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Research Article

ANTIMICROBIAL PROPERTIES OF METHANOL EXTRACT OF *BASSIA MURICATA* GROWING IN ARID ZONES IN QASSIM, SAUDI ARABIA

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ABSTRACT
Areal parts of a neglected desert plant; *Bassia muricata* was investigated for its antimicrobial properties during the flowering season. Antimicrobial investigation was carried out using disc diffusion method against 4 gram-positive and 3 gram-negative bacterial strains, besides 1 fungal and 1 yeast strains. Results revealed that the crude methanol extract of *Bassia muricata* leaves exhibited moderate to weak antibacterial activity on gram-positive bacteria (11.5±1.5 to 8.5±1. 5 mm zone of inhibition), weak antibacterial activity on gram-negative bacteria (8.5±0. 5 to 7.5±0.5mm zone of inhibition) and no antifungal effect on the tested fungal and yeast strains. These findings would open up the scope for further analysis using different plant parts, solvents and sample collection from different seasons to explore more bioactive properties of this desert plant.

Keywords: *Antibacterial, Antifungal, Bassia muricata, Crude, Extract*

INTRODUCTION
The use of natural products and medicinal plants as drugs are dates back to the first human community appeared on Earth since 60,000 years ago (Fabricant and Farnsworth, 2001). Human being since that time used plants in nutrition, building of houses, knitting clothes, medication, ceremonies, cosmetics, and even in magic (Elsharkawy *et al*., 2018). The era of modern drugs arisen in the beginning of the nineteenth century, initially depended on medicinal plants and then turned to synthetic chemical drugs which led to noticeable decline in the use of plants in modern medicine (Yuan *et al.,* 2016). Recently, under the consequent failures and serious side effects of modern synthetic drugs, the interest and use of raw medicinal plants, herbal prescriptions and food supplements are rapidly growing all over the world, regardless any concern about their possible risk factors and the misuse of these natural products (Ekor, 2013). Accordingly, screening of medicinal plants to understand more about their bioactivity are of great value. Plant produced many phytochemical compounds known as secondary metabolites, these compounds have important roles in its defense mechanisms against herbivorous animals, insects, and microorganisms (Bennett and Wallsgrove, 1994). *Bassia muricata* (*B. muricata*) is a desert plant belong to family Chenopodiaceae, little is known about this plant, however some studies reported that it is used in traditional medicine to treat renal and rheumatic diseases (Kamel *et al*, 2001). *B. muricata* is rich in some bioactive phytochemical compounds such as polyphenols and flavonoids (Abu Ziada *et al.,* 2015). The current study aimed to investigate the potential antimicrobial activity of a neglected dessert plant (*B. muricata)* from an arid zone in Arabia peninsula (Qassim, KSA) during the short rainy season.

MATERIALS AND METHODS
A) Collection of plant materials
Fresh aerial parts of *B. muricata* were collected manually from the field after botanical identification by the first author (Alaa M.M. Sadeek), It was collected in the rainy winter season on October 2016, from the arid areas near Al-Rass town in Qassim district, Saudi Arabia where *B. muricata* grown wild and profusely during that season.

B) Plant extraction

Aerial parts of *B. muricata* were dried in shade, dried leaves were crushes and ground into a powder, the dried powder was extracted with 80% methanol (400 ml MeOH/100 H2O) using Soxhlet extractor for 16 hours at 90°C. The polar extract was evaporated at low pressure to collect crude methanol extract and the semi-solid crude extract was left in oven for around 10 hours at 40 °C to get a solid crude, which was kept in a dark container for antimicrobial analysis.

c) Antimicrobial test

The antimicrobial activity of the crude extract of *B. muricata* was estimated using Kirby-Bauer disc diffusion method as mentioned in (Abdallah *et al*., 2017) with minor change, against different gram-positive bacteria, gram-negative bacteria and fungi, namely; *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 49461, *Enterococcus faecalis* ATCC 29212, *Bacillus cereus* ATCC 10876, *Escherichia coli* ATCC 35218, *Klebsiella pneumoniae* ATCC 700603, *Klebsiella pneumoniae* ATCC 27736, *Aspergillus niger* ATCC 6275 and *Candida albicans* ATCC 10231. The tested microbial strains were first sub-cultured in Mueller-Hinton broth for 24 hours for bacteria or Sabouraud dextrose broth for fungi for 48 hours, then the growing broth were kept in the refrigerator (4^{o}C) until used within the same day to test the microorganisms during their exponential phase. On the other side, bottles containing 20 ml of Mueller-Hinton agar for bacteria or Sabouraud dextrose agar for fungi were autoclaved and poured hot on sterile Petri-dishes (diameter of the plate is 90 mm) and left at room temperature until solidified. Sub-cultured microbial strains were adjusted to McFarland standard, 100 µl from each microbial strain was poured on a Mueller-Hinton plate and spread over the agar using sterile cotton swap. Discs saturated with about 15 µl/disc of the reconstituted crude extracts (500 and 250 mg/ml) of *B. muricata* were placed above the seeded agar plates. Gentamicin disc (10µg/disc) and clotrimazole (15µg/disc) was added to plates containing Mueller-Hinton and Sabouraud dextrose agar, respectively. The antibiotic discs served as positive control. As well, discs saturated with 10%DMSO which was used to reconstitute the crude was placed above the tested plates and served as negative control. Plates were incubated for 24 hours at 37°C for bacteria or for 48 hours at 25-28 ^{o}C for fungi. After incubation, plates were inspected for a clear inhibition zones around the discs, which are measured in nearest millimeter and recorded. The test was repeated twice and mean ± standard error of means was calculated.

RESULTS AND DISCUSSION

Results of the antimicrobial properties of the crude extract of *B. muricata* are represented in (Table 1) and (Figure 1), which indicated moderate to weak antibacterial activity at the concentration 500 mg/ml of the crude methanol extract against the gram-positive bacteria, weak antibacterial activity against the gram-negative bacteria and no activity against the fungal strains, compared to the tested antibacterial and antifungal drug. These average effects of *B. muricata* are decreased at the lower concentration (250 mg/ml). However, among the gram-positive bacteria, *Enterococcus faecalis* was the most susceptible (11.5±1.5 mm), followed by *Staphylococcus aureus* (11.0 ±1.0 mm), *Staphylococcus epidermidis* (10.5±0.5 mm) and *Bacillus cereus* (8.5±1.5 mm), respectively. Whereas, *Klebsiella* spp. recorded weak susceptibility ranged between 8.5±0.5 to 8.0±0.0 mm. Whereas, *Escherichia coli* recorded the weakest susceptibility (7.5±0.5 mm). On the other side, *Aspergillus niger* and *Candida albicans* exhibited no susceptible at all against the crude methanol extract of *B. muricata*. These results can be clearly seen when omitting the diameter of the paper disc (6 mm), as shown in (Figure 1). Further studies are needed to isolate the bioactive components of this plant. Perhaps, the antibacterial compounds are present in low quantity in the methanol crude and isolation if pure compounds might exhibit greater antibacterial activity. In general, little is known about the antimicrobial properties of this desert plant. Moreover, earlier reports on the antimicrobial activity of *Bassia muricata* showed different conflicting results; Bouaziz *et al.* (2009) reported that *Bassia muricata* showed no antimicrobial activity against different bacterial and fungal strains, except with hexane extract and ethyl acetate extracts, where only

Table 1: Antimicrobial activity of crude methanol extract of *Bassia muricata* against different microorganisms

Tested compound	Mean zone of inhibition (mm)*								
	Gram-positive bacteria				Gram-negative bacteria			Fungi	
	Sa	Se	Ef	Bc	Kp1	Kp2	Ec	As	Ca
B. Muricta 500mg/ml	11.0 ±1.0	10.5 ±0.5	11.5 ±1.5	8.5 ±1. 5	8.0 ±0.0	8.5 ±0.5	7.5 ±0.5	6.0 ±0.0	6.0 ±0.0
B. Muricta 250mg/ml	8.5 ±1.5	9.5 ±0.5	8.0 ±2.0	7.5 ±0.5	6.75 ±0.25	7.0 ±1.0	6.0 ±0.0	6.0 ±0.0	6.0 ±0.0
Gent. 10µg/disc	25.0 ±0.0	22.0 ±1.0	21.5 ±0.5	25.5 ±0.5	11.5 ±0.5	16.0 ±2.0	22.0 ±0.0	NA	NA -
Chlot. 15µg/disc	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	20.5 ±1.5	27.5 ±0.5
10% DMSO	6.0 ±0.0	6.0 ±0.0	6.0 ±0.0	6.0 ±0.0	6.0 ±0.0	6.0 ±0.0	6.0 ±0.0	6.0 ±0.0	6.0 ±0.0

*6.0 mm zone inhibition = no activity (diameter of paper disc), Gent.= Gentamicin (antibacterial), Chlot.= Chlotrimazole (antifungal), DMSO= Dimethyl sulphoxide, NA= Not applicable Sa=*Staphylococcus aureus* ATCC 25923, Se=*Staphylococcus epidermidis* ATCC 49461, Ef= *Enterococcus faecalis* ATCC 29212, Bc=*Bacillus cereus* ATCC 10876, Kp1=*Klebsiella pneumoniae* ATCC 700603, Kp2=*Klebsiella pneumoniae* ATCC 27736, Ec=*Escherichia coli* ATCC 35218, As=*Aspergillus niger* ATCC 6275, Ca=*Candida albicans* ATCC 10231

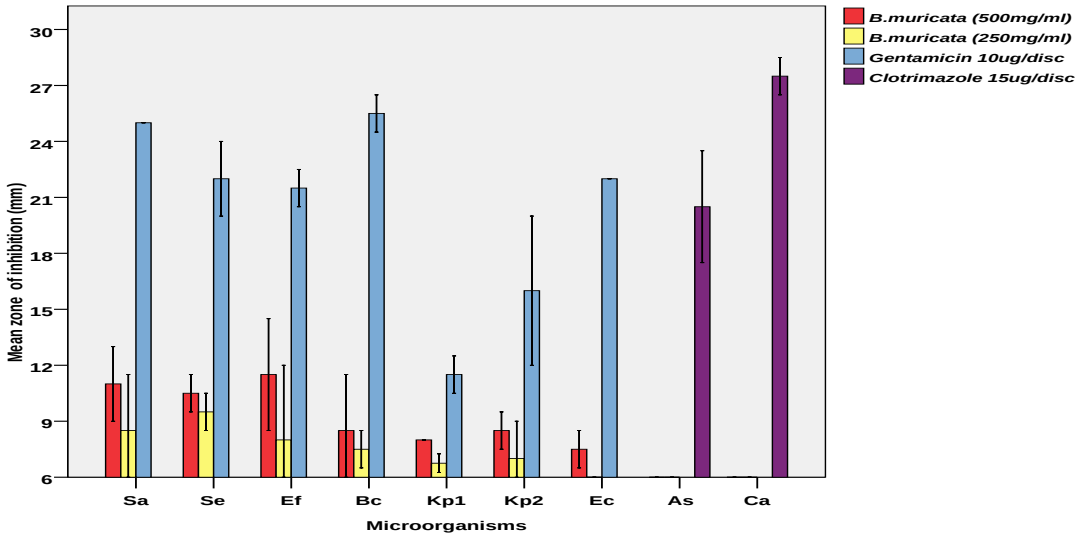

Figure 1: Susceptibility of different microorganisms to crude methanol extract of *Bassia muricata**

***** Sa=*Staphylococcus aureus* ATCC 25923, Se=*Staphylococcus epidermidis* ATCC 49461, Ef= *Enterococcus faecalis* ATCC 29212, Bc=*Bacillus cereus* ATCC 10876, Kp1=*Klebsiella pneumoniae* ATCC 700603, Kp2=*Klebsiella pneumoniae* ATCC 27736, Ec=*Escherichia coli* ATCC 35218, As=*Aspergillus niger* ATCC 6275, Ca=*Candida albicans* ATCC 10231

Pseudomonas aeruginosa recorded 11mm inhibition zone and with methanol extract *Escherichia coli* was only the susceptible one (10 mm zone of inhibition), and *Aspergilllus niger* (11 mm) were also susceptible. Chemsa *et al*. (2016) published that ethanol, butanol and ethyl acetate extracts of *B. muricata* showed varied degrees of antibacterial activity, however butanol extract possessed best antibacterial activity on both of gram-positive and gram-negative bacteria ranging from 9 to 0 mm zone of inhibition. Al-Yahya *et al.*, (1990) cited that the ether and benzene extracts of *B. muricata* have antimicrobial properties. In agreement with our findings, Chemsa *et al*. (2016) claimed that butanol extract (low polarity) of *B. muricata* was better in its antimicrobial activity than ethyl acetate extracts (high polarity). So, it is logic to accept that methanol extract, as a semi polar and higher in polarity than butanol, has a moderate antibacterial activity. Accordingly, we assume that the bioactive phytochemical components of this plant may vary; based on the solvents used in the extraction, geographical locations and seasons. A systematic and integrated study on *B. muricata* is badly needed.

Conclusion

On the basis of the results obtained in the current study, we conclude that the methanol extract of *Bassia muricata* showed no antifungal activity, and varied degrees of antibacterial activity ranging from moderate to weak activity. The study was conducted in the flowering rainy season, so another complementary study in the dry season is required which assumed to show different results.

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Conflict of interest
None

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