



## Identification Of Bioactive Compounds in Selected Seeds of Medicinal Plants

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The present study was conducted to evaluate the phytochemical and antimicrobial potential of forty different local plant seeds collected from the local market of Haripur district. Seeds were washed with water, sorted, graded, grounded, and stored at room temperature for further analysis. The sample was analyzed for total phenolic content (TPC), total flavonoid content (TFC), antioxidant activity, and antimicrobial activity against three bacterial isolates i.e, Escherichia coli, Staphylococcus aureus, and Bacillus subtilis. The results indicated that the highest phenolic compounds were found in Pista maghz (40.80 mg GAE/gram) and Kaju (1.27 mg GAE/gram) had the lowest amount for TPC. Total flavonoid content was noted as highest in Tukham-e-taboos (55.40 mg QE/gram) and Til said (7.26 mg QE/gram) has the lowest amount of total flavonoid. The antimicrobial activity results showed that the crude extract of seeds Tukhme gajar and Tulasi exhibited great potential against multi-drug resistance strain Escherichia coli showed activity about 21 mm and 20 mm, respectively. The crude extract of seed Mako and Til said exhibited great potential against multi-drug resistance strain S. aureus with zones of inhibition of 21 mm and 20 mm, respectively. The crude extract of seed Mako and Black pepper exhibited great potential against the multi-drug resistance strain Bacillus Subtilis with a zone of inhibition of 21mm and 20mm, respectively. Seed extracts Soya, Nalon seed, Safaid mirch, Pista maghaz, and Til said exhibited the scavenging activity where percentages of inhibition of free radicals of extract were recorded as 41%, 33%, 34%, 29%, and 28% respectively. Ascorbic acid (control) exhibited percentage inhibition of 55%, 52%, 51%, 50%, and 48% respectively. According to our results, the desired seed extract shows remarkable antibacterial, antioxidant, total phenolic content, and total flavonoid content. All the selected medicinal seeds have a good number of phytochemicals as well as antimicrobial activity.

**Keywords:** Phytochemicals, antimicrobial, seed extract, and total flavonoid.

**Introduction:**

Medicinal plants have been valued for centuries for their healing properties, primarily because they contain bioactive compounds like phenolics, flavonoids, alkaloids, and terpenoids [1][2][3]. These phytochemicals exhibit a wide range of biological activities, including antioxidant, antimicrobial, anti-inflammatory, and anticancer effects [4][5][6]. Seeds of medicinal plants, in particular, are a rich yet underexplored source of these bioactive constituents [7][8][9]. Amid the rising threat of antibiotic-resistant pathogens and the growing demand for natural alternatives to synthetic drugs, there is a resurgence of interest in exploring plant-derived compounds for their therapeutic potential [10][11][12].

*Aconitum heterophyllum*, often referred to as ash, is a flowering plant species belonging to the *Aconitum* genus in the *Ranunculaceae* family [13]. It is a robust herb characterized by a thick stem and fibrous roots. Various types of this plant can be distinguished based on their morphological and anatomical features. Among the different types of *Aconitum heterophyllum*; Yellow, Black, Red, and White, the white variety is the most commonly available and is considered the best. Its tuberous roots, which exhibit a pale yellow hue, appear plump and fleshy in texture [14].

A perennial herbaceous plant in the *Zingiberaceae* family is *Amomum subulatum*, also called Black Cardamom, big Cardamom, Hills Cardamom, Winged Cardamom, Large Cardamom, Indian Cardamom, Bengal Cardamom, or Brown Cardamom. Its seed pods have a solid, camphor-like taste, derived from the drying process, with a smoky character. It is named (*ba'ī ilāichī*) in Hindi. Much like green Indian cardamom pods, these pods are used as a seasoning, though they offer a unique and distinctive flavor. This spice, unlike green cardamom, is never used in sweet dishes. Its pungent flavor and scent come from conventional drying practices over open fires. Some people can describe black cardamom as an unfavorable substitute for green cardamom.

The cashew plant derives mostly from Brazil and develops primarily in the Cerrado biomes, Caatinga, and Amazon. It belongs to the *Anacardiaceae* family and in the genus *Anacardium* there are around 23 species [15].

The standard soluble solids of cashew apple juice are 10-12 Brix, which decreases the sugar content by 8.7 grams/100 grams and the overall sugar content by 9.10 grams/100 grams [16]. In terms of reducing sugars, the primary components are glucose (4.8 g per 100 grams) and fructose (4.9 g per 100 grams) [17]. A major source of ascorbic acid is Cashew apple juice (145-161 mg/100 mL). The antioxidant ability of cashew apple juice was primarily due to its ascorbic acid content, based on kinetic studies. There are also malic, tartaric, and citric, acids in cashew apple juice (434-578, 238237, respectively, 2127 mg /100 mL) [18]. The gross dietary fiber content of the cashew apple is 306 g/kg, most of which is insoluble (89 percent). This fiber has polyphenols that are non-extractable and more numerous than extractable polyphenols. These non-extractable dietary fiber-related polyphenols can enter the colon and boost the status of colonic antioxidants [15].

*Melia azedarach* is a perennial plant originating in the Mahogany family *Meliaceae*, or the Chinaberry, Proud of India, beads trees, caps lilac, clover syringes, lilac, Indian lilac, and white cedar. It has a native tree in Indomalaya and Southeast Asia [18].

The *Meliaceae* family in plants is known for its different chemicals which show insecticidal, antifeedant, development, and growth-modifying characteristics [19]. Mazedarch extracts from different parts of the plant have been shown to affect many pests [20]. Many terpenoids are defined as ectiaid behavior in plants including its *Meliaceae* family. Three *Meliacarpin* derivatives were confirmed to be isolated from Mazedrach leaves [20]. The effectiveness of *melicarpine* compounds in chronic feeding bioassays has been examined on phytophagous insect larvae *Spodoptera littoralis*. The findings of Kelecom et al., 1996 also suggested similar to the standard biopesticide *azadirachtin* from the neem tree for its insecticidal effectiveness. The crude ethanol seed extract of Bazilian Mazedarach showed both

phagoinhibitory and anti-molting behavior, which was one of the agents of Dengue Disease, the hematophagous bug *Rhodnius prolixus*

*Argemone mexicana* (Mexican poppy) is a poppy plant from Mexico that is abundantly found in different parts of the world, Mexican prickly poppy, flowering hibiscus [21], cardo, or cardosanto. The wine is resistant to drought and poor soil, an extraordinarily resilient pioneering wine that is often the only defense against new paths. It is a bright latex of yellow. It is rarely consumed directly, poses no toxicity to grazing animals, and is utilized in various traditional medicinal practices, including those in its native region as well as in parts of the western United States, Mexico, and several regions of India. The colorful Holika Dahan festival is a tribute for adults and children in India and this plant flowering during March when the Holi Festival is conducted. In India, it is also known as "kateli ka Phool."

*Eruca vesicaria*; Syns *Eruca Sativa* Mill., *E. vesicaria* subsp. *Sativa* Thell. Rocket, as well as arugula (known in American English) (*Brassica eruca* L.), is an annual fit for human consumption plant used for its clean, bitter, bitter, and spicy taste as a leaf vegetable in the family Brassicaceae. Garden rocket is commonly known by several names across different regions, including "rockets" in South Africa, Britain, Ireland, and New Zealand. It is also referred to as *Eruca*. Other popular names include "rucola," "rucoli," "colewort," "ruchetta," and "roquette" [22]. From Portugal and Morocco in the west to Egypt Syria, and Lebanon, Turkey in the east, *Eruca sativa* is a genus of *Eruca* native to the Mediterranean, widely known as salad vegetables [23].

The herb in the family of Apiaceae is an annual herb. It is often named the Chinese parsley or the Chinese cilantro (/s'lantro -l'n-/). Coriander (*Coriandrum sativum*) is safe to consume, with both its fresh leaves and dried seeds being widely used in cooking. The fresh leaves are typically used as an herb, while the dried seeds are commonly used as a spice. Some pick out the flavor of coriander like unpleasant, lemon/calcareous, but the leaves are almost a quarter of those surveyed who are associated with a gene that recognizes unique aldehydes often used as odorants in soaps and detergents [22].

Coriander leaves, Chinese parsley, fresh coriander, and sometimes cilantro are named herbs in the US and commercially in Canada Coriander may be replaced by cilantro into the similar family (Apiaceae) as cilantro (*Coriandrum sativum* L.), (*Eryngium foetidum* L.). Culantro, a more active volatile leaf oil, has a markedly different fleshy look and a higher fragrance. The leaves, with citrus overtones, taste distinct from the seeds [21]. Fresh leaves are part of a lot of foods such as chutney and salads, guacamole, and a common broth, fish, and beef garnish [23]. Coriander leaves are mostly raw or applied to a dish just before eating as the heat reduces its taste. They are used in significant quantities in Indian and Central Asian cuisine and are fried before the taste decreases. When extracted from the grape, the leaves easily spoil and lose their scent when dry and frozen.

Coriander seeds are the dried berries of the plant, and in culinary contexts, the term "coriander" often specifically refers to these seeds when used as a spice, rather than to the entire plant. When grounded, the bean has a lemon citrus odor due to the pinene linalool and terpenes. It is classified as spicy warm, and flavored with orange. For drug development projects in the pharmaceutical industry, natural ingredients play a significant role. However, it also recognized the value of traditional medicine and focused extensively on the production of botanical techniques, recommendations, and standards [24].

Recently there has been a rising research interest in medicinal plants as plant-related medications are increasing in efficacy and concern about the side effects of modern medicines. Because of the continued proliferation of drug-resistant species and adaptations of microbial pathogens to common antimicrobials, existing antimicrobial agents have decreased in effectiveness. There has remained a significant source of commercial medicines in the quest for new drugs from plants [25].

**Materials and Methods:**

This study was conducted in the Department of Food Technology, University of Haripur Pakistan. Forty selected seeds were collected from District Haripur. Listed in Table 1 given below. The seeds were washed with water, followed by sorting and grading, and then stored at room temperature until further analysis.

**Table 1.** List of selected medicinal plant seeds

| S. No. | Local Name                       | Botanical Name                   |
|--------|----------------------------------|----------------------------------|
| 1      | Atees, Sharangi, Chitijari       | <i>Aconitum heterophyllum</i>    |
| 2      | Moti elaichi, Bari elaichi       | <i>Amomum subulatum</i>          |
| 3      | Kaju, Kaji sabzi                 | <i>Anacardium accidents</i>      |
| 4      | Drake                            | <i>Melia azedarach</i>           |
| 5      | Soya, Tukhme Shibbat             | <i>Anthemum graveolens</i>       |
| 6      | Baramdamdi, Pila Dhatura, Kateli | <i>Argemone Mexicana L</i>       |
| 7      | Rai Dana, Sarson                 | <i>Brassica campestris</i>       |
| 8      | Sya zeera                        | <i>Bunium bulbocastamum</i>      |
| 9      | Tara meera, usoon                | <i>Eruca vesicaria</i>           |
| 10     | Dhania                           | <i>Coriandrum sativum</i>        |
| 11     | Safaid zebra                     | <i>Cumiumum cyminum</i>          |
| 12     | Choti elaichi, Sabz elaichi      | <i>Elettaria cardamomum</i>      |
| 13     | Zaminekand                       | <i>Dioscorea bulbifera</i>       |
| 14     | Saunf                            | <i>Foeniculum vulgare</i>        |
| 15     | Aalsi                            | <i>Azadirachta indica</i>        |
| 16     | Koonch Kawanchah                 | <i>Mucuna pruriens</i>           |
| 17     | Nalon seed                       | <i>Lepidium sativum</i>          |
| 18     | Kalonji                          | <i>Nigella sativa</i>            |
| 19     | Tulasi, Tulsi                    | <i>Ocimum tenuiflorum</i>        |
| 20     | Khashkhash said                  | <i>Papaver somniferum</i>        |
| 21     | Safaid mirch                     | <i>Piper nigrum</i>              |
| 22     | Tukhme ajar                      | <i>Daucus carota</i>             |
| 23     | Pista maghaz                     | <i>Pistacia vera</i>             |
| 24     | Badam                            | <i>Prunus amygdalus</i>          |
| 25     | Anar dana, Durni                 | <i>Punica granatum</i>           |
| 26     | Loung                            | <i>Syzygium aromaticum</i>       |
| 27     | Til said                         | <i>Sesamum indicum</i>           |
| 28     | Mako, Kasoori musk               | <i>Solanum minimum</i>           |
| 29     | Ajwain                           | <i>Trachyspermum ammi</i>        |
| 30     | Methi                            | <i>Trigonella foenum-graecum</i> |
| 31     | Timer, Timber                    | <i>Zanthoxylum armatum</i>       |
| 32     | Paneer Doda                      | <i>Vithania coagulants</i>       |
| 33     | Mustard                          | <i>Brassica campestris</i>       |
| 34     | Walnut                           | <i>Juglans regia</i>             |
| 35     | Magaz kheer                      | <i>Cucumis sativus</i>           |
| 36     | Khanor                           | <i>Aesculus indica</i>           |
| 37     | Black pepper                     | <i>Piper nigrum</i>              |
| 38     | Tukhm-e-mooli                    | <i>Raphanus raphanistrum</i>     |
| 39     | Tukhm-e-Tarbooz, hindrance       | <i>Citrullus vulgaris</i>        |
| 40     | Bartang, Chamcha Patar           | <i>Plantago major</i>            |

The selected seeds of different plants were sun-dried for 2 to 3 days. For phytochemical analysis, 10 grams of the dried seeds were powdered. Approximately 50 grams of dry seeds were placed in an electric grinder and ground for 5 to 10 seconds.

#### **Phytochemicals Analysis:**

Ten-gram sample of seeds was placed in 50 ml of 70% methanol and concentrated in a water bath till 10 ml remained. The mixture was centrifuged at 3000 rpm for 10 min. The supernatant was decanted and the residue was again dissolved in 10 ml methanol and centrifuged at 3000 r/m for 5 min, then both the supernatants were combined. This aliquot was used for the determination of TPC, TFC, and DPPH radical scavenging activity.

#### **Total Phenolic Content (TPC):**

For this test, 1 ml of previously prepared aliquot was oxidized with 2.5 ml of Folin-Ciocalteu's reagent (10 %). This oxidized sample was then neutralized with 2 ml Na<sub>2</sub>CO<sub>3</sub> (7.5 %). The mixture was kept in a dark place for ¾ hours and then its absorbance was measured at 765 nm wavelength using a spectrophotometer with Gallic acid as standard [9].

#### **Total Flavonoid Content (TFC):**

For TFC, 1 ml of already prepared aliquot was mixed with 0.3 ml NaNO<sub>3</sub> (5%) and the mixture was left for 5 minutes. Then 0.6 ml of AlCl<sub>3</sub> (10%) was added and mixed it. After 5 minutes, 2 ml of 1 M NaOH was added to the mixture. In the end, the absorbance at 510 nm wavelength was measured using a spectrophotometer taking quercetin as the standard [6].

#### **Antioxidant Activity:**

In order to find free radical scavenger activity of the aliquot 1, 1-diphenyl 1-2-picrylhydrazyl (DPPH) was used [22]. The aliquot was added at an equal volume, to methanolic solution of DPPH (0.2 mM). The absorbance was noted at 517 nm at room temperature after 30 minutes. Radical scavenging activity is expressed as the inhibition percentage and calculated using:

$$\text{Radical scavenging activity (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

Where, A<sub>control</sub> and A<sub>sample</sub> are absorbance of control and sample, respectively.

#### **Anti-Bacterial Activity of Seed:**

Approximately fifty grams of the powder were soaked in 200 ml of ethanol and kept at room temperature in the dark for one week, with continuous stirring throughout the process. After a week the extract was filtered by filter paper and dried in a rotary evaporator at 40 °C. Methanol evaporated and the extracts were stored in the dark at room temperature for analysis of anti-bacterial activity. Agar-well plate method was used for the analysis of anti-bacterial activity. Firstly, the media was prepared. Nutrient agar was used for media preparation. Media was poured into the glass plate. The plates were left for 30 minutes to solidify. Clinical isolates were spread on the plate using a spreader one by one. A well was created on the media plate through a borer (5mm) and sealed with molten nutrient agar. Samples were added to the well and the plates were placed in incubators maintained at 37°C for 25 hours. The diameter of the zones cleared by the action of antibacterial substances on clinical isolates was measured. For seeds samples plates and wells were prepared by the above-mentioned method and seeds were poured into sealed wells for analysis of antibacterial properties. All the readings were taken in three replications.

#### **Statistical Analysis:**

Data was analyzed by application of multivariate data analysis using principle component analysis. Principle component analysis (PCA) was performed with the SIMCA-P software (v. 12.0, Umea, Sweden) by unit variance (UV) scaling method.

#### **Results and Discussion:**

##### **Phytochemical Analysis:**

##### **Total Phenolic Content:**

The seed extract of Pista maghz 40.80 mg GAE/ gram, Nalon seed 34.18 mg GAE/gram, Soya 30.41 mg GAE/gram, Saunf 27.53 mg GAE/gram, and Moti elachi 27.22 mg GAE/gram showed excellent phenolic compounds. The phenolic compounds shown in above Table 2, Sabz elachi 6.73 mg GAE/gram, Drek 5 mg GAE/gram, Saya zebra 4.62 mg GAE/gram, Badam 3.62 mg GAE/gram, and Kaju 1.27 mg GAE/gram have the lowest amount for TPC. Phenols are chemical compounds that have a hydroxyl group (-OH) that is bonded with aromatic hydrocarbon groups. Plants can produce phenolic compounds under specific conditions. Those plants that produce phenolic compounds can be used as medicinal plants. Phenolic compounds are soluble but some are volatile. These compounds also work as antimicrobial, antioxidant, and antifungal and can help in increasing the shelf life of fish meat [26][27].

#### **Total Flavonoid Content:**

The seed extract of Tukham-e-taboos 55.40 mg QE/gram, Paneer doda 51.37 mg QE/gram, Kalongi 49.36 mg QE/gram, Ajwain 48.49 mg QE/gram, Bartang 47.14 mg QE/gram, Baramdamdi 45.19 mg QE/gram and Black pepper 43.36 mg QE/gram Shows remarkable flavonoid contents. Similarly, the lower amount for TFC-containing seeds was Badam 26.95 mg QE/gram, Tulsi 26.68 mg QE/gram, Khashkhash 24.29 mg QE/gram, Pista magaz 21.20 mg QE/gram, Magaz kheer 23.23 mg QE/gram, Tukham-e-mooli 21.48 mg QE/gram and the lowest one till said 7.26 mg QE/gram. According to our results, the desired seed extract shows remarkable antibacterial, antioxidant, total phenolic content, and total flavonoid content. Flavonoids are the naturally occurring plant compounds responsible for the colors and pigments in flowers. These also can inhibit some disease-causing microorganisms [28][29]. These also have anti-allergic, anti-inflammatory, and antioxidant properties. Flavonoids are responsible for the activation of antioxidant enzyme radicals. These are important components of diet but are not considered as nutrients [30][31].

#### **Antioxidant Activity of Plant Seeds:**

##### **DPPH Free Radical Scavenging Activity of Seed Extract:**

Seed extracts Soya, Nalon seed, Safaid mirch, Pista maghaz, and Til said exhibited the scavenging activity where percentages of inhibition of free radicals of extract were recorded as 41%, 33%, 34%, 29%, and 28% respectively. Seed extracts Saunf, Tukhm-e-mooli, Tulsi, Tukhm-e-Tarbooz, and Koonch Kawanchah exhibited scavenging activity where percentages inhibition of free radicals of extract were recorded as 26%, 25%, %, 24%, 22 %, and 19% respectively. The seed extracts of Kaju, Walnut, Saya Zeera, Drek, and Dhania (SMS1) exhibited antioxidant activity, with the recorded percentages of free radical scavenging being 1.2%, 3%, 4%, 5%, and 7%, respectively. Ascorbic acid (control) exhibited percentage inhibition of 55%, 52%, 51%, 50%, and 48% respectively. Antioxidants can protect the lipids and oils in food from oxidative degradation. When these are added to the food these control rancidity developments and also stop the toxic oxidation product development. As well as these help to maintain the nutritional quality of food hence increasing the shelf life of the products. Nowadays synthetic antioxidants are used as food preservatives because of safety concerns. Radical scavenging activity was used to determine the free radical properties of seed extracts [32][33].

**Table 2.** Total Phenolic Content, Total Flavonoid Content, and Antioxidant Activity of medicinal seeds

| S. No. | Seeds Name                 | Total Phenolic Content | Total Flavonoid Content | Antioxidant Activity |
|--------|----------------------------|------------------------|-------------------------|----------------------|
| 1      | Atees, Sharangi, Chitijari | 19.74±1.15             | 34.41± 0.78             | 27.38±1.69           |
| 2      | Moti elaichi, Bari elaichi | 27.22±1.36             | 31.31±1.20              | 26.43±2.40           |
| 3      | Kaju, Kaji sabz            | 1.27±0.64              | 21.76±1.77              | 1.27±0.64            |
| 4      | Drake                      | 5.00±0.66              | 41.60±1.03              | 5.00±0.65            |

|    |                                     |            |            |            |
|----|-------------------------------------|------------|------------|------------|
| 5  | Soya, Tukhme Shibbat                | 30.41±1.90 | 31.42±1.20 | 41.41±3.59 |
| 6  | Baramdamdi, Pila<br>Dhatura, Kateli | 1.64±0.64  | 45.19±0.92 | 1.64±0.64  |
| 7  | Rai Dana, Sarson                    | 5.99±0.84  | 27.29±0.38 | 4.98±2.57  |
| 8  | Sya zeera                           | 4.62±1.70  | 39.71±0.52 | 4.61±1.70  |
| 9  | Tara meera, usoon                   | 16.76±1.48 | 34.30±0.91 | 12.42±5.02 |
| 10 | Dhania                              | 7.22±1.70  | 31.25±1.02 | 7.21±1.70  |
| 11 | Safaid zeera                        | 9.67±1.15  | 31.92±1.54 | 8.33±2.80  |
| 12 | Choti elaichi, Sabz elaichi         | 6.73±1.03  | 38.69±1.12 | 9.06±3.20  |
| 13 | Zaminekand                          | 4.40±1.15  | 27.22±1.85 | 5.73±2.80  |
| 14 | Saunf                               | 27.53±1.26 | 39.71±1.52 | 26.52±2.92 |
| 15 | Aalsi                               | 3.62±0.84  | 31.41±1.35 | 4.61±2.57  |
| 16 | Koonch Kawanchah                    | 19.05±1.78 | 37.74±1.39 | 19.05±1.77 |
| 17 | Nalon seed                          | 34.18±1.16 | 41.12±1.73 | 33.84±1.42 |
| 18 | Kalongi                             | 21.76±1.74 | 49.36±1.46 | 7.75±3.05  |
| 19 | Tulasi, Tulsi                       | 27.04±1.58 | 26.68±1.12 | 24.70±2.56 |
| 20 | Khashkhash said                     | 16.03±1.39 | 24.29±1.56 | 18.36±3.42 |
| 21 | Safaid mirch                        | 26.91±3.21 | 32.28±0.67 | 34.91±3.39 |
| 22 | Tukhme gajar                        | 17.25±3.58 | 29.23±1.34 | 17.24±3.57 |
| 23 | Pista maghaz                        | 40.80±2.31 | 21.20±1.02 | 29.46±2.50 |
| 24 | Badam                               | 3.62±0.84  | 26.95±1.16 | 15.02±4.22 |
| 25 | Anar dana, Durni                    | 14.03±2.52 | 33.08±1.49 | 15.02±3.57 |
| 26 | Loung                               | 17.36±0.75 | 38.02±1.90 | 15.02±3.58 |
| 27 | Til said                            | 19.86±1.10 | 7.26±1.02  | 28.86±0.98 |
| 28 | Mako, Kasoori mhushk                | 15.39±2.96 | 30.90±1.28 | 15.38±2.95 |
| 29 | Ajwain                              | 13.17±2.94 | 48.49±3.54 | 9.83±4.48  |
| 30 | Methi                               | 18.75±2.95 | 36.75±2.84 | 18.74±2.95 |
| 31 | Timer, Timbar                       | 17.35±1.77 | 38.35±0.87 | 14.34±3.99 |
| 32 | Paneer doda                         | 16.80±2.45 | 51.37±2.03 | 16.13±3.57 |
| 33 | Mustard                             | 10.92±1.92 | 36.33±2.53 | 10.92±1.92 |
| 34 | Walnut                              | 3.13±1.93  | 38.07±2.58 | 3.12±1.93  |
| 35 | Magaz kheera                        | 10.19±2.32 | 23.23±1.96 | 10.19±2.32 |
| 36 | Khanor                              | 15.95±1.67 | 34.54±2.08 | 14.28±3.34 |
| 37 | Black pepper                        | 8.77±2.23  | 43.36±2.00 | 8.77±2.23  |
| 38 | Tukhm-e-mooli                       | 25.05±2.83 | 21.46±1.93 | 25.05±2.82 |
| 39 | Tukhm-e-Tarbooz,<br>hindrance       | 23.46±1.86 | 55.40±2.28 | 22.46±3.40 |
| 40 | Bartang, Chamcha Patar              | 21.50±2.89 | 47.14±1.14 | 21.16±2.03 |

Values are means of triplicate and different values means  $\pm$  S.D ( $P \leq 0.05$ ).

#### **Antibacterial Activity of Extract Against Mdrs Strains:**

The screening of antibacterial activities of crude extract was determined by the agar well diffusion method. The activity was screened against 3 MDRS (Escherichia coli, Staphylococcus aureus, and Bacillus Subtilis).

#### **Activity of Different Bacterial Extracts Against Escherichia Coli:**

The results revealed that the crude seed extracts of Tukhme Gajar, Tulasi, Safaid Zeera, Badam, and Zaminekand demonstrated significant antibacterial activity against a multidrug-resistant strain of Escherichia coli, with inhibition zones measuring approximately 21 mm, 20 mm, 19 mm, 18 mm, and 17 mm, respectively. In comparison, the extract from

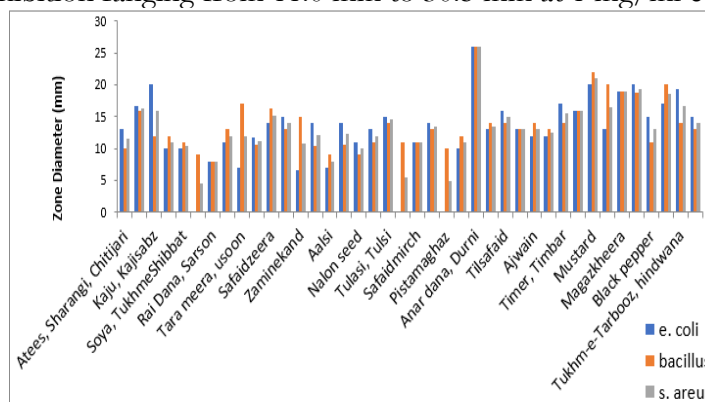
Kaju showed moderate activity with an inhibition zone of about 8 mm, while Moti Elachi exhibited minimal activity, producing a zone of only 6 mm.

### The activity of Different Bacterial Extracts Against *S. Aureus*:

The results showed that the crude extracts of Mako seed, Til Safaid, and Khanor exhibited strong activity against the multidrug-resistant strain of *S. aureus*, with zones of inhibition measuring 21 mm, 20 mm, 19 mm, 18 mm, and 17 mm, respectively. In contrast, extracts from Safaid Mirch showed moderate activity (8 mm and 7 mm), while Rai Dana showed minimal activity with a 3 mm zone of inhibition.

### Activity of Different Bacterial Extracts Against *Bacillus Subtilis*:

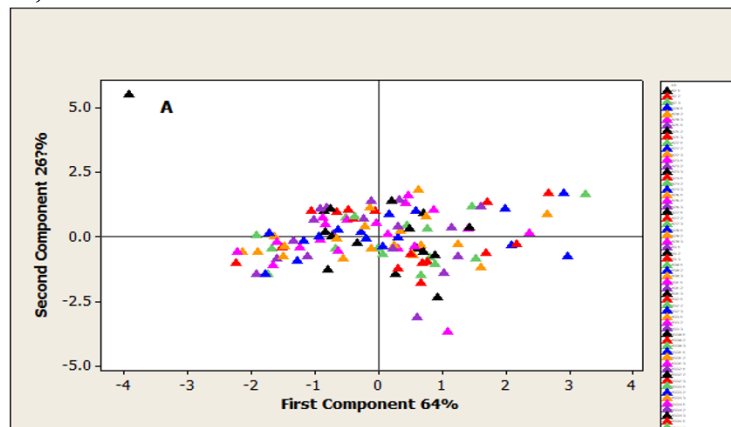
The results showed that the crude extract of seed Mako, Black pepper, and Tukhm-e-Tarbooz, exhibited great potential against multi-drug resistance strain *Bacillus Subtilis* with a zone of inhibition of 21 mm, 20 mm, 19 mm, 18 mm, 17 mm, other extracts *Dhania* showed activity about 8 mm and *Badam* showed less zone of inhibition of 3 mm against *Bacillus Subtilis*. Our results revealed that the methanol extract showed the highest antibacterial activity with a zone of inhibition ranging from 11.0 mm to 30.5 mm at 1 mg/ml concentration.



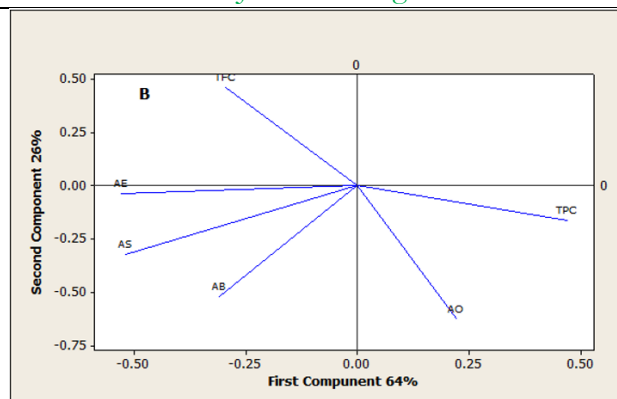
**Figure 1.** Antibacterial activity of medicinal seeds.

### Principal Component Analysis (PCA):

Data were analyzed using principal component analysis to find out the relationship between all treatments of plant seeds and the qualitative parameters studied. The scores were generated through Principal Component Analysis (PCA) for all samples, as illustrated in Figure 12. The distribution of the parameters within the space defined by the first and second principal components is depicted in Figure 13. The sum of principal components 1 and 2 (PC1 and PC2) accounted for 90% of variations among treated samples. PC1, the first component contributed to 64% of the total variation and the second component accounted for 26% of the total variation. PC1 was positively correlated with TFC while negatively correlated with AE, TPC, AO, AB, and AS.



**Figure 2.** PCA for selected medicinal plant seeds



**Figure 3.** PCA for correlated results of medicinal seeds

### Conclusion:

This study determined the promising antimicrobial, and antioxidant activities, total phenolic content, and total flavonoid content. These studies may provide significant data about the types and bioactivity of extract obtained from different seeds. Further studies are needed for the identification of these active seed extracts and to separate the active substances from different medicinal plant seeds. Further studies on extracts may result in the discovery of bioactive compounds that may lead to the development of safer and more potent drugs in the future.

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