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RESEARCH ARTICLE

Evaluation of Antimicrobial Activity of *Acacia etbaica* Water Extract Leafs against Some Pathogenic Microorganisms

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ABSTRACT

The antimicrobial activity about water extract of *Acacia etbaica* was examined by using agar well diffusion methods against five gram-positive and negative bacteria [*Staphylococcus aureus* (ATCC653-8), *Pseudomonas aeruginosa* MTCC2453, *Bacillus cereus* (ATCC6633), *Escherichia coli* MTCC739, and one local isolate (*Staphylococcus epidermidis*)] in addition to *Candida albicans* (ATCC2019). this results designated that the water extract of *Acacia etbaica* possess antimicrobial efficacy against all tested microorganisms either (gram-positive and negative bacteria) or fungi (*Candida albicans*). Whereas the gram-positive bacteria (*S. aureus*, *S. epidermidis* and *B. cereus*) with inhibition zones (21, 19.5 and 16.5) respectively was more sensitive than gram-negative bacteria (*P. aeruginosa* and *E. coli*) and *C. albicans* with inhibition zones 16 and 14.5 respectively. The antimicrobial effect was directly proportional with concentration of water extract where the highest inhibition zone at higher concentration 30% equal 21mm against *S. aureus* and the lower inhibition zone at lower concentration 5% equal 10mm against *C. albicans*

INTRODUCTION

The *Acacia etbaica* Schweinf subspecies *etbaica*, family: *Leguminosae* is a one of the most widespread plant in Yemen, locally known as 'Qarad' [1-3] has been reported that several *Acacia* species have been used to prepare disinfectant for microorganisms and for hands washes. Yemen has a rich culture of medicinal herbs, but only very few have been studied chemically and pharmacologically for their potential medicinal value [1]. Very few works has been carried out on the leaves of this plant toward documenting its ethnomedicinal uses and establishing its chemical constituents [4]. In Yemen it used as medicinal plants in traditional healthcare and well known in rural areas in many developing countries [5]. According to World Health Organization medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. Therefore, such plants should be investigated to better understand their properties, safety and efficiency. For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies [6]. Medicinal plants produce a variety of compounds such as phenolics, flavonoids, alkaloids, glycosides, saponins essential oils, mucilages and tannins in all of their parts (root, stem, bark, leaves, seeds) which possess a healing physiological effect in the treatment of various

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diseases of animals and humans [7]. The tribal people use the plants in their unique ways for various purposes mostly for the treatment of various diseases [8]. It has been reported that the plant *Acacia sp* was used to treat swelling, ring worm infection, hemorrhoids, scabies, fire burn, eye infection of livestock and anthrax by the community of Kilde Awulaelo District of Tigray Region, Ethiopia [9]. The fresh plant parts of different *Acacia* species are considered as astringent, spasmolytic, demulcent, anthelmintic and abortifacient in Indian traditional medicine system [10,11]. The *Acacia* species used to treat a variety of disease including malaria, leprosy and most concerning cancer [12]. Acacias have been cultivated in many countries for various purposes whereas it have a valuable wood that can be used for industries, decorations and as an important sources for gum (Arabian gums), tannins, perfumes, ink, proteins and paint [13,14].

This research aimed to investigate the potential antimicrobial activity of *Acacia etbaica* to ascertain the rationale for its use in traditional medicine.

MATERIALS AND METHODS

Preparation of sample

Sample collection: The *Acacia etbaica* leaves were collected from Yemeni rural - Bani Alhusam -Taize government and identified according to guideline of the [15,16]. The collected plant leaves were dried in an open air protected from direct sun light. The dried plant samples finely ground by using an electrical grinder to a fine powder and made ready for extraction.

Preparation of extract: The fine powder of *Acacia etbaica* was extracted by using 1:10 in purified water at room temperature for 48 hours with occasional shaking. Then filtered by using filter paper (Whatman No 1.5, whatman Ltd., England). The water extract was evaporated to dryness in oven at 45°C to yield dry crude extract. The obtained crude extract was filled to amber tight closed bottle glass in refrigerator until uses.

Antimicrobial assay

Microorganisms used include: The tested microorganisms have gotten from Science college, Sana'a university, Republic of Yemen include: *Escherichia coli* MTCC739 Mar.2001, *Pseudomonas aeruginosa* MTCC2453 Mar.2001, *Staphylococcus aureus* (ATCC653-8), local isolate (*Staphylococcus epidermides*), *Bacillus cereus* (ATCC6633) and *Candida albicans*(ATCC2019).

The bacterial strains were maintained on Trypton Soya Gar Medium (TSDA), and the fungal strains were maintained on Sabouraud dextrose agar (SDA) medium.

Antimicrobial and Antibiotic assay: The Antimicrobial and Antibiotic assay was performed by using Agar Well Diffusion Method according to (USDA, 2007 and Mahesh & Satish, 2008).

Antimicrobial assay: Four different concentrations of *Acacia etbaica* water extract 300 mg/ml (30%), 200 mg/ml (20%), 100 mg/ml(10%) and 50 mg/ml(5%) were dissolved in sterile purified water to be used in antimicrobial assay test. Extract solutions were prepared directly before the test. Antimicrobial activity of the extracts was determined by agar well diffusion method as described by (USDA, 2007 and Mahesh & Satish, 2008) on Tryptone soya Agar (TSA) (Figure 1).

In each of these plates four wells were cut out by using a standard cork borer (8 mm). About 60µl of each extract was added into different wells (triplicate each concentration). A positive control antibiotic disc was placed in the other plate. All the plates were incubated for 24h at 37°C. After incubation bioactivity was evaluated by measuring zone of inhibition. All the experiment was performed in triplicates.

Antibiotic assays: The Antibiotic assay was performed by using antibiotic standard disc diffusion. The antibiotic standard disc was get from HiMedia & oxoid company which are Aztreonam (AT30), Ceftazidin (CAZ30, Gentamycin (Gen10), Nystatin (Nys100IU), Norfloxacin (NOR5) and Vancomycin (VA30).

RESULTS

Antimicrobial Activity of water extract

The results in present study showed that the water extract to *Acacia etbaica* possess high antimicrobial efficacy against tested microorganisms (gram positive and negative bacteria) in addition to *C. albicans*. results appeared that the tested gram-positive bacteria more sensitive than tested gram-negative bacteria and *C. albicans* where the highest effect was against *S. aureus* with inhibition zone equal 21 mm at high concentration(30%) followed by *S. epidermides* and *B. cereus* with inhibition zone 19.5 and 16.5mm respectively. whereas the gram negative bacteria (*P. aeruginosa* and *E. coli*) as well as *C. albicans* was low sensitive to these extract with inhibition zone 16 and 14.5mm respectively. results illustrated that the highest concentration (30%) was more effect against *S. aureus* and lower effect at lower concentration (5%) against *C. albicans*. these effected results showed in table 1.

DISCUSSION

In water extract results showed a high antimicrobial activity at all concentrations against *S.aureus*. While, showed a low antimicrobial activity against *E.coli* at all concentrations. This results were agreed with previous study conducted by [17-19] who reported that the highest activity against *S.aureus*. Regarding to *E.coli* was agree with the results conducted by [18,19] while disagree with results by [17]. In recent study, *B.cereus* as well as *P.aeruginosa* showed sensitive to water extract of *A.etbaica* and this results agree with study conducted by [17] while disagree with results conduct

Figure 1 Appear Inhibition zone diameters of *Acacia etbaica* water extract against tested microorganisms.

Table 1: Water extract activity of *Acacia etbaica* against tested microorganism at different concentrations.

Type of microorganism	Concentration %				Name of antibiotic standard					
	5%	10%	20%	30%	AT ₃₀	CAZ ₃₀	NOR ₅	Gen ₁₀	VA ₃₀	Nys _{100IU}
	Zone of inhibition in mm									
<i>Staphylococcus aureus</i>	16	16.5	20	21	0	23	20	22	21	NT
<i>Staphylococcus epidermidis</i>	15	16	19	19.5	0	19	20	23	22	NT
<i>Bacillus cereus</i>	12	12.5	15	16.5	0	24	30	27	30	NT
<i>Pseudomonas aeruginosa</i>	12	12.5	14	16	24	21	12.5	21	0	NT
<i>Escherichia coli</i>	11.5	12	15	16	29	27	26.5	19.5	12	NT
<i>Candida albicans</i>	10	12	14	14.5	0	0	0	0	0	13.8

by [18] who evaluated other species of *Acacia* (*A. nilotica*). Mechanism that the crude plant extract acting against gram-positive and gram-negative bacteria by changes in membrane potential leading to hyperpolarization and decreasing cytoplasmic pH [20]. This changes that occurred in the internal pH and Hyperpolarization has been suggested to be one of the primary indicators of membrane damage of bacteria cells [21,22]. The differences in sensitivity of tested microorganisms may be return to different in concentration of extracts and microorganisms strains [19,23] was attributed the difference in the sensitivity of microorganisms to the differences in the ability of the active ingredients in the plant to penetrate through the cell wall and cell membrane of the tested microorganisms. In the former study by [4] on the phytochemical constituents of *Acacia etbaica* who evaluated that many of phytochemical compound like saponins, flavonoids, coumarins, tannins, triterpenes, sterols, amino acid and protein, sterols, triterpenes, carbohydrates and glycosides found in all extracts including water extract. This

chemical compounds play essential role in its bioactivity. The antibacterial activity of flavonoids is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls [24]. Terpenes are biologically active molecules and are considered to be part of plants defence systems and as such have been included in a large group of protective molecules found in plants named as 'phytoprotectants' [25]. The results of this study revealed that the gram-negative bacteria as well as *C. albicans* was more resistance to *A. etbaica* water extract than gram-positive extract these differences in sensitivity return to variations in chemical composition of microbial cell wall where the major components of the outer surface of gram-negative bacterial cell wall compositions is lipopolysaccharide layer with the combination of proteins and phospholipids, where heavy lipopolysaccharide layer hindered of most compounds from Access to the peptidoglycan layer of the cell wall [26,27] has reported that other genus of *Acacia* (*Acacia nilotica*) contain all the essential amino acids in good proportions

comparable to egg protein. Mechanism of action plant may be the formation of ion channels in the microbial membrane through diverse molecular modes and bind to chitin or increasing the permeability of the fungal membrane and cell wall. This defense system of plants provide protection them against potential microbial invaders. Among these other plants secreted compounds several proteins or peptides and carbohydrates have a specific defense mechanism against phytopathogenic microbes by inhibiting their growth [24].

CONCLUSION

The high antimicrobial clear efficiency to water extract of *Acacia etbaica* leaves against many of microbial strains that causes different important diseases to human and animal confirming the traditional herbal medicines usages and provide a scientific basis for traditional medicine uses of water extract of *Acacia etbaica* leaves in primary health care.

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