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Qualitative phytochemical analysis of stem, leaves and fruits of *Withania somnifera* L. in different solvents

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Abstract

Withania somnifera L. member of family Solanaceae abundantly found in sub-tropical regions of the world, it is a multipurpose medicinal plant. Phytochemical screening of *Withania somniferra* L. was carried out to know about the medicinal potential of same plant. A qualitative analysis was performed to detect various bioactive compounds. The stem, leaves and fruits were individually subjected to phytochemical analysis, revealing the presence of carbohydrates, alkaloids, saponins, flavonoids. proteins, Anthocyanin, Quinones and Tanins which enable it for the developing of new drugs.

KEYWORDS

Withania somnifera L., Phytochemical screening, bioactive Compounds.

1.0 INTRODUCTION

The abundance of chemical variety is unparalleled in plants [1]. Plant extracts are natural materials that provide countless possibilities for the development of novel drugs, whether they are pure chemicals or standardized extracts. Over 80% of people worldwide get their primary healthcare from conventional medicine, according to the World Health Organization (WHO). Asia is a symbol of the long history of human engagement with plants throughout human history. The nature and strong bioactive components of plants make them useful for treating both viral and chronic disorders, [2] With every new day the mutations and development of resistance in the microbes to the chemically synthesized drugs, men turn to plants for the synthesis of new drugs and found that there are thousands of bioactive compounds that can be safely used against resisting microbes with less adverse effects.

The value of biologically active compounds with therapeutic characteristics found in medicinal plants is widely recognized [3]. Many plants have been reported to have antimicrobial, antioxidant, antidiarrheal, anticancer, analgesic, and wound-healing activity [4]. Traditionally mankind has been assigning many health benefits to plants since time

immemorial but there is a great need to extract and asses bioactive compounds for their activities at the clinical stage [5]. The pharmacokinetics, bioavailability, efficacy, safety, and medication interactions of recently produced bioactive chemicals have been the primary focus of these clinical studies, and their formulations (extracts) need careful examination [6]. These trials have mainly been designed to investigate the safety, short time and long-term health effects of these plant-derived drugs. WHO has divided the world into 12 mega biodiversity countries comprising of 91 conventional countries, these biodiversity-rich areas have around 20000 medicinally important plants. For the proper screening of important phytochemicals, some cardinal steps are extraction, pharmacological screening, isolation, molecular characterization, toxic activity assessment, and finally clinical evaluation. This paper is focused on providing a short introduction of commonly used methods of extraction, separation, and characterization of bioactive secondary metabolites from plant extracts. The current paper focuses on an in-depth discussion of the extraction, isolation, and characterization of bioactive compounds from plant extract by using a phytochemical screening assay.

2.0. MATERIALS AND METHODS

2.1. Collection of Plant

The plant of *Withania somnifera L.* was collected from Haripur road near Hattar Industries District Haripur. Care was taken to select healthy and normal plant organs. The plant organ was collected during the period from September to November 2021. The collected plant was identified with the help of Flora of Pakistan. The carefully identified plant and selected fruit, leaves, and stem were used for further investigation i.e., Phytochemical analysis.

2.2. Preparation of Extract

20 grams of dried powder of fruit, stem, and leaf extracts *Withania somnifera L* were soaked in methanol, acetone, and distilled water. The powder was mixed with solvents in reagent bottles individually and kept at room temperature for 72 hours until the soluble matter was dissolved with frequent agitation. The extracts were filtered with Whattman filter paper and the filtrates were collected and stored at 4^0C till further use.

2.3. Phytochemical Analysis

Different extracts were used for the phytochemical screening to detect various bioactive compounds such as alkaloids, flavonoids, saponins, phenolic compounds, tannins, quinones, carbohydrates, and Anthocynin.

2.4. Alkaloids Test

Mayer's Test

To 1-2 ml of each extract, a few drops of Mayer's reagent were applied along the test tube's sides. The presence of alkaloids is indicated by the formation of a green precipitate. [7].

2.5. Test for Flavanoids

Acid Test:

To a few ml of extract, few drops of dilute H2SO4 were added. Orange color indicates the presence of flavonoid [8].

2.6. Saponins Test

Foam Test: Twice as much distilled water (H2O) as two milliliters of each extract were shaken hard. Stable foam formation suggests the presence of saponins [9].

2.7. Test for Phenolic Compounds

Phenol Test

FeCl3 solution was added in between three and four drops for each extract. The presence of phenols is shown by the formation of a bluish-black color [10].

2.8. Test for Quinones

Hydrochloric acid Test

1 ml of each extract and 1 ml of the conc. HCl was added. The formation of a yellow color indicates the presence of Quinones [11].

2.9. Test for Tannins

Test for Lead Acetate

Three to four drops of a 1% lead acetate solution were added to each extract. Tanning agent presence is shown by the formation of white precipitate [7]

2.10. Test for Carbohydrates

Molisch's Test

2 ml of Molisch reagent was added with 2 ml of each extract and 2 ml of Conc.H_2SO_4. The formation of a reddish ring indicates the presence of Carbohydrate [12].

2.11. Fehling's Test

2ml of the extract was treated with a few drops of Fehling's solution A and then Fehling's solution B, which was then subjected to boiling. The appearance of reddish-brown precipitates confirms the existence of Reducing Sugars [13].

2.12. Anthocyanin

Test for Sulphuric Acid

Two milliliters of the extract were mixed with a few drops of strong sulfuric acid. The development of a yellowish-orange hue signifies the existence of anthocyanins [14].

2.13. Test for Protein

Xanthoproteic Test

2ml of plant extract was treated with few drops of concentrated nitric acid. Formation of yellow colour confirms the presence of protein [15].

3. RESULTS AND DISCUSSION

The significance of medicinal plants for the well-being of both people and communities has been demonstrated [16]. Naturally occurring phytochemicals in medicinal plants contain defensive mechanisms that shield against a range of diseases. Primary and secondary chemicals are known as phytochemicals. Primary contents include proteins, common sugars, and chlorophyll; secondary constituents include terpenoids, alkaloids, and phenolic chemicals [17]. The initial stage in herbal medicine research is phytochemical screening to find bioactive and new lead chemicals. Plant material is made up of many distinct types of natural compounds, each having a different polarity and hence a varied mode of solubility [18]. In this study qualitative phytochemical screening of was carried out using different solvents and the results revealed the presence of various bioactive compounds. Methanolic extract of stem confirmed the presence of Carbohydrates, Alkaloids, Phenols, Quinones, Tanins, Saponins, Tanins, Proteins, Saponins, Flavonids, and Anthocyanin. Acetone extract indicated the presence of phytoconstituents such as Alkaloids, Flavonids, Saponins, Phenols, Proteins, Quinones, Anthocyanin, Tannins, and Carbohydrates. Aqueous extract showed the presence of Alkaloids, Flavanoids, Proteins, Carbohydrates, Tannins, Quinones, Anthocyanin, and Saponins, which is revealed in **Table-1**. The qualitative analysis of bioactive compounds was carried out in various solvent extracts of the leaf of *Withania somnifera* L. which is shown in **Table 2**. Methanolic extract showed the presence of Carbohydrates, Alkaloids, Quinones, Tanins, Saponins, Proteins, Flavonoids, and Anthocyanin. Acetone extract indicated the existence of phytoconstituents like Proteins, carbohydrates, Alkaloids, Quinones, Tannins, and Saponins. Aqueous extract showed the presence of Alkaloids, Flavonoids, Tannins, Phenols, Proteins, Carbohydrates, Saponins. Quinones. In **Table 3** the qualitative analysis was carried out in fruit parts of *Withania somnifera* L. The methanolic extract confirmed the presence of Carbohydrates, Alkaloids, Phenols, Quinones, Tanins, saponins, Flavanoids, Anthocyanin, and Protein. Acetone extract showed the presence of phytoconstituents like Proteins, Carbohydrates, Saponins, Phenols, Quinones, Alkaloids Tanins, Flavonoids, and Anthocyanin. Aqueous extract indicated the presence of Alkaloids, Carbohydrates, Quinones, Tanins, Proteins, Anthocyanin, Flavonoids, and Saponins.

Table 1: Results of phytochemical screening of stem of *Withania somnifera* L. in different solvents

	Phytochemicals	Tests	90% Methanol	90% Acetone	Distilled Water
1	Carbohydrates	Molisch's test	+	+	+
		Fehling test	+	+	+
2	Alkaloids	Mayer's test	+	_	_
		Wagner's test	_	+	+
3	Phenols	FeCl3 test	+	+	_
4	Quinones	HCL acid test	+	+	+
5	Tanins	Lead acetate test	+	+	+
6	Saponins	Foam test	+	+	+
7	Flavonoids	Acid test	+	+	+
8	Anthocyanin	Sulphuric Acid test	+	+	+
9	Protein	Xanthoproteic test	+	+	+

Table-2: Results of phytochemical screening of leaves of *Withania somnifera* L. in different solvents

	Phytochemicals	Tests	90% Methanol	90% Acetone	Distilled Water
1	Carbohydrates	Molisch's test	+	+	+
		Fehling test	+	+	+
2	Alkaloids	Mayer's test	+	+	+
		Wagner's test	_	_	+
3	Phenols	FeCl3 test	_	-	+
4	Quinones	HCL acid test	+	+	+
5	Tanins	Lead acetate test	+	+	+
6	Saponins	Foam test	+	+	+
7	Flavonoids	Acid test	+	+	+
8	Anthocyanin	Sulphuric Acid test	+	-	_
9	Proteins	Xanthoproteic test	+	+	+

Table-3: Results of phytochemical screening of fruits of *Withania somnifera* L. in different solvents

	Phytochemicals	Tests	90% Methanol	90% Acetone	Distilled Water
1	Carbohydrates	Molisch's test	+	+	+
		Fehling test	+	+	+
2	Alkaloids	Mayer's test	+	-	-
		Wagner's test	-	+	+
3	Phenols	FeCl3 test	+	+	-
4	Quinones	HCL acid test	+	+	+
5	Tanins	Lead acetate test	+	+	+
6	Saponins	Foam test	+	+	+
7	Flavonoids	Acid test	+	+	+
8	Anthocyanin	Sulphuric Acid test	+	+	+
9	Protein	Xanthoproteic test	+	+	+

4. CONCLUSION

Medicinal plants contain bioactive compounds that have been utilized for thousands of years to treat a wide range of human diseases and to promote healing. Withania somnifera L. has a variety of bioactive compounds. Phytochemicals are the name for the bioactive compounds that are utilized as therapeutic agents. Folk medicine frequently uses Withania somnifera L. to cure a variety of conditions, including fever, cancer, asthma, diabetes, ulcers, hepatitis, eyesores, arthritis, heart issues, and hemorrhoids. The plant is well-known for its ability to prevent cancer, relieve low back discomfort, and build stronger muscles.Based on the findings, it can be inferred that the stems, leaves, and fruits of Withania somnifera contain a significant number of phytochemicals, including proteins, carbohydrates, flavonoids, alkaloids, and phenols.

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CONFLICT OF INTEREST

The authors declare no conflict of interest."

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