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Morpho-biochemical Analysis of Underutilized Wild adlay (*Coix lacryma-jobi* L.) For Medicinal Value

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A B S T R A C T

Coix lacryma-jobi L. (Adlay) is an economically valuable annual crop in temperate and subtropical areas, but a perennial in warmer climates where frost is mild or absent. The purpose of this research was to study the morpho-biochemical analysis of *Coix lacryma-jobi* L. (Adlay) and its medicinal value. The experiment was carried out at National Agricultural Research Center (NARC), Islamabad. Experimental material comprised seeds of wild Adlay collected from Botanical Conservatory of National Herbarium Program (PGRI, NARC), Islamabad. The biochemical characterization of Adlay leaf and seed extract was carried out. The qualitative phytochemical screening of Adlay leaves methanolic extracts revealed the presence of steroids, alkaloids, tannins, carbohydrate, phenols, glycosides, saponins, and flavonoids, whereas in seeds extract, steroids, flavonoids, tannins, glycosides, and phenols exhibited higher antimicrobial activity. For medicinal properties, methanolic extract of seeds and leaves were examined against six different bacterial strains. In case of Adlay leaf extract the highest inhibitory activity was shown against *Staphylococcus aureus* (32.83mm at 50mg/ml) in methanol solvent while, *P. aeruginosa* showed no inhibition at any concentration of leaf extract. Moreover, in case of seed extract *Staphylococcus aureus* showed highest inhibition (31.53mm-50mg/ml), while *P. aeruginosa* and *E. coli* did not show any inhibition in case of seed extract. According to the findings of this research, methanolic leaf and seed extracts of Adlay revealed a broader range of efficacy against both gram negative and gram-positive bacteria, indicating that they can be utilized as precursors of antibiotic substances for medicine formulation to manage bacterial disorders like diarrhea, urinary tract infection, pneumonia, gastrointestinal infections, and respiratory tract infection.

Keywords: Methanolic extracts,*Staphylococcus aureus*, Coix, Phytochemical screening, Medicinal value.

INTRODUCTION

Microbial infections and the emergence of drug-resistant microbes have become one of the biggest challenges worldwide (Rajendrasozhan *et al.*, 2021). The use of medicinal plants against the treatment of many diseases is now widely under consideration and is being used as a secure choice for many diseases (Mehmood *et al.*, 2021). World Health Organization has emphasized the use of traditional medicines and reported that about 80% of the population from developing countries relies on medicinal plants for their primary health care(Adnan *et al.*, 2014). It is believed that more than 8,000 plant species in South Asia carry medicinal properties, of which 1000 exists in Pakistan (Bashir *et al.*, 2015).

Coix lacryma-jobi L., commonly known as Job's tears, is a broad-leaved, grain-bearing tropical plant of the family Poaceae, which is considered a nutritious health food in Asian countries such as China, India, Bangladesh, Pakistan, Sri Lanka, Malaysia, Japan, the Philippines, Burma, and Thailand (Das *et al.*, 2017).In Pakistan, it is growing in Khyber Pakhtunkhwa (KP), Punjab, and Sindh. In KP, it is found in Hazara Region below Sathan Gali and Siran Valley, in Attock District and surrounding areas of Wah (Flora of Pakistan). Job's tears have been widely studied and reported to contain thiamine, riboflavin, niacin and ascorbic acid, comprising 10.83% moisture, 13.05 g protein (protein content approximately double that of rice), 5.45 g fat, 68.60 g carbohydrate, 0.36 g fiber, and 1.3 g ash, and Job's tears flour contained 2.25% amylase (Chhabra and Gupta, 2015). Coix seeds contain phenols, flavonoids, polysaccharides, proteins, fibers, vitamins, and oils (Devaraj *et al.*, 2020). The distinctive

elements of Adlay among cereals are coixol, lactams (Chung *et al.*, 2011), coixin, and coixenolide (Ottoboni *et al.*, 1990). The purpose of the current research is to assess Adlay's antibacterial effectiveness in vitro. It also helps to promote the usage and consumption of Adlay grains as natural food products that contain edible polyphenols as antioxidants. With its medical application, the demand for Adlay seeds is growing day by day, yet data on genetic diversity and quality evaluation of Adlay seeds is still insufficient. Therefore, the aim of present study was to find out the diversity of bioactive compounds among seed and leave extracts of Adlay and to investigate their antibacterial potential.

MATERIALS AND METHODS

Sample preparation (Soxhlet extraction) (For seeds)

The experiment was conducted at the Plant Genetic Research Institute (PGRI), National Agricultural Research Center (NARC), Islamabad, Pakistan. The seeds of wild Adlay were collected from the Botanical Conservatory of National Herbarium, PGRI, (NARC), Islamabad. 100 grams of seed powder was refluxed for 2-8 hours in a Soxhlet assembly was then filtered through filter paper and transferred to a rotary evaporator to evaporate the solvent to obtain dry extract. The dry extracts were kept in the refrigerator at 4 ° C for analysis. The protocol was adapted as described by Hassan *et al. (*2020) with some changes.

Sample preparation (For leaves)

The maceration process was used to make a crude extract of selected plant leaves (Chen *et al,* 2013) with some modifications. The leaves were washed and dried for 2 weeks in shade at room temperature. The leaves of the plant were then separated and crushed in an electric grinder before being stored separately in sealed plastic bags until needed for the extraction procedure. After that, 30 grams of powdered leaves were soaked in 150ml of methanol in a conical flask and shaken at room temperature for 48 hours on a shaker. Under a fume hood, the filtrate was filtered through muslin cloth and then again through Whatman No.1 filter paper. The filtrate was then dried in a rotary evaporator to eliminate all of the solvent and leave only the dry extract. The dry extract was then maintained at 4 ° C for analysis.

Screening of bioactive compounds

Tannins, terpenoids, flavonoids, alkaloids, phenols, carbohydrates, anthraquinones, steroids, glycosides, and saponins were determined in Adlay leaf and seed extracts qualitatively using standard phytochemical analytical procedures (Siddique *et al.*, 2013).

Evaluation of antibacterial activities

Antibacterial activity of Adlay leaves and seed extracts (methanol) was evaluated by the disc diffusion method (Kausar *et al.*, 2021).

Test microorganisms

In the present research, six different bacterial strains were studied. Four of them were Gram-positive, i.e., *Bacillus subtilis* (ATCC 23857), *Listeria monocytogenes* (ATCC 7644), *Staphylococcus aureus* (ATCC 23235), and *Bacillus cereus* (ATCC 11778). Out of six bacterial strains, two were Gram-negative, namely *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853).

Preparation of samples

To make the nutrient broth medium (8 g/L), nutrient broth (Merck) was dissolved in distilled water. In a 100ml flask, 50ml of media was dispensed at pH= 7.0 and autoclaved. Individual bacterial cultures were inoculated and kept at 37°C in a shaker incubator at 150 rpm overnight. For positive control, standard antibiotic discs, namely Ciprofloxacin (5µg), Ampicillin (10µg), and Amoxicillin/Clavulanic Acid 2:1 (30µg) were used. The negative control was pure DMSO.

Preparation of agar plates

A conical flask was used to prepare the media, which was made by dissolving 28 g of nutrient agar in 1 liter of distilled water. Shake the flask to completely dissolve the media in distilled water and adjust the pH to 7.0. Use an autoclave to sterilize the media for 15 minutes at 121 °C. Each Petri plate received 25ml of media, which was poured and solidified.

Extract loading

By using the pour plate method, bacterial broth culture was transferred to nutrient agar plates. Rotate the plate 60 degrees each time to ensure that the inoculum is evenly distributed. Different dilutions of extract (leaves and seeds of Adlay) were prepared to give concentrations of 3.14, 6.25, 12.5, 25, and 50 mg/ml. After this, four Whatman No. 1 filter paper disks were placed on the plate, and on the second plate, 2 filter paper disks were placed in different concentrations of extracts with the help of forceps. In the center of one plate, a standard antibiotic disc was placed, while on the other plate, two standard antibiotic discs were placed, and one disc of pure DMSO for negative control was placed.

Minimum inhibitory concentration

Using the disc diffusion method, the MIC of Adlay plant

extracts was calculated. For 24 hours, the plates were incubated at 37°C. The diameter of clear zones with no bacterial growth around each disc was measured after 24 hours of incubation. For each extract, triplicate plates were made. The standard deviation of the mean zone of inhibition was determined.

RESULTS

Preliminary phytochemical analysis

Using simple chemical assays, crude extracts of wild Adlay leaf and seed (in methanol) were tested for basic phytochemical analysis (Table 1). Alkaloids, glycosides, flavonoids, phenols, carbohydrates and terpenoids were present in both seeds and leaves, while saponins, steroids and tannins were only present in leaves. Anthraquinones were absent in both.

Table 1. Phytochemical analysis of leaf and seed extract of Adlay.

Phytochemicals	Leaf extract	Seed extract
Alkaloids	+	+
Saponins	+	_
Glycosides	+	+
Flavonoids	+	+
Steroids	+	_
Tannins	+	_
Phenols	+	+
Anthraquinones	_	_
Carbohydrates	+	+
Terpenoids	+	+

Antibacterial activity of leaf and seed extracts against gram-positive bacteria

The current research examined the antibacterial properties of a medicinal plant *Coix lacryma jobi* for the first time. Four gram-positive strains, i.e. *Bacillus subtilis* (ATCC 23857), *Listeria monocytogenes* (ATCC 7644), *Staphylococcus aureus* (ATCC 23235), and *Bacillus cereus* (ATCC 11778), were used in this study. *Bacillus subtilis* showed 25.5mm, *Listeria monocytogenes* 20.73mm, *Staphylococcus aureus* 32.83mm and *Bacillus cereus* 25.5mm at 50mg/ml which is highest concentration of extract. *B. subtilis* showed 0mm, *L. monocytogenes* 1.26mm, *S. aureus* 3.4mm and *B. cereus* 4.86mm at 3mg/ml which is lowest concentration of leaf extract. The leaf extract of Adlay showed excellent results against all the tested gram-positive strains. In case of Adlay seed extract *Bacillus subtilis*, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Bacillus cereus*showed 26.5mm, 30.5mm, 31.53mm and 22.83mm inhibition zones, respectively, at a concentration of 50mg/ml. *Bacillus subtilis* and *Listeria monocytogenes* showed 0mm, *Staphylococcus aureus* 10.33mm and *Bacillus cereus* 9.03mm zones. The results obtained against the tested bacteria are shown in Figures 1 and 2.

Antibacterial activity of leaf and seed extracts against gram-negative bacteria

Two gram-negative strains, namely *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) were used in this study. The Adlay leaf extract was found to be ineffective against *Pseudomonas aeruginosa* and did not possess potential inhibition activity. On the other hand, *E. coli* showed 28.53mm at 50mg/ml, while at lowest concentration 3mg/ml it showed 10.2mm zone of inhibition. The Adlay seed extract does not show any activity against gram-negative bacteria. This means that the seed extract was ineffective against gram-negative strains and didn't control growth of the pathogen even at the highest concentration used.

The standard antibiotic discs, namely Ampicillin (10 µg), Amoxicillin/Clavulanic Acid 2:1 (30 µg) and Ciprofloxacin (5µg) were also used for comparison. The standard antibiotic disc, AMC and Adlay leaf and seed extracts at 50mg/ml showed good results against all the strains used in the study, and they inhibited the growth

of the pathogen. The negative control disc was also used, which showed maximum bacterial growth. The results obtained against the tested bacteria at concentrations of 3, 6, 12, 25 and 50mg/ml were shown in Figures 3 and 4.

The results were further analyzed by Tukey pairwise comparison. The Analysis of variance showed that all the bacteria and treatments were significant $p<0.000$ and mean values were significantly different. The one-way ANOVA Tukey test was used, and the results provide the grouping information using the Tukey method with a 95% confidence interval. The p-value was < 0.000 highly significant. All the mean values are significantly different from each other as they don't share common letters.

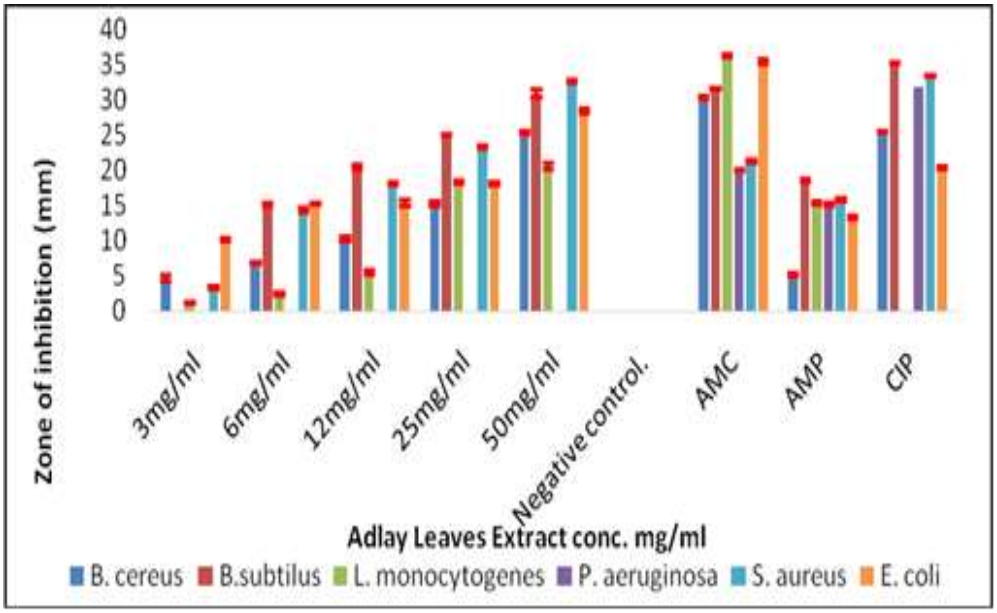


Figure 1. Antibacterial activity of leaf extract against gram-positive and gram-negative bacteria.

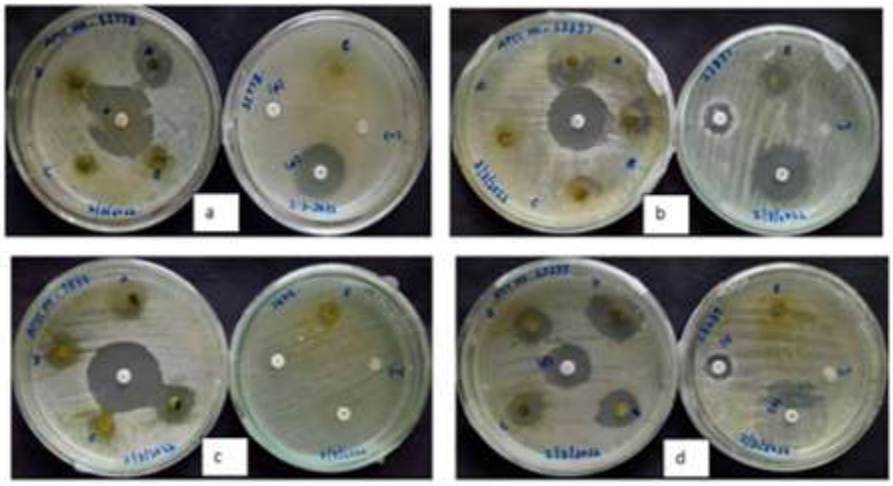


Figure 2 (a). (a, b, c and d f): MIC of Adlay leaf extract against *B. cereus*, *B. subtilis*, *L. monocytogenes*, *S. aureus*, *P. aeruginosa* and *E. coli*, respectively.

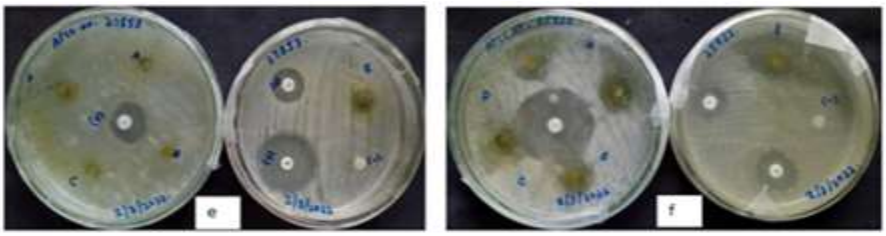


Figure 2(b). (e and f): MIC of Adlay leaf extract against *B. cereus*, *B. subtilis*, *L. monocytogenes*, *S. aureus*, *P. aeruginosa* and *E. coli*, respectively.

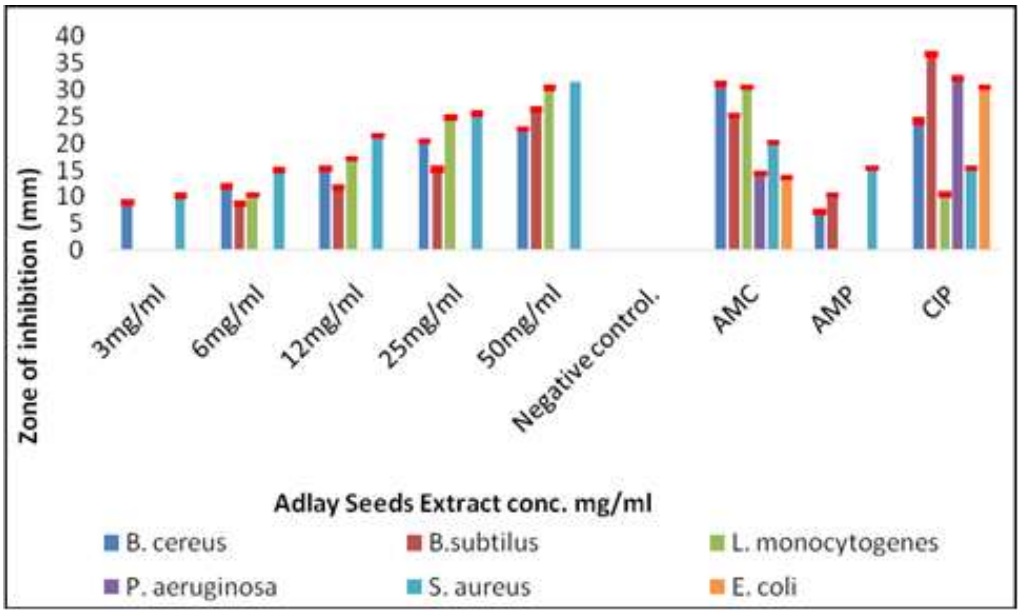


Figure 3. Antibacterial activity of seed extract against gram-positive and gram-negative bacteria.

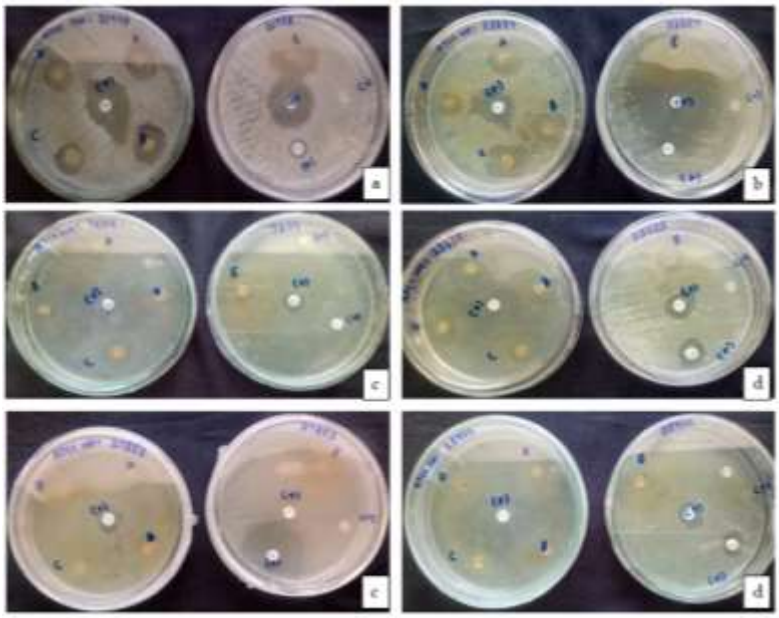


Figure 4. (a,b,c,d,e and f): MIC of Adlay seed extract against *B.cereus*, *B. subtilis*, *L. monocytogenes*, *S. aureus*, *P. aeruginosa* and *E. coli*, respectively.

DISCUSSION

Coix lcryma jobi. L is an important medicinal plant and has a lot of potential to contribute to health, nutrition, food, and income generation for the well-being of people. In the present study the screening of some important and active phytochemicals carried out from plant extracts i.e., crude extract of leaves of Adlay plant leaves was prepared by maceration method(Chen *et al.*, 2013) and grains extract prepared as described by (Hassan *et al.*, 2020). The results showed that alkaloids, glycosides, flavonoids, phenols, carbohydrates, and terpenoids were present in both seeds and leaves extracts, while saponins, steroids and tannins were only present in leaves. Anthraquinones and phlobatannins were absent in both. The presence of phenolic content in a plant implies that it has the ability to treat inflammatory disorders and may be helpful in wound healing (Kaparekar and Anandasadagopan, 2022) . This study may partially be responsible for the health benefits because of the diverse group of phytochemicals are present in Adlay leaf and seed extracts. It was also observed that methanol extract showed highest inhibitory activity because of its high polarity and it allows extracting all the phenolic compounds. These antimicrobial agents kill microorganisms by degrading cell wall protein components, interfering with enzymatic activity, and interrupting DNA and RNA replication (Bakal *et al.*, 2017). Das *et al. (*2017) reported that chloroform extract of Adlay leaves showed a wide range of antimicrobial activity against Gram-positive and Gram-negative bacteria compared to the seeds extract. The range of zone of inhibition was within 9-14mm in chloroform leaf extract, whereas in case of seed extract the zone of inhibition ranging from 6-8mm. In agreement with this finding, (Devi *et al.*, 2015) reported that antibacterial activity against *K. pneumonia* and *P. aeruginosa* showed maximum efficiency in the chloroform extract followed by petroleum ether extract, aqueous extract, hexane extract, and methanol extract, whereas antibacterial activity tested against *E.coli* did not show any activity which is similar to the present study. Less antibacterial activity of seeds extract in the present study may be due to presence of less bioactive components or the composition of samples used and the extraction process. The results of present study revealed that methanolic extracts of leaf and seed of Adlay plant were found to have acceptable antibacterial activities with respect to reference antibiotic discs. The antimicrobial activity of plants may be the consequence of presence of wide range of bioactive compounds. Plant extracts comprise many polyphenols, flavonoids and alkaloids which could be antimicrobial representatives. Many studies show the association between antimicrobial activities of plants and the phytochemicals present in them. Flavonoids are well known for their antiviral, antimicrobial and spasmolytic activities. Likewise, alkaloids extracted from plants have also shown antimicrobial activity. The inhibition of microbial growth may result from the binding of bio-compounds to the cell wall (Mehmood and Murtaza, 2018).

CONCLUSION

Coix lacryma–jobi L. (Adlay) is a widely grown and studied crop in Asian countries, but in Pakistan it is one of the underutilized grains and finds applications in food and pharmaceutical industries in a broader prospect if given attention. The preliminary screening of Adlay leaf and seed methanolic extracts showed that they had great fiber content in addition to good content of phenolic compounds, alkaloids, flavonoids, tannins, and saponins. During the study, it was noted that due to the presence of a wide range of phytochemicals, it is used as an antimicrobial agent for the treatment of different diseases. Despite common belief, the current study demonstrates that Adlay, a non-traditional food crop, outperforms main crops like rice, maize, wheat, etc. in terms of their medicinal and nutritional properties. Additionally, the plant has a large genetic diversity that can be used for sustainable and productive purposes.

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CONFLICT OF INTEREST

The authors affirm that the research was conducted without any commercial or financial affiliations that could be perceived as potential conflicts of interest.

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