



Determination of phytochemicals, antibacterial and phytotoxic potential of Desert cotton (*Aerva javanica*)

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Abstract

Traditional medicine plants include various chemicals that can be exploited to treat chronic and infectious conditions. The *Aerva javanica* (Burm. f.) Juss. ex Schult. is commonly called kapok bush or desert cotton. It is a perennial herb distributed in Pakistan and elsewhere in the world (Middle East, Africa, and tropical Asian countries). Due to the vast indigenous use, *Aerva javanica* was analyzed for its chemical constituents using GC-MS and EDX. *Aerva javanica* extracts (n-hexane, methanol, ethyl acetate, and chloroform) were also examined for antibacterial (*Salmonella typhi*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*) and allelopathic activity. The results revealed that 20 mg/mL of each tested plant extract inhibited the growth of all pathogenic bacterial strains. The crude ethyl acetate extract inhibited the growth of *S. typhi*, followed by *Pseudomonas aeruginosa*. Whereas to limit the growth of *E. coli*, methanolic extract of the plant was most suitable. The n-hexane fraction against *S. typhi* also showed a moderate level of inhibition while it showed resistivity against chloroform extract even at higher concentrations. The extracts also exhibited phytotoxic potential and significantly reduced the % germination, shoot length, and root length of *Lactuca sativa* Linn. In the GCMS analysis, 8 compounds were detected including 2-Bromooctadecanal (RT: 6.78), Methyl cedryl ether (RT: 11.76), Phenol, 2,6-bis(1,1-dimethylethyl)methyl (RT: 12.28), 1H-Indene, 2-butyl-5-hexyloctahydro (RT: 15.22), Lamotrigine (RT: 19.00), 1,54-dibromo tetrapentacontane (RT: 21.47), Pentatriacontane (RT: 22.02) and Stearic acid, 3-(octadecyloxy)propyl ester (RT: 24.40). EDX analysis shows that *A. javanica* contains different elements, of which Cu was the highest and Ni was the lowest. The current investigation identified novel biologically active phytochemicals in *A. javanica*. This plant can be exploited against several antibiotic resistant microorganisms due to its remarkable antibacterial potential. Furthermore, the phytotoxic application of this plant allowed it to be consume to combat a variety of weeds.

Keywords: Bioactive compounds, Chemical proliferation, Desert cotton (*Aerva javanica* (Burm. f.)), EXD, GC-MS

List of Abbreviations: WHO = World Health Organization; GCMS = Gas Chromatography-Mass Spectrometry; EDX = Energy Dispersive X-Ray; Ni = Nickel; Cu = Copper; Zn = Zinc; RT = Retention time; L. sativa = *Lactuca sativa*

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Introduction

The *Aerva javanica* (Burm. f.) Juss. commonly called desert cotton had a diverse effect on human society (Boobalan & Kamalanathan, 2019; Karela & Dedar, 2022). According to WHO, about 80% of plants are used in herbals (Kazmi et al., 2019a; Shereen et al., 2021; Ehsan et al., 2022). Pakistan has a diverse zone and biodiversity due to which it contains about 6000 species of the plant, out of which about 400 -600 are considered essential for

medicinal uses (Ahmed, 2015; Rahman et al., 2022; Birjees et al., 2022). The plant extracts have been developed and proposed for use as antimicrobial substances. Plants used in traditional medicine contain a vast array of substances that can be used to treat chronic and infectious diseases (Kazmi et al., 2019c; Kazmi et al., 2019b; Mashwani et al., 2022). The demand for more and more drugs from plant sources is continuously increasing. Therefore, it is essential to systematically evaluate plants of medicinal value for various ailments used in traditional medicine (Anand et al., 2022).

Hence, medicinal plants need to be screened for their promising biological activity. From back to ancient history, the plants have been used to treat infection and disorders and the making of medicine called medicinal herbs (Abiri et al., 2021; Shahrajabian et al., 2022).

In the world, particularly in advanced countries, the appreciable population prioritizes using the traditional herbal system against many fatal diseases (Cheesman et al., 2017; Shereen et al., 2021; Ehsan et al., 2022). For the production of practical and potent drugs, several hundred genera of plants are used as critical sources (Majolo et al., 2019). As far as western medicine is concerned, the formation of drugs is dependent mainly upon medicinal plants. The trends have changed, and the natural medicinal system has gotten more attention than the modern medicinal system (Jamshidi-Kia et al., 2018). Herbal preparations have received increased attention in order to meet the healthcare demands of Germany and Europe, and almost 1400 herbal remedies are now utilised by the European Union (Jamshidi-Kia et al., 2018; Ansari, 2021). The herbal preparation can also be utilized in various cosmetic industries, such as (anti-wrinkling agents, skin tissue regenerators, and anti-age creams) which can lead to an increase in importance (Gamage et al., 2021; Albuquerque et al., 2022).

The *Aerva javanica* (Burm. f.) is commonly called kapok bush or desert cotton. *Aerva javanica* (Burm. f.) is a perennial herb distributed in Pakistan and elsewhere in the world (middle east, Africa, and tropical Asian countries) (Karakilcik & Kalyar, 2014; Mouhoub et al., 2018; Saleem et al., 2021). The plant *Aerva javanica* (Burm. f.), belongs to the Amaranthaceae family. This plant is used as Pasanabheda in India, meaning "one who breaks the kidney stone" (Movaliya & Zaveri, 2014). The roots and flowers are believed to have anti-rheumatoid potential and treat kidney complications (Movaliya & Zaveri, 2014; Arbab et al., 2016; Karela & Dedar, 2022). The plant contains several chemical constituents such as sterol triterpene, stearic acid, linoleic acid, palmitoleic acid, glycoside, flavonoids, oleic acid, phenol, canine and siphoning (Amin et al., 2021; Shahin et al., 2021; Srinivasan et al., 2021). The objective of the current research work was to explore the potent chemicals in *Aerva javanica* (Burm. f.) through GC-MS and EDX. Furthermore, the antibacterial and phytotoxic potential of the various extracts of the *Aerva javanica* (Burm. f.) was also investigated.

Material and Methods

Assembling and dehydrating certain plants materials

The locally available plant species "*Aerva javanica* (Burm. f.)" was collected from District Dir, Pakistan. Plant samples were collected and washed, followed by shade drying, ground to make their fine powder, and shifted to a cool, dry place before extraction (Shi, 2020).

Extraction and fractionation

The cold maceration method was used to form extracts; 2 litres of ethanol (70%) and 1.5 kg of plant powder were taken, dissolved, and stored for five days at 25 °C (Afu et al., 2020). After five days, a clear filtrate Whatman filter paper was taken, and the solution was passed three times. Using a rotary evaporator, the filtrates were allowed to evaporate at 40 °C to obtain the whole filtered plant.

Fractionation of dried filtrates based on solubility

The fractionation or Elution was carried out by isolating first nonpolar and then polar eluent. Then, the dried filtrate was taken to dissolve 100 ml of distal water. With the help of a separating funnel, various fractions (n-hexane, methanol, ethyl acetate, and chloroform) of the extract were formed (Tiwari et al., 2020). The solution was then shifted to the separating funnels while adding n-hexane in the ratio of 2:1. For making distinct phases of water and n-hexane, the solution in a funnel was vigorously shaken and left for a while to get proper stratification. As we know, water is denser than n-hexane, so the water remains at a lower portion while the n-hexane hangs around as an upper layer in the separating funnel. The n-hexane layer was left in a funnel by separating the aqueous phase, and the hexane was dried over a hydro rug MgSO₄ and concentrated in vacuo. In the same way, the distilled water layer was successfully fractionated with chloroform and ethyl acetate following the procedure described above.

Antibacterial activity

The agar thriving diffusion technique was executed for antibacterial activity against *Salmonella typhi*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* bacterial strains by using 6 mg/ml, 12mg/ml, and 20 mg/ml concentration of each extract (n-hexane, methanol, ethyl acetate, and chloroform) (Wang et al., 2020). Ciprofloxacin was used as a model antibiotic. The experimental procedures were performed in replicates, and the average zone of inhibition was recorded (mm).

Phytotoxicity activity

The sandwich method of (Abe et al., 2020) with slight modifications to study the allopathic effect of different plant extracts (n-hexane, methanol, ethyl acetate, and chloroform). 15 grams of agar was dissolved in distilled water (1 litre) and autoclaved. Then the agar solution was transferred into sterilized Petri plates and solidified at room temperature under a sterile environment. In different concentrations (0mg/ml (control), 10 mg/ml, 20 mg/ml, and 40 mg/ml), the tested plant (dried materials) were taken and were spread over the agar plate. Then dried materials were again covered with agar solution. Ten seeds of *Lactuca sativa* Linn. were placed on agar gel on the plate when the agar solution completely solidified. Every plate was wrapped with the flexible sticky tape and incubated at 24 °C for 72 h under dark conditions. The

%germination, shoot, and root length of *Lactuca sativa* Linn. were measured to determine the phytotoxic effects of the selected plant.

For data analysis, ANOVA was used using Statistix software and MS excel.

$$\% \text{ Germination} = \frac{\text{No. of Seeds Germinated}}{\text{No. of Seeds Sown}} \times 100$$

Results

GC-MS analysis

The documentation of phytochemical components was executed using a Hewlett-Packard 5890 Series II gas chromatograph, equipped with an HP-5971 mass selective detector and capillary column HP-5 (25 m × 0.2 mm × 0.33 μm diameter). The chloroform fraction was used by following available literature, indicating that the selected fraction contains the best GC-MS analysis results.

Antibacterial potential of the crude extract of *Aerva javanica* (Burm. f.)

To elucidate the antibacterial potential, various extracts of *Aerva javanica* (n-hexane, Methanol, Ethyl acetate, and Chloroform) were applied to *Salmonella typhi*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* along with Ciprofloxacin for a better comparison at various concentrations (6, 12, and 20 mg/ml). This study revealed that 20 mg/mL of each tested plant extract inhibited the growth of all the pathogenic bacterial strains. The crude ethyl acetate extract inhibited the growth of *S. typhi* with a 30±5.56 mm zone of inhibition, followed by *Pseudomonas aeruginosa* (29.5±4.04 mm zone of inhibition). Whereas to limit the growth of *E. coli*, methanolic extract of the plant was most suitable at 20 mg/ml (29±2.65 zone of inhibition), followed by *Staphylococcus aureus*. The n-hexane fraction also showed a moderate level of inhibition against *S. typhi* (27±3.60 mm zone of inhibition) and *Staphylococcus aureus* (23±4.04 mm zone of inhibition) when applied at 20mg/ml. The chloroform fraction was effective only against *E. coli* (27.5±6.11mm zone of inhibition) at higher concentrations. *Salmonella typhi* showed resistivity against chloroform extract even at higher concentrations (Table 1; Fig. 1).

EDX analysis

The Model Perkin Elmer AA Analyst 700 (Energy-dispersive X-ray spectrometer) was used to demonstrate the quantitative analysis of elements (macro and micro), which is installed in the University of Peshawar (Centralized Resource Laboratory). The CRL was given 3g powder of the selected plant for analysis.

Statistical analysis

Table 1 Average zone inhibition potential of various extracts of *Aerva javanica* against different common bacteria

Bacterial species	Average zones of inhibition (mm)				
	Extracts				
	n-hexane	Methanol	Ethyl acetate	Chloroform	Ciprofloxacin
	Concentration (6 mg/ml)				
<i>Salmonella typhi</i>	18±1.00	11±2.64	21±3.00	6±1.73	21±3.46
<i>Escherichia coli</i>	12±0.58	20±3.00	10±2.64	18±2.52	22±2.00
<i>Pseudomonas aeruginosa</i>	12±1.52	17±1.52	20±3.51	12±2.08	23±1.74
<i>Staphylococcus aureus</i>	16±2.00	17±1.15	13±3.21	10±0.58	19±2.08
	Concentration (12 mg/ml)				
<i>Salmonella typhi</i>	21±5.56	15±2.00	25.5±4.04	10±1.00	24±3.61
<i>Escherichia coli</i>	17±2.08	23±2.52	14.5±1.53	21±3.44	26±5.03
<i>Pseudomonas aeruginosa</i>	15.5±2.64	19±1.53	24±3.60	16.5±2.00	26.5±3.05
<i>Staphylococcus aureus</i>	19.5±3.05	22±2.64	18±3.51	17±3.46	23.5±4.36
	Concentration (20 mg/ml)				
<i>Salmonella typhi</i>	27±3.60	21±3.61	30±5.56	17.5±3.06	28.5±4.62
<i>Escherichia coli</i>	20.5±2.51	29±2.65	20±4.58	27.5±6.11	31.5±5.69
<i>Pseudomonas aeruginosa</i>	19±2.00	23±3.79	29.5±4.04	21±3.60	30±6.25
<i>Staphylococcus aureus</i>	23±4.04	26±4.00	22.5±3.21	23±2.65	25.5±4.73

Phytotoxicity

To determine the phytotoxic potential of the *Aerva javanica*, n-hexane, methanolic, ethyl acetate, and chloroform extracts of the *Aerva javanica* were applied to the *Lactuca sativa* Linn. (model plant) at different

concentrations. The results revealed that compared to control, the % germination of *Lactuca sativa* Linn. was reduced with the increase in concentration. The n-hexane and ethyl acetate extract significantly reduced the seed germination at higher concentrations, followed by methanolic extract (30% and 40%, respectively). Minimal reduction in seed germination was

observed in seeds exposed to chloroform extract (Fig. 1; Fig. 2A). Similarly, the *Lactuca sativa* Linn. seeds germinated in the presence of allelochemical stress conditions had retarded growth. The extracts at higher concentrations significantly reduced the seedlings' root length and shoot length. Dramatically, the chloroform extract of *Aerva javanica* did not much affected the seed germination but significantly restricted the shoot and root lengths of the *Lactuca sativa* Linn. The seeds germinated under control conditions and had a 27.8 mm root length. In contrast, the seeds germinated under a higher concentration

(40 mg/ml) of chloroform extract had a minimum root length (10.1mm), followed by ethyl acetate (12.3 mm). The n-hexane and methanolic extracts of *Aerva javanica* also limited the root development of *Lactuca sativa* Linn. at 40 mg/ml (Fig. 2B). Similarly, the shoot development and elongation were also restricted by the extracts of *Aerva javanica* at higher concentrations (20 and 40 mg/ml). Under the control environment, the maximum shoot length was observed (19.75 mm). In contrast, the minimum shoot length was observed in seeds germinated in the presence of ethyl acetate extract (9 mm), followed by chloroform extract (10.7 mm) (Fig. 2C).

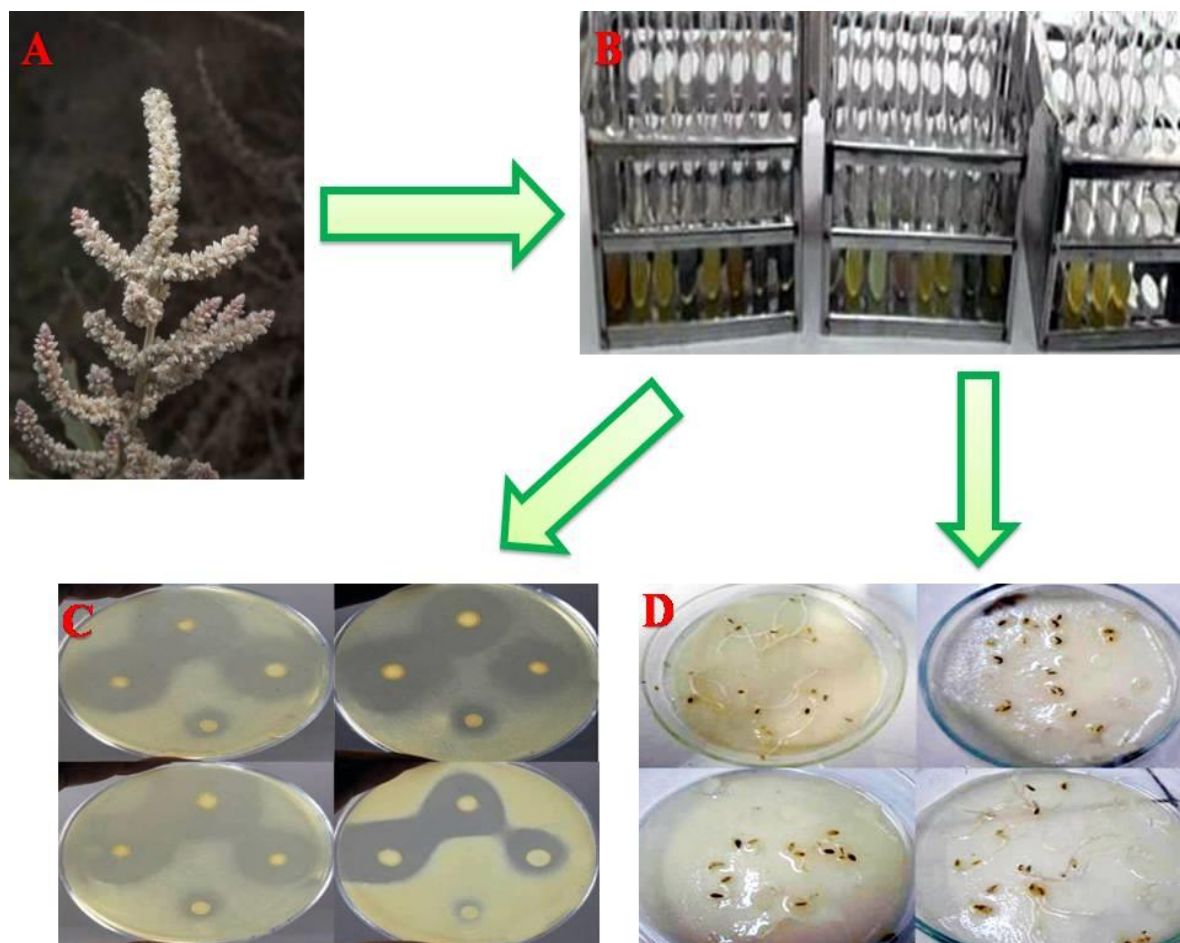


Fig.1 (A) *Aerva javanica* (Burm. f.) plant (B) Different extracts of *Aerva javanica* (C) Antibacterial activity of various extracts of *Aerva javanica* (D) Phytotoxic analysis of different extracts of *Aerva javanica* against *L. sativa*.

Table 2 The details of compound detected in *Aerva javanica* (Burm. f.) after GCMS analysis

Compound name	Retention Time	Formula	Probability
2-Bromooctadecanal	6.78	C ₁₈ H ₃₅ BrO	8.33
Methyl cedryl ether	11.76	C ₁₆ H ₂₈ O	12.17
Phenol, 2,6-bis(1,1-dimethylethyl) methyl	12.28	C ₁₅ H ₂₄ O	19.56
1H-Indene, 2-butyl-5-hexyloctahydro	15.22	C ₁₉ H ₃₆	10.44
Lamotrigine	19.00	C ₉ H ₇ C ₁₂ N ₅	84.34
1,54-dibromo tetrapentacontane	21.47	C ₅₄ H ₁₀₈ Br ₂	16.21
Pentatriacontane	22.02	C ₃₅ H ₇₂	36.03
Stearic acid, 3-(octadecyloxy)propyl ester	24.40	C ₃₉ H ₇₈ O ₃	30.22

EDX analysis

The elemental composition of the plant was carried out on powder in both 'EK' and 'control'. It was observed that Ni, Cu, and Zn were present in both plant powder samples. The elemental composition percentage demonstrated that Ni is 11.84%, Cu is 0.45%, while the Zn is 0.55% present

in the control plant powder (Fig. 3). At the beginning of the analysis, Zn and Ni have less percentage composition than Cu. The EDX analysis demonstrated that elemental composition percentages were significantly increased by 0.55% for Cu, 0.42% for Ni, and 0.90% for Zn. The highest value of plant absorption is 0.27% for Ni in plant powder, leading to the mobility of some aspects through the medium.

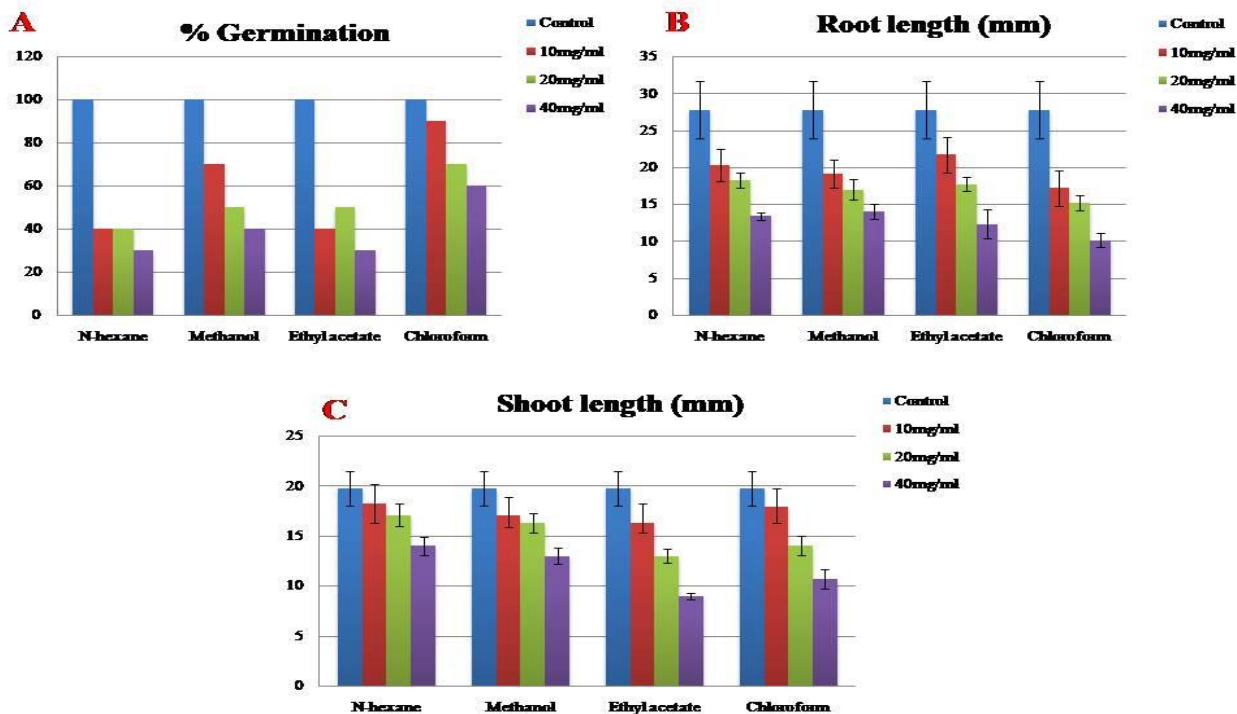


Fig.2 Phytotoxic potential of various extracts of *Aerva javanica* against *L. sativa*. (A); % germination inhibition, (B); phytotoxic effect root development, (C); Phytotoxic potential against shoot development

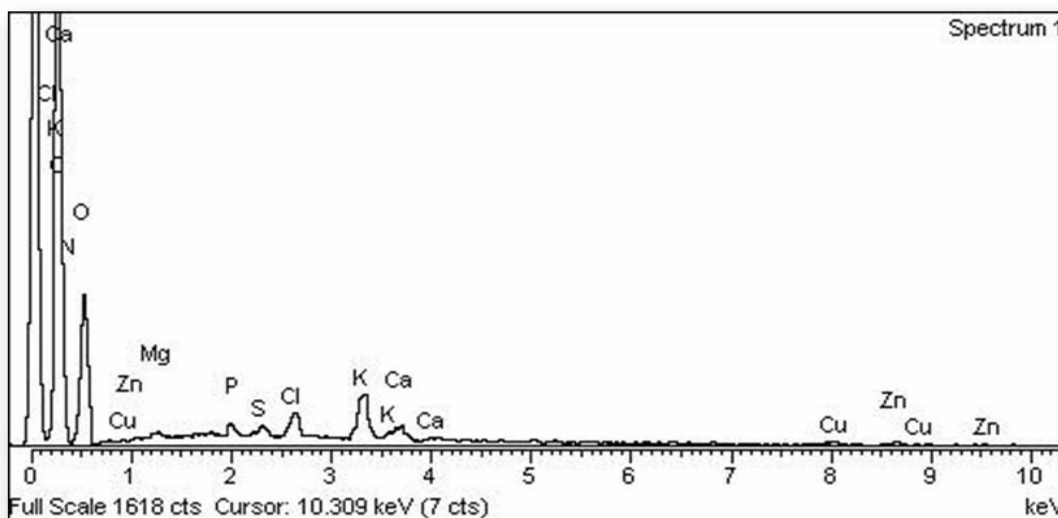


Fig. 3 Result of Energy Dispersive X-ray (EDX) analysis representing the concentration of different elements present in *Aerva javanica* (Burm. f.)

GC-MS analysis

The GC-MS study of *Aerva javanica* (Burm. f.) demonstrates that the plants comprise various important secondary metabolites (phytochemicals) that subsidize the plant's therapeutic potential (Table 2, Fig. 4). The key constituents which are existing in the plant *Aerva javanica*

were 2-Bromooctadecanal (RT: 6.78), Methyl cedryl ether (RT: 11.76), Phenol, 2,6-bis(1,1-dimethylethyl)methyl (RT: 12.28), 1H-Indene, 2-butyl-5-hexyloctahydro (RT: 15.22), Lamotrigine (RT: 19.00), Tetrapentacontane 1, 5,4dibromo (RT: 21.47), Pentatriacontane (RT: 22.02) and Stearic acid, 3-(octadecyloxy)propyl ester (RT: 24.40).

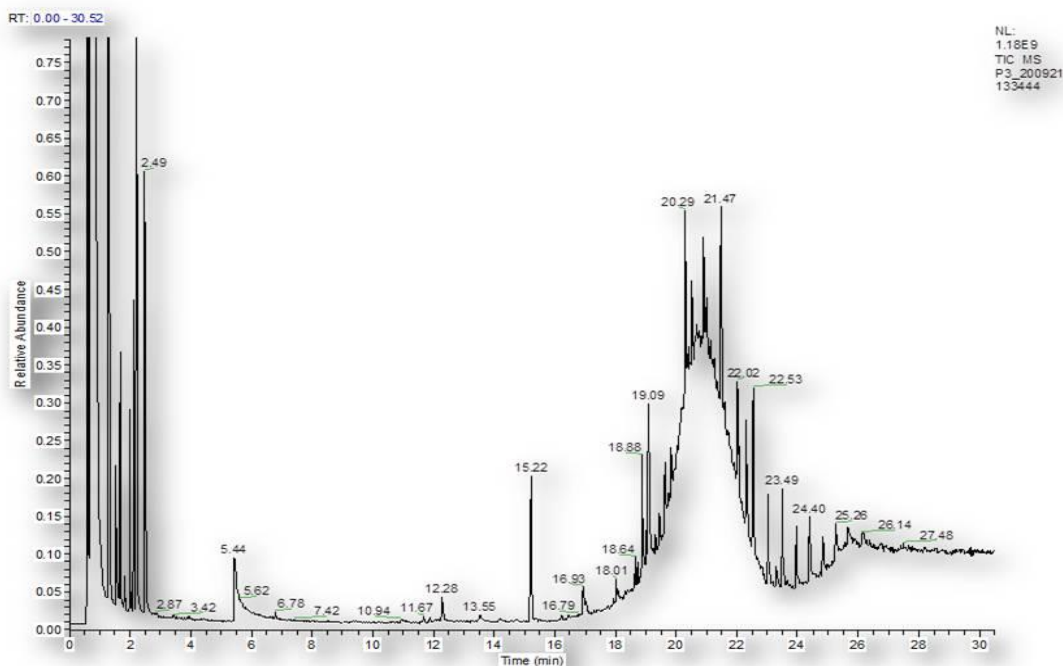


Fig. 4 Results of Gas Chromatography Mass Spectrometry (GCMS) analysis of *Aerva javanica* (Burm. f.)

Discussion

The genus *Aerva* is medicinal (Saleem et al., 2021), with numerous species shown to have biological activities such as anthelmintic, antioxidant, antimalarial, hypoglycemic, antivenin, and analgesic properties (Shahin et al., 2021; Karela & Dedar, 2022). *Aerva javanica* is a medicinal and commercially valuable plant. Ayurvedic medicine considers being one of the most acceptable sources of natural treatments, especially treating kidney and bladder stones (Movaliya & Zaveri, 2014; Arbab et al., 2016; Thasneem et al., 2022). *Aerva javanica* is a shrub (or small tree) that produces essential oils and is found in tropical and subtropical arid environments. Its blossoms have a variety of folk uses in traditional herbal therapies (Azhar et al., 2022).

The availability of essential oils in the *Aerva javanica* might be the reason for meaningful antibacterial activity. The development of all pathogenic bacterial strains in this investigation was strongly suppressed by 20 mg/mL of each examined plant extract. The crude ethyl acetate extract was the most effective against *S. typhi* (30 ± 5.56 mm

zone of inhibition), followed by *Pseudomonas aeruginosa*. Whereas to limit the growth of *E. coli*, methanolic extract of the plant was most suitable at 20 mg/ml (29 ± 2.65 zone of inhibition), followed by *Staphylococcus aureus*. The n-hexane fraction against *S. typhi* and *Staphylococcus aureus* also showed moderate inhibition. *Salmonella typhi* showed resistivity against chloroform extract even at higher concentrations (Table 1). In a previous study, the petroleum ether, ethyl acetate, and methanol extracts of leaves of *Aerva javanica* (Burm. f.) were exploited against *Bacillus subtilis*, *Bacillus cerus*, *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris*, *Shigella dysenteriae*, and a few fungal species including *Aspergillus flavus*, *Aspergillus niger*, and *Candida albicans*. All the extracts exhibited significant antibacterial and antifungal potential (Srivivan & Reddy, 2008; Al-Ghamdi, 2022).

To the best of our knowledge, it is the first study on the determination of the phytotoxic potential of the *Aerva javanica*. The results have indicated that *Aerva javanica* exhibit significant phytotoxic potential by limiting the % germination, shoot length, and root length of *L. sativa* at higher concentration. At a higher level, n-hexane and ethyl acetate

extract drastically inhibited seed germination, followed by methanolic extract (30% and 40%, respectively). Seeds subjected to chloroform extract showed a minor decrease in germination (Fig. 1; Fig. 2). Previously, the essential oils from the flower of the *Aerva javanica* have been reported for considerable antioxidant activity (Shahin et al., 2021; Afzal et al., 2022b; Afzal et al., 2022a).

Their growth was halted when *Lactuca sativa* Linn. seeds germinated in the presence of allelochemical stress. The chloroform extract of *Aerva javanica* dramatically affected the shoot and root lengths while having little effect on seed germination. Seeds grown with a greater concentration of chloroform extract (40 mg/ml) had the lowest root length (10.1mm), followed by ethyl acetate (12.3mm). The minimum shoot length was observed in seeds germinated in the presence of ethyl acetate extract (9mm), followed by chloroform extract (10.7mm) (Fig. 2). As the atomic number of Ni is the lowest equated to Cu and Zn, it licenses a further hasty movement of this element over the soil than the other two elements (Boschi & Willenbring, 2021). The existence of electrical influences enhanced the probability of plant absorption and the mobility of elements. The plants situated at the bank of river soil will uptake the heavy metal (Bi et al., 2011; Maurya et al., 2018), which is improved with the help of EK-assisted phytoremediation, as demonstrated in the results. The current study detected Ni, Cu, and Zn in plant powder samples. The elemental composition percentage demonstrated that Ni is 11.84%, Cu is 0.45%, and Zn is 0.55% present in the plant powder (Fig. 3).

In the present study, eight different compounds were detected in *Aerva javanica* with several applications, such as 2-Bromooctadecanal, which is used as medicine, and several species of insects as pheromones (dos Santos Neta et al., 2021). Phenol, 2,6-bis(1,1-dimethylethyl)methyl; used as an insecticide, dyestuff, and fungicides (Fauziati et al., 2016), 1H-Indene, 2-butyl-5-hexyloctahydro; used in petroleum and they are derivative and the synthesis of sulofenour (Feklistova et al., 2018), Lamotrigine; used to control the seizure, bipolar disorder controlling and antiepileptic drugs (Evora et al., 2019). 1,5,4-dibromo tetrapentacontane; as gas is used for fuel (Salem, 2019); Pentatriacontane; is used as binding for chewing gum, wax, and as a flavor (Greene & Richards, 1940; Onu et al., 2016; Adhisatrio & Pradana, 2020). Stearic acid, 3-(octadecyloxy)propyl ester is used in paint and coating, soap forming, and plastic (Mohamed, 2018) (Table 2). In recent research, 29 distinct volatile components were extracted from *Aerva javanica* flowers, with angustione being the most prominent constituent (Shahin et al., 2021; Thasneem et al., 2022).

Conclusion

Aerva javanica (Burm. f.) is a xerophytic medicinally important plant that is traditionally utilized for the treatment of renal complications. The current study revealed that the plant contains several secondary

metabolites (phytochemicals). The detected chemicals are utilized for various purposes such as insecticide, dyestuff, fungicides, fuel, wax, paints, and coating. Due to the availability of such metabolites in *Aerva javanica*, it evolved high antibacterial and phytotoxic activity. Further chemical and molecular investigations are required to explore the plant's future to obtain the desired chemicals with enhanced production and manufacture various drugs that can be utilized against multi-drug resistant pathogens. Furthermore, detecting metals in the plant through EDX suggested that the plant can also be exploited for phytoremediation.

Conflict of interest: The authors of the study declare no competing interest

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