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## CHEMICAL ANALYSIS OF THREE SAUDI MEDICINAL PLANTS AND THEIR ANTIBACTERIAL ACTIVITY AGAINST MULTI-DRUG RESISTANT BACTERIA

Leena Zino<sup>1</sup>, Abdunaser Alsharaa<sup>\*2</sup>, Sahar Al-Dossary<sup>1</sup>, Afnan Alwadey<sup>1</sup> and Basheer Chanbasha<sup>2</sup>

Department of Biology<sup>1</sup>, University of Dammam, Dammam 31454, Saudi Arabia.

Department of Chemistry<sup>2</sup>, King Fahd University of Petroleum and Minerals, Dhahran 31261, Saudi Arabia.

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### Correspondence to Author:

**Abdunaser Alsharaa**

Chemistry Department, KFUPM  
Dhahran 31261, Saudi Arabia.


**E-mail:** asharaa@kfupm.edu.sa

**ABSTRACT:** The present work aims to examine the antimicrobial activity of three medicinal plant extracts grown in Saudi Arabia namely *Pulicaria crispa*, *Achillea fragrantissima* and *Cleome Africana* against 10 clinical isolates of multi-drug resistant bacteria (MDR) that belong to four species, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Klebsiella pneumonia*. The antibacterial potential of the methanolic plant extracts was assessed by the agar well diffusion method and expressed in terms of diameters of inhibition zone. The outcomes of these experiments indicated that each extract has shown an activity against four resistant isolates at least, with maximum zone of inhibition 19 mm recorded by *P. crispa* extract against *S.aureus* strain (1). As a new finding, it was observed that *C. Africana* extract has bioactivity as antibacterial agent against maximum number of resistant isolates (seven out of ten tested strains) including all tested isolates of *S.aureus* (ranged between 7 and 14 mm). Methanolic extract of *A. fragrantissima* demonstrated two considerable growth inhibition against one isolate of both *S.aureus* and *Ps. aeruginosa*. The chemical analysis of the three plants extracts was carried out using Gas Chromatography-Mass Spectrometry instrument (GC-MS). The results showed the presence of many active groups of phytochemicals such as Flavonoids, Glycerides, Tannins, Alkaloids and Steroids. The good activity of medicinal plants was originated from presence of these compounds.

**INTRODUCTION:** Over the past decades, many types of gram-negative bacteria have developed adaptation techniques against wide range of pharmacological antibiotics and chemical drugs<sup>1,2</sup>. Multi-drug resistant bacteria (MDR) are responsible for Nosocomial infection, tuberculosis and other serious health problems. In addition, using combination of antibiotics regimes has more disadvantages such as toxicity risks and economical trammels<sup>2</sup>.

Consequently, controlling the use of antibiotics became a major need in conjunction with developing new efficient treatments. For the mentioned reasons; the interest has increased to investigate the antimicrobial properties of the herbal products as an alternative therapy<sup>3</sup>.

For centuries, medicinal plants have been used as therapeutic agents for human diseases. They are considered as the best curing method with wide availability and less side effects. However, only 1% of the traditional medicinal herbs are recognized and examined by new scientists, while more than 10% of earth plants have been used as remedies in ancient times<sup>4</sup>. Many antibacterial activities have been recorded in many plant extracts with variety percentages of inhibition. For instance, extracted

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oils from *Thymus vulgaris* and *Eucalyptus globulus* proved that medicinal plants could be in vitro effective against MDR bacteria especially Methicillin resistant *Staphylococcus aureus* (MRSA) <sup>5</sup>. Another reported study about the antibacterial activity of (Clove and Jambolan) extracts as medicinal plants, which could be used in daily meals, presented the great potentials of these plants as antimicrobial agents against resistant bacteria species like *Pseudomonas aeruginosa* and *Staphylococcus aureus* <sup>3</sup>. In 2013, miyasaki et al, identified the inhibition ability of norwogonin compound extracted from *Scutellaria baicalensis* plant against distinct isolates of resistant *Acinetobacter baumannii* <sup>6</sup>. A group of Cameroonian medicinal plants have also been tested and giving promising results against many types of resistant bacteria, which could be combined with antibiotics to increase their efficiency <sup>7</sup>. In light of previous evidences, research groups started to screen their domestic medicinal plants and identify their biological and pharmacological activities as a base for new safe medicines.

Recently many reported studies demonstrated the antioxidant, anti-inflammatory and antifungal properties of different extracts of *Achillea fragrantissima* <sup>8-10</sup>. In addition, many cancer chemopreventive and antileukemic elements were proved in *pulicaria* species <sup>11</sup>. Moreover, antihelicopacter properties have also been discovered in many plant extracts including *Cleome Africana* with high inhibition ability<sup>12</sup>.

In this study, three medicinal plants grown in Saudi Arabia have been selected, *P. crispa*, *A. fragrantissima* (family: *Asteraceae*) and *C. Africana* (family: *Cleomaceae*) which have been used widely as common folk remedies in the Middle East for healing many illnesses <sup>13-15</sup>. The inhibition behavior of these plant extracts was tested against different types of MDR bacteria that record the highest rates of infections in local hospitals. Moreover, chemical analysis of the selected plants have taken place using GC-MS instrument to determine the active groups of phytochemicals that might be responsible for the antimicrobial behavior.

## MATERIALS AND METHODS:

### Plant materials:

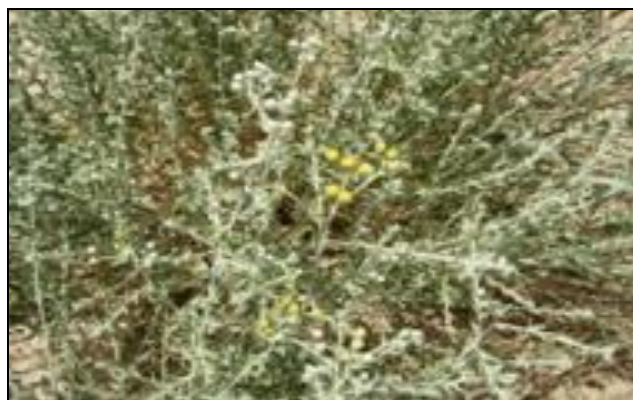
*Pulicaria crispa* (Forssk)

**Common name:** Gethgath

**Family:** *Asteraceae*

**Used parts:** Aerial parts

**Folkloric medicinal usage:** Anti-inflammation and anti-insect agent and herbal tea



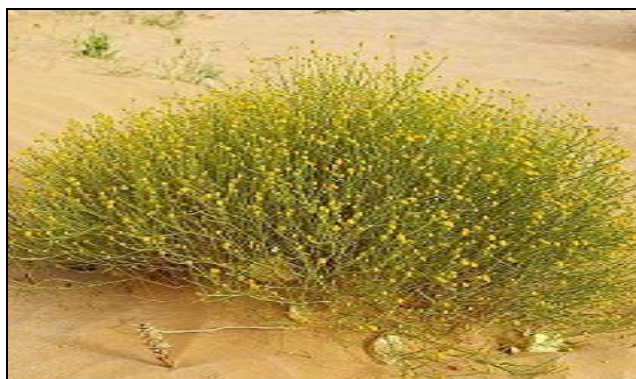
*Achillea fragrantissima*: (Bostch)

**Common name:** Qaisoom

**Family:** *Asteraceae*

**Used parts:** Aerial parts

**Folkloric medicinal usage:** Sterilizer, cuts and epidermal illnesses, cough, muscle strain



*Cleome Africana*:

**Common name:-**

**Family:** *Cleomaceae*

**Used parts:** leaves

**Folkloric medicinal usage:** Pain reliever for intestinal and arthritis.



FIG. 1: ETHNO-BOTANICAL DATA OF *PULICARIA CRISPA*, *ACHILLEA FRAGRANTISSIMA* AND *CLEOME AFRICANA*.

Both *P. crispa* and *A. fragrantissima* (Aerial parts) were collected from the desert lands near the Riyadh-Dammam highway, Eastern region, Saudi Arabia. While *C. Africana* (Leaves) was obtained from Talh Valley, Hafr Albaten, Saudi Arabia. All Plants were collected in May 2014 and then identified botanically in the library of the Biology Department Herbarium, University of Dammam, Saudi Arabia.

#### The herbal extracts:

The different parts of the selected plants were cleaned first with tap water and then with distilled water. Washed parts were dried at room temperature (25°C) for 21 days under controlled environment. Then these parts were grounded into coarse powder using electrical blender. For extraction, 10 grams of each plant material were soaked in 100 ml of 99.9% methanol and kept for 96 hours over a shaker. The next step was using the centrifuge for the mixture (5000rpm for 5 minutes). Extracts were filtered using Whatman filter paper (No. 1) and then subjected to rotary evaporator at 60°C for 8 hour to get concentrated extracts. Finally, the extracts were sterilized by using bacterial filters with 0.45µm pores (Fisher scientific, Loughborough, LE11 5RG, UK) and kept chilled at 4 °C until used<sup>16</sup>.

#### Bacterial samples:

All the bacterial isolates were taken from human clinical samples and identified in the microbiology laboratory at the King Fahd Hospital, Alkhobar, Saudi Arabia. The tested strains of bacteria were, 5

strains of *S. aureus* (MRSA) (numbered from 1 to 5), two strains of *P. aeruginosa* (numbered as 6 and 7), two strains of *A. baumannii* (numbered as 8 and 9) and one strain of *K. pneumonia* (numbered as 10). All bacterial Samples were duplicated by subculturing the isolates on Muller–Hinton agar and incubated at 37°C for 48 hours and then kept at 4°C until further use.

#### Antimicrobial susceptibility test:

The Antibacterial potential of the three methanolic extracts was checked by antimicrobial susceptibility test<sup>17</sup>, via using agar well diffusion method<sup>18</sup>. To activate the microorganisms, 2-3 colonies were transferred into nutrient broth (10 ml) and incubated overnight at 25°C on a rotary shaker. In order to predict the approximate number of bacteria, turbidity at 0.5McFarl and standard ( $1 \times 10^8$  CFU/ml) was applied using nutrient broth (4 ml) for the preparation of the bacterial inoculum. Sterile swabs were used to inoculate petri dishes contained commercial Muller-Hinton agar media in strict clean conditions. Each 0.6 mm well in the culturing plates was created by a sterile cork borer and filled with one extract. Methanol (99.9%) was filled in the wells for the negative control samples. After that, all plates were incubated on 37°C for 18-24 hours. At the end of the incubation period, diameters of the inhibition zones formed on media were expressed in millimeters.

#### GC-MS instrument:

Analyses were carried out using GC-MS (Shimadzu technologies, QP 2010 ultra system). HP-1 methyl siloxane column (Shimadzu Rxi-5Sil MS; 30.0 m × 0.25 mm × 0.25 µm thickness) was used. Carrier gas was helium with high purity (>99.999%) and a constant flow of 1.0 mL min<sup>-1</sup> was used for analyzing samples. The following temperature program was used for the analyses: initial temperature of column was 60 °C and it was held for 5 min and then increased to 220 °C at 3 °C min<sup>-1</sup> and held for 5 min. The injection port, ion source and interface temperatures were 200 °C, 220 °C, and 200 °C, respectively. For qualitative determinations, scan mode was operated from m/z 50 to 550. Identification of the compound groups was done through comparison with the instrument library directly.

**RESULT AND DISCUSSION:****Evaluation of the antimicrobial potential of plant extracts:**

The three medicinal plants of interest have shown a promising inhibition activity against 10 isolates of resistant bacteria. Generally, **Table 1** shows that each tested extract has an activity against four resistant strains at least. The results exhibited the strong possibility of using medicinal plants as alternative therapies or antibiotic complementary. In more details, *P. Crispa* extract (Aerial parts) gave the widest spectrum of antibacterial potential against all studied isolates of *S. aureus* (MRSA) except *S.aureus* (2) with inhibition zone varying between 11-19 mm. In this direction, it is important to mention that the cell wall of *S. aureus* contains high amount of lipids which responsible usually for the high resistant behavior<sup>19</sup>.

Moreover, other characteristics such as enzyme modification, decreasing sensitivity by target site alteration and changes in membrane permeability shrink the number of antibiotics which could be applied against MRSA every day<sup>20</sup>. For the above, *P. Crispa* extract demonstrated a considerable potential against methicillin-resistant *S. aureus* which give a high motivation for scholars for further studies about the chemical nature and the mechanism of the herbal extract interaction with this bacteria to investigate the possibility of using it as a therapeutic product. On the other hand, there was no growth inhibition recorded against other bacterial isolates by the *P.Crispa* extract. The methanolic extract of *A. fragrantissima* (Aerial parts) showed good activity against *S. aureus* (1)

and *Ps. aeruginosa* (2) with inhibition zone 10, 12 mm respectively.

As well as the latest extract presented little ( $\leq 8$  mm) or no effect with other isolates. Since there are very limited reported work studied the bioactivity of *C. Africana* extract<sup>12, 15</sup>, good antibacterial potential was investigated in the present study. As depicted in **Table 1**, *C.Africana* methanolic extract (leaves) gave promising inhibition ability against all tested isolates of *S. aureus* with inhibition zone ranged from 7 to 14 mm. Moreover, the *C. Africana* extract registered a noticeable antibacterial behavior against *A.baumannii* which has increasing rates of infection worldwide<sup>6</sup>, and the extract also showed low bioactivity (5 mm) against resistant *K. pneumonia* that spread widely in patients at the local hospitals in Saudi Arabia<sup>21</sup>. Among all the bacterial isolates of interest, *Ps. Aeruginosa* (1) and *A. baumannii* (2) did not interact with any of the tested extract, which could be explained by the nature of the outer membranes in gram-negative bacteria that separate between the bacterial cells and the active plant substances<sup>22</sup>. In contrast, *A.fragrantissima* extract had an antibacterial potency against one isolate of *Ps. Aeruginosa* (1) which was a promising result with this resistant type. These results point out the urgent need for increasing the clinical researches in the field of herbal materials and resistant bacteria to ensure the using of appropriate herbal or chemical antibiotic and the effective concentration against each type of resistant bacteria on the way of controlling infectious diseases.

**TABLE 1: EVALUATION OF THE ANTIMICROBIAL POTENTIAL OF THREE METHANOLIC EXTRACTS AGAINST MDR BACTERIA BY WELL DIFFUSION ON AGAR**

Microorganism	Mean Zones of Inhibition (mm)		
	<i>P. crispa</i>	<i>A.fragrantissima</i>	<i>C.Africana</i>
<i>S.aureus</i> strain (1)	19	10	14
<i>S.aureus</i> strain (2)	-	-	12
<i>S.aureus</i> strain (3)	11	8	8
<i>S.aureus</i> strain (4)	15	4	7
<i>S.aureus</i> strain (5)	19	-	14
<i>Ps. aeruginosa</i> strain (1)	-	-	-
<i>Ps. aeruginosa</i> strain (2)	-	12	-
<i>A. baumannii</i> strain (1)	-	5	8
<i>A. baumannii</i> strain (2)	-	-	-
<i>K. pneumonia</i>	-	-	5

Diameter >8 mm considered positive result.

**GC-MS analysis:**

**Table 2** shows the GC-MS analysis of methanolic extracts for the studied plants. As clearly seen, the chemical analysis identified existence of major bioactive phytochemicals such as Tannins, Flavonoids, Alkaloids, Steroids and Glycerides. The presence of Flavonoids along with Glycerides were noted in all extracts, while Alkaloids and Tannin were only found in *A. fragrantissima*. Moreover, Steroids were shown in both *A. fragrantissima* and *C. Africana*. Different reported works proved the role of Flavonoids and Steroids as strong growth inhibitors mostly against gram-positive bacteria<sup>23, 24</sup>. The analysis of *A.*

*Fragrantissima* extract confirmed the presence of Tannins similar to many published researchers studied the chemical composition of this plant species<sup>10, 25, 26</sup>. It has been proven by Ribeiro et al.,<sup>22</sup> that the presence of Tannins in the plant extracts is a major reason for the antimicrobial activity against gram-positive bacteria such as *S.aureus*. In addition, many published work confirmed the high bioactivities of Alkaloids as effective antimicrobial agents<sup>27, 28</sup>. According to the previous explanations, the positive result (12 mm inhibition zone) of *A. Fragrantissima* extract with MDR isolate *Ps. aeruginosa* (2) could be understood clearly.

**TABLE 2: GC-MS ANALYSIS FOR THE ACTIVE PHYTOCHEMICALS OF THE TESTED EXTRACTS**

	Flavonoid	Glyceride	Tannin	Alkaloid	Steroid
<i>P. Crispa</i>	+	+	-	-	-
<i>A. Fragrantissima</i>	+	+	+	+	+
<i>C. Africana</i>	+	+	-	-	+

**CONCLUSION:** This study demonstrated the antibacterial activity of *Pulicaria crispa*, *Achillea fragrantissima* and *Cleome Africana*, against different species of multi-drug resistant bacteria including gram-positive and gram-negative bacteria. The methanolic extract of *P. crispa* showed the highest activity compared with other extracts, while the *C. Africana* was the only extract that inhibited all isolates of *S.aureus*. Furthermore, the GC-MS analysis of the three methanolic extracts explained the antimicrobial activity due to the presence of many phytochemicals that usually responsible for the bioactive behavior in plants. Scientifically, the outcomes of the study consider as an evidence for using the medicinal plant as alternative antimicrobial drugs.

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