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Phytochemical screening of *Salvia moorcroftiana* plant extracts qualitatively

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Abstract

Qualitative and quantitate evaluation is essential for finding phytochemicals in medicinal plants. Plants exhibit therapeutic potential by virtue of the availability of certain functional ingredients. The utilization of herbal remedies for purpose of research is a fundamental and essential aspect in achieving competence in study findings. The extraction and discovering of the quantity and type of bioactive constituents in a plant for medicinal reasons is the main element of the analysis. Alkaloids, tannins, flavonoids and phenolic compounds are among the most prominent of these bioactive plant ingredients. The current study goal was to perform a phytochemical constituent's investigation of the Lamiaceae family, particularly the specie, Salvia moorcroftiana, which is known for its numerous applications all over the world. The *Salvia moorcroftiana* plant has mostly been recognized as a tenacious weed in most temperate places all over the world. The recent research examines at the key bioactive constituents of the medicinally valuable herb. Fruit, roots, and leaves extracts comprised primary phytochemicals such as carbohydrates, reducing sugar, amino acids, hexose sugar, and lipids. Tannins and anthraquinones are examples of secondary phytochemicals. Phytoconstituents were recognized in fruit, root and leaf extracts respectively. The results revealed that *Salvia moorcroftiana* contains valuable plant chemical components that can be used in the pharmaceutical and naturopathic fields.

Key words

Medicinal plant, Primary phytochemicals, *Salvia moorcroftiana,* Secondary phytochemical

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1. Introduction

Medicinal plants are extremely crucial to human health, the therapeutic potential of these plants is attributed to the presence of certain chemical substances that have a specific physiological influence on the human body [1]. Active ingredients such as tannins, flavonoids and phenolic chemicals are the most prominent plant bioactive constituents. Medicinal plants are a dominant contributor of novel pharmaceuticals and healthcare products [2]. Plant extracts are getting prominence as natural antibacterial and antioxidant agents, particularly in the dietary, medical and agrochemical industries [3]. For hundreds of years, plants have served as a significant medicinal source, especially nowadays, the World Health Organization claims that up to 80% of people still use traditional remedies such as herbs for the therapies of various diseases [4]. Medicinal plants have now been examined for their potential use in the treatment of a wide variety of diseases triggered by oxidative stress, significant bacterial and viral infections, and other factors. Plant products contain a broad range of biologically active molecules [5], the majority of whom are phenolic and these components have been found to have a variety of biological effects [6]. Due to the resistance that microorganisms have acquired towards antibiotics, scientists have been interested in important bioactive chemicals that come from plant species against destruction of harmful microbes [7].

The utilization of plants containing bioactive components in humans has become one of the foremost fundamental yet conservative therapeutic medicinal techniques. Despite the fact that over 6,000 medications have been synthesized from diverse plant sources [8]. Phytoconstituents have a substantial influence on the human physiology, phytochemicals in the body interact with proteins and nutrients to protect the body from ailments and stress conditions [9]. Tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids are among the chemical components found within medicinal plants that have physiological effects on the body of organisms. Natural substances have long been utilized in medicinal herbs. Barks, leaves, flowers, roots, fruit and seeds might all be used to produce medicines [10]. *Salvia moorcroftiana* is a perennial herbaceous plant found in the Himalayan regions in Pakistan. It may grow to be 2.5 feet height and has long-stemmed basal leaves with a serrated border that appears to be covered in white wool [11]. The flowers are supported by several inflorescences that grow above the leaves. The blooms are surrounded by a hairy calyx, and the shrub is complemented with brilliant green-veined bracts. Under growth, it requires full light, loose soil, and regular watering [12]. *Salvia moorcroftiana* is the biggest and most diverse genus in

the Lamiaceae family [13]. Fruit, whole grains, nuts and the seeds of the *Salvia moorcroftiana* plant contain phytochemicals [14]. Phytoconstituents are natural bioactive molecules that are classified into two types based on their roles in plant metabolism, primary and secondary constituents [15]. Carbohydrates, amino acids, proteins and chlorophyll are the major ingredients, whereas alkaloids, terpenoids, steroids and flavonoids are the minor constituents [16].

People in Pakistan's living in rural areas use medicinal plants to cure all sorts of diseases [17]. It is believed that 80% of the world's population relies on herbal medicine for basic health care. Salvia moorcroftiana is one of the most important genera in this regard, of which 9 species are medicinal in nature [18]. *Salvia moorcroftiana* roots are used to treat colds, coughs, gastrointestinal disorders and stomach troubles [19]. The leaves are used to treat guinea worm and itching and they are put on wounds as an ointment. In addition to medication effects, plant species have an important role in the growth of people's economic standing. *Salvia moorcroftiana* leaves are used as a plaster for wounds, while the seeds are used to treat coughs and colds [20]. Fresh leaves are picked, washed and dried in the sunlight before being ground into powder. This powder is employed to treat coughs with honey and diarrhoea with water [21]. *Salvia moorcroftiana* has traditionally been utilized to treat bronchitis, asthma, cough, digestive and circulatory illnesses, oral and throat infection and other ailments all over the world. Some species also have anti-diabetic, anti-tumor, antibacterial and antioxidant properties [22].

Fig 1. Image of *Salvia moorcroftiana*

2. Material and methods

2.1. Plant sample

Salvia moorcroftiana plant parts such as Roots, leaves and fruit were collected from the Tehsil Dargai District Malakand in the month of December 2020. The botanical name of the plant was verified by Lecturer Mr. Ibrahim Khan at the Department of Botany GPGC Dargai Malakand KPK. The roots, leaves, and fruit were washed with water and dried for 15 days in the shade at room temperature (18 °C). After drying, the fresh roots, leaves, and fruit were cut into smaller pieces using scissors and pulverised with a crusher.

2.2. Solvent Extraction Process

An ethanol solvent is used in the extraction method. 60 gm of leaves was soaked in 450 ml of ethanol, 40 gm of roots in 300 ml of ethanol, and 60 gm of fruit in 300 ml of ethanol for 15 days at room temperature. After 15 days, the extracts were filtered. After that, the filtrate was allowed to evaporate at room temperature for 20 days.

2.3. Reagents

Benedict Reagent, Millions Reagents, Wagner Reagent, Selwinoff’s Reagent, Mayer’s Reagent, Hager’s Reagents and Molish Reagents.

2.4. Test for phytochemical

2.4.1. Test for primary phytochemical

2.4.1.1. Carbohydrate Tests

The presence of carbohydrates was tested using the Molish test, in which 3 ml of the plant extract sample and a few drops of Molish's reagent were added, the mixture was thoroughly stirred, and then 2 ml of concentrated H_2SO_4 was gently poured over the test tube's edge. The existence of carbohydrate was shown by the formation of a purple interfacial ring.

2.4.1.2 Test for Reducing Sugar

The existence of reducing sugar was assessed using the benedict test, which involved heating an equivalent amount of sample extract and Benedict's reagent in a water bath for five minutes. The

solution will have coloured, green, yellow or red depending on the amount of reducing sugar in the test solution.

2.4.1.3. Test for Monosaccharide

The presence of Monosaccharide was determined with the help of Barfoeds test in which a roughly equal amount of sample extract and Barfoeds reagent were combined. These solutions were boiled for 2 minutes in a water bath before being cooled. The development of reddish precipitate confirmed the presence of monosaccharide.

2.4.1.4. Test for Hexose Sugar

The existence of Hexose Sugar was evaluated employing Cobalt Chloride procedure, which involved mixing 3 mL of sample extract with 2 mL of cobalt chloride. After that, it was boiled and cooled. Following the cooling phase, a few drops of NaOH solution were then added. Solution of greenish blue (glucose) or purple (fructose), or top layer greenish blue and bottom layer purplish (mixture of glucose and fructose). The presence of Hexose Sugar was also determined with the help of Selwinoff's Chloride test in which a boiling water bath was used to heat 3 ml of Selwinoff's reagent and 1 ml of test sample extract for 1-2 minutes while waiting for the red colour to appear.

2.4.1.5. Test for Non-Reducing Polysaccharides (Starch)

The appearance of Non-Reducing Polysaccharides (Starch) was tested using the Iodine test, which involved mixing roughly 3 ml of sample extract with a few drops of weak iodine solution. The presence of starch is indicated by the blue colouration.

2.4.1.6. Test for Protein

The presence of Protein was determined with the help of Biuret test in which about 3 ml sample extract and 4% NaOH solution was added in a test tube. After the addition of few drops of 1% $CuSO_4$ solution, Violet or pink colour will have appeared. The presence of Protein was determined with the help of Million's test as well, in which 3 ml of sample extract and 5 ml of Million's reagent were combined then white port was displayed. On heating, the white precipitate becomes brick red or dissolves with a green tinted solution.

2.4.1.7. Tests for Amino Acids

The presence of Amino Acids was determined with the help of Ninhydrin test in which 3 ml of sample extract was cooked in a boiling water bath for 10 minutes with 3 drops of 5% Ninhydrin solution. The purple or blue colour revealed the presence of Amino Acids.

2.4.2. Test for secondary phytochemical

2.4.2.1. Test for Steroids and Phytosterols

The presence of Phytosterols was determined with the help of Sulphuric acid test in which One millilitre of plant extract, one millilitre of chloroform, and few drops of strong sulphuric acid were added. The presence of steroids is shown by the creation of a brown ring, whereas the presence of phytosterols indicated by the production of a blue green tint.

2.4.2.3. Test for Cardiac Glycosides

Cardiac Glycosides were detected employing reagents in which approximately 5 ml of each extract was treated with 2 ml of glacial acetic acid comprising one drop of ferric chloride solution. This was followed by 1 cc of concentrated Sulphuric acid. Cardenolide's deoxysugar property is indicated by a brown ring at the contact.

2.4.2.4. Test for Anthraquinones Glycosides

The existence of Anthraquinones Glycosides was tested using the Borntragers test, which involved heating and filtering 3 ml of extract sample with 3 ml of dil. H_2SO_4. To cool the filtrate, an equivalent volume of benzene and chloroform were added. The organic layer emerged after carefully stirring the mixture, then ammonia was introduced, an ammonical layer of pink or red coloured appeared indicted the presence of Anthraquinones Glycosides.

2.4.2.5. Test for Saponins Glycosides

The presence of Saponins Glycosides was determined with the help of Foam Test in which an equal volume of the extract sample and water was shaken vigorously. Persistent foam observed showed the presence of Saponins Glycosides.

2.4.2.6. Test for Cyanogenetic Glycosides

The presence of Cyanogenetic Glycosides was determined with the help of Sodium Picrate Test in which the filter paper was first immersed in 10% picric acid to check for the presence of Cyanogenetic glycosides. Then it was dried in a 10% solution of sodium carbonate. After that, we took our sample

extract and placed it in a conical flask with filter paper. The filter paper became brick red indicates the presence of Cyanogenetic Glycosides.

2.4.2.7. Test for Alkaloid

The existence of alkaloid was tested using Hager's Test, which involved adding 3 mL of Hager's reagent to 1 mL of sample extract (saturated aqueous solution of picric acid). The presence of alkaloids was demonstrated by the production of yellow precipitate.

2.4.2.8. Test for Tannin

In order to identify Tannin, add 5 mL of sample extract in 20 mL of chloroform then a few drops of 0.1% ferric chloride ($FeCl_3$) solution were mixed in. The prevalence of tannins was shown by the development of a brownish colour after filtering.

2.4.2.9. Test for Saponins

To identify Saponins, 5 ml of each extract sample was boiled in 20 ml of chloroform in a water bath and filtered. Following filtering, 10 mL of the filtrate was mixed with 5 mL of distilled water and vigorously shaken to form a stable, sustained foam. Before looking for the formation of an emulsion, the foam was mixed with three drops of olive oil and vigorously stirred.

2.4.2.10. Test for Flavonoids

Before being treated with concentrated H_2SO_4, a portion of the filtrate from each plant extract and chloroform was treated with 5 ml of ammonia solution. A golden coloration in each extract indicated the amount of flavonoids.

2.4.2.11. Test for Phlobotanin

Two millilitres of sample extract were poured to two millilitres of 1% HCl, which was then boiled. The existence of Phlobotanins was shown by the formation of a crimson precipitate.

2.4.2.12. Test for Terpenoids

By gently adding 5 mL of the sample extract to 2 mL of chloroform and 3 mL of concentrated Sulphuric acid, a layer of a reddish brown foam was formed, a reddish brown colour confirmed Terpenoids.

3. Results and discussion

The phytochemical active components of Salvia moorcroftiana were qualitatively analysed individually for roots, leaves and fruit. At different stages of the screening procedure, alkaloids, tannins, saponins, flavonoids, terpenoids, glycosides, and phenols all produced different findings. The word positive (+) denotes the existence of a phytochemical, whereas negative (-) indicates the absence of a phytochemical. Reducing sugar, Hexose sugar, Protein, Steroids and Phytosteroids, Cardiac Glycosides, Anthraquinone glycosides, Saponin glycosides, Saponins, Cyanogenetic glycosides, Tannin, Terpenoids showed positive results while carbohydrates, non-reducing polysaccharides, Amino Acids, Alkaloids, Phlobotanins, Flavonoids showed negative results.Reducing sugar, Hexose sugar, Protein, Steroids and Phytosteroids, Cardiac Glycosides, Anthraquinone glycosides, Saponin glycosides, Saponins, Cyanogenetic glycosides, Tannin, Terpenoids showed positive results while carbohydrates, non-reducing polysaccharides, Amino Acids, Alkaloids, Phlobotanins, Flavonoids showed negative results. Phytochemical study of the Salvia moorcroftiana plants under investigation's roots. Reducing sugar, Hexose sugar, Protein, Steroids and Phytosteroids, Cardiac Glycosides, Anthraquinone glycosides, Saponin glycosides, Saponins, Cyanogenetic glycosides, Tannin, Terpenoids examined positive tests. Carbohydrates, non-reducing polysaccharides, Amino Acids, Alkaloids, Phlobotanins and Flavonoids showed negative tests.

S.No	Primary Phytochemical	Test	Fruit	Root	Leaves
1	Test for carbohydrates	Molish's test	Negative	Negative	Negative
2	Test for reducing sugar	Benedict's test	Positive	Positive	Positive
		Fehling's test	Positive	Positive	Positive
3	Test for hexose sugar	Cobalt chloride test	Positive	Positive	Positive
		Selwinoff's test	Positive	Positive	Positive
4	Test for non-reducing polysaccharide's	Iodine test	Negative	Negative	Negative
5	Test for protein	Biuret test	Positive	Positive	Positive
		Million test	Positive	Negative	Positive
6	Test for Amino Acids	Ninhydrin test	Negative	Positive	Negative
7	Test for fixed oil and lipids	Filter paper test	Positive	Positive	Positive

Table 1. Primary Phytochemicals screening of fruit, Roots and Leaves extracts of *Salvia moorcroftiana*

S.No	Secondary phytochemical	Test	Fruit	Root	Leaves
1	Test for steroids and Phytosteroids	Sulphuric Acids	Positive	Positive	Positive
2	Test for Cardiac Glycosides	Acetic acids test	Positive	Positive	Positive
3	Test for Anthraquinone glycosides	Borntragers test	Positive	Positive	Positive
		Modified Borntragers test	Positive	Positive	Positive
4	Test for Saponin glycosides	Foam test	Positive	Negative	Positive
5	Test for Saponins	Foam test	Positive	Positive	Positive
6	Test for Cyanogenetic glycosides	Sodium picric acids test	Positive	Negative	Negative
7	Test for Alkaloids	Hagers test	Negative	Negative	Negative
		Wagners test	Negative	Negative	Negative
		Harborne test	Negative	Negative	Negative
8	Test for Tannin	Ferric chlorides test	Positive	Positive	Positive
9	Test for Phlobotanins	HCL test	Negative	Negative	Negative
10	Test for Flavonoids	Sulphuric acids test	Negative	Negative	Negative
11	Test for Terpenoids	Chloroform test	Positive	Positive	Positive

Table 2. Secondary Phytochemicals screening of fruit, Roots and Leaves extracts of *Salvia moorcroftiana*

4. Conclusion

The result gathered in this study suggests that the *Salvia moorcroftiana* plant has pharmacological characteristics. It is utilised as a traditional treatment all over the world to treat various bodily complications. Plant extracts from *Salvia moorcroftiana* shown antibacterial, antioxidant, antidiabetic, and anticancer activities. When the phytochemical analyses of different parts of the Salvia moorcroftiana were compared, it was discovered that they contain almost the same type of phytochemical since their results were identical. As a result, we concluded that the fruit, leaves, and roots of Salvia moorcroftiana contained the same phytochemical moieties.

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Recommendation

In this era of antibiotic resistance, the current study proposes more phytochemical screening and clinical trials to create new antimicrobial medicines.

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Conflict of Interest:

The authors declare that they have no potential competing interest.

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