more than 600 known species of Aloe (Family Liliaceae) (Kawai *et al*., 1993) [16], many of which have been used as botanical medicines in many countries for thousands of years. The efficacy of Aloe liquid as an antibacterial agent is shown to have a wide range against Gram positive and Gram-negative bacteria. The antimicrobial agents of *Aloe vera* gel was reported to effectively kill or greatly reduce or eliminate the growth of *Staphylococcus aureus, Klebsiella pneumoniae, Streptococcus pyogenes, Pseudomonas aeruginosa, Escherichia coli, Propionibacterium acne, Helicobacter pylori* and *Salmonella typhi*. (Zawahry *et al*., 1973; Bashir *et al*., 2011) [12, 13].

For example, *Aloe emodin* (AE), a naturally derived from plant anthraquinone, is described to have potential ant proliferative activity in different cancer cell lines (Suboj *et al*., 2012) [17]. A number of reports are available on the microbial activity of the hexane, ethanolic, methanol, acetone, petroleum ether; ethyl acetate extracts *A. vera* gel and leaves. Although a lot of works have been carried out on the biological activity of *A. vera* gel, there is a lacuna on the exercise of DMSO as solvent for the phyto-constituents (active compounds) extraction. Dimethyl sulfoxide (DMSO) is an organosulfur compound. It is an important polar aprotic solvent that dissolves both polar and nonpolar compounds and is miscible in a wide range of organic solvents as well as water.

There are no studies and examination of these *A. castelorum* and *A. pseudorubroviolacea* in terms of uses, but the species of the genus Aloe are used globally in medicines. and cosmetology. There are a lot of research and published papers that has addressed the importance of Aloe species, especially widely known species Aloe vera (Qadir, 2009; Yadav, 2017) [18, 19].

About 50 Aloe species where distributed in the Arabian Peninsula and many of them are endemic to Arabian Peninsula, among the remarkable studies on Aloes of Arabia (Lavranos, 1965) [20]. *Aloe castellorum* of the genus Aloe belong to the family Asphodelaceae was found to be highly endangered in the Arabian Peninsula. It has rich medicinal properties and also very important in cosmetic industries. It was found to be threatened species and remain only at eleven localities and highly endangered in the Arabian Peninsula.

Aloe pseudorubroviolacea is one of the 14 endemic Aloes of Saudi Arabia, recorded from Jabal Radhwa, Raidah and near Ajalta (Collenette, 1999) [3], while *A. castellorum* is recorded from north Abha and Jabal Fayfa (Collenette, 1999) [3]. *Aloe pseudorubroviolacea* is closely contact with local community, two species near the settlement zones such as the endemic *A.pseudorubroviolacea* and *A.castellorum* are extremely threatened in Albaha, Saudi Arabia. Here, in this study, we described regionally endangered plant of *A. castellorum* and *A. pseudorubroviolacea* of morphological

characters and antimicrobial properties (Aklabi *et al*., 2021; Al-Khulaidi *et al*., 2018) [1].

Materials and methods

Plant sampling

Endangered plant specimens *A. castrollum* and *A. pseudorubroviolacea* were collected from Al-Baha region of Saudi Arabia its GPS Latitude and Longitude coordinate is about 19.8800, 41.5790, 19.8345°0′N 41°30′E. The plant was identified by Taxonomist, at Department of Biology, College of Arts and Sciences, Al - Baha University.

Plant Extraction

The selected plant leaves were dried at room temperature. Appropriate quantities (5000 mg) of the leaves and in plant were using various solvent (aqueous, ethanol, acetone and methanol) extraction with 5 times. The collected extract was centrifuged at 5000*g* for 10 min at room temperature, then the supernatant of methanol was prudently pipetted out transferred to sterilized eppendorf tubes without disturbing the inter-phase residues for further analysis (Ahmed *et al*., 2013).

Bacterial strains

Bacterial strains of *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27584), *Salmonella typhimurium* (ATCC 14028), *Staphylococcus aureus* (ATCC 29213), *Staphylococcus epidermidis* (MTCC 3615) MRSA (ATCC 43300). All the identified cultures were transferred on nutrient agar medium and kept at −20°C for further study.

Antibacterial assay

The aliquots were prepared with respective solvents and tested for their antibacterial activity against human pathogens according to the agar diffusion assay described by Idhayadhulla. In brief, sterile Mueller Hinton agar was dispensed into Petri plates. For the antimicrobial assay, inoculums were prepared from overnight cultures by the direct colony method. Distinct colonies were picked up directly from the plate with a sterile wire loop and suspended into sterile 0.85% saline. The turbidity of suspension to be inoculated was adjusted in line with 0.5 Mc Farland standard. Afterward, test organisms were uniformly swabbed over the Mueller-Hinton agar (Hi-Media, Mumbai) surface. Five-millimeter diameter well was made on the seeded surface using a sterile Cork borer and 50 µL of the appropriate extract of a known concentration (5 mg/ml) was placed inside the wells. Organic solvents used for the extraction were used as negative controls. The Petri plates were incubated for 24 hr at 37 ◦C and the inhibitory activity was measured by calculating diameter (millimeter) of inhibition zone around the wells. The zone of inhibition ≥ 8 mm was considered active. The assays were conducted in triplicate to validate the findings statistically.

Determining the minimum inhibitory concentration and minimum bactericidal concentrations

The broth dilution method was used for finding the minimum inhibitory concentrations and minimum bactericidal concentrations.

The dosing range of plant extracts was calculated by a factor of 2 (antilog 0.3). MICs were recorded as the lowest concentration of plant extracts that prevented visible growth as indicated by the absence of turbidity in line with the control. To measure the minimum bactericidal concentration.

Results and Discussion

Antibacterial activity

The present study provides evidence that Aloe exhibits antibacterial properties, the work was carried out for *Aloe castellorum* (AC) and *Aloe pseudorubroviolacea* (AP) against gram positive and gram negative bacteria (*Escherichia coli*¸ *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *MRSA*) various solvent extract were used screening such as aqueous, ethanol, Acetone, and methanol for this screening, positive control was used for comparison. (Table 2, Fig2, Fig 3). All extracts were respectable active against Gram-positive/negative bacteria Among them methanol extract was highly active against Escherichia coli compared with other solvent extract with MIC values 2 ul. (Table 2).

The average antibacterial activity against bacterial strains ranged from 8 to 23 mm (the zone of inhibition).The maximum zone of inhibition was obtained at 50 µL of Methanol Extraction of *Aloe castellorum.* The various solvent extract of *Aloe castellorum* showed zone of inhibition such as 14, 10, 12, and 23 mm corresponding to aqueous, ethanol, acetone and methanol extract against *E. coli* compared with positive control. Similarly, the maximum zone of inhibition was obtained at 50 µL of Methanol Extraction of *Aloe pseudorubroviolacea*. The Ap highest zone of inhibition noted 18 for *S aureus*.

Therefore, overall results, the methonal exacta of *Aloe castellorum* and *Aloe pseudorubroviolacea* highly active in all bacterial species.

Aloe pseudorubroviolacea was also reasonable active against all bacterial (Fiq. 2). The active of the *Aloe castellorum*, the methanol exacted of *Aloe castellorum* was highly active against E. *coli*,23mm (MIC 2 ul), Methanol exact of *Aloe castellorum* was also highly active (16mm; MIC: 8 ul, 12mm; MIC: 8ul) against *S.aureus* and *Staphylococcus epidermidis*. The active of the *Aloe pseudorubroviolacea*, the methanol exacted was highly active E. coli, (19mm; MIC: 2ul) compared with positive control and also highly active against *Staphylococcus aureus* (18mm; MIC: 4 ul) than positive control.

Fig 1a. *Aloe pseudorubroviolacea*

Fig *1b. Aloe castellorum*

Fig 1: Morphology of *Aloe Castellorum* and *A. Pseudorubroviolacea*

Table 1: Morphological comparison of *A. castellorum and A. pseudorubroviolacea*

Characters	*A. castellorum*	*A. pseudorubroviolacea*
Color of tender branches	pale green to darken green	The foliage is red/purple and blooms in the summer/spring
Tree trunk	The stiff, barely visible trunk 3 feet height	2 foot wide rosettes of thick andheavy stems and inflorescences of orange-red flowers.
Petiole	Absent	Absent
Pedicel (in flower)	spikes rise about 2 feet above the leaves bearing waxy greenish yellow flowers that at first point outwards as a bud then lie flat against as spike.	The flowers are produced in inflorescences of orange-red flowers.
Distribution	Saudi Arabia, Yeman,	Yemen to north of Yanbu al-Bahr(John Lavranos in 2000)

Table 2: Zone of inhibition of *Aloe castellorum and Aloe pseudorubroviolacea*

		Aloe Castellorum					*Aloe Pseudorubroviolacea*			
		PC	Aqu	Etha	Ace	Meth	Aqu	Etha	Ace	Meth
Gram-negative	*E. coli*	35	14	10	12	23	10	8	8	19
	P. aeruginosa	22	8	-	10	12	-	-	6	8
	S typhimurium	35	12	9	12	14	12	6	12	10
Gram-positive	*S aureus*	22	10	8	10	16	-	6	-	18
	S epidermidis	24	6	10	6	12	10	8	6	16
	MRSA	35	10	8	12	14	-	10	8	14

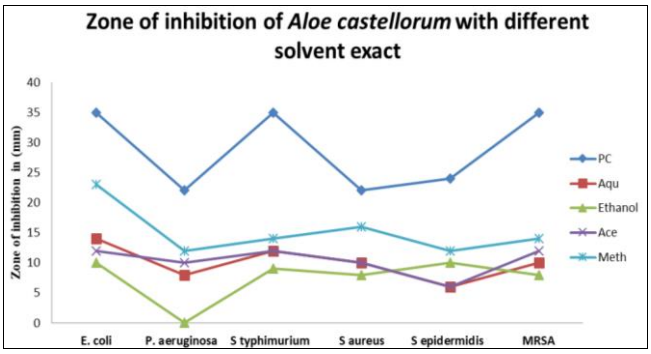

Fig 2: Zone of inhibition of *Aloe castellorum* with different solvent exact

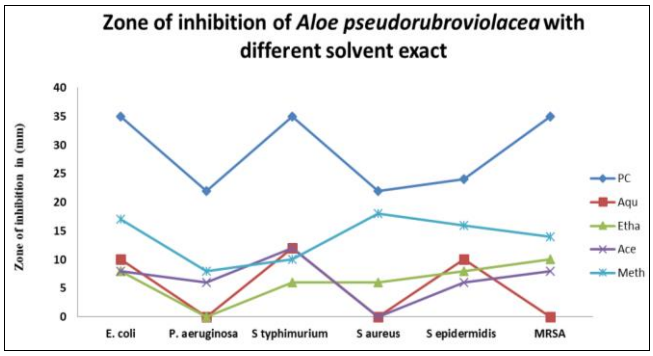

Fig 3: Zone of inhibition of *Aloe pseudorubroviolacea* with different solvent exact

Conclusion

The two plant morphologically identified and extracts having significant antibacterial activity, meanwhile the methanolic extract of *Aloe castellorum* shows higher antibacterial activity than *Aloe pseudorubroviolacea* extract. Therefore, the methanolic extracts of medicinal plants *Aloecastellorum* and *Aloe pseudorubroviolacea* having potential antimicrobial chemical constituents itself for the development of new era for drugs. As per our literature reports, till date there is no scientific investigations found on species *Aloe castellorum* and *Aloe pseudorubroviolacea*.

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