CHAPTER-3

THE PRELIMINARY PHYTOCHEMICAL ANALYSIS OF *NIGELLA SATIVA* L. *SMILAX ZEYLANICA* L. & *WITHANIA SOMNIFERA* L. (DUNAL) PLANT PARTS.

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Summary

Phytochemicals are plant-based chemicals with unique chemical, structural, and molecular properties. It is accepted that plant products are one of the major requirements of the world, from kitchens to laboratories plants are nature-gifted materials. This is because of plant chemicals. Plant-based chemicals play a crucial role throughout the world. So before using plants as our nutrition or medicine, it is mandatory to know their chemical content. The preliminary phytochemical analysis method allows us to understand the chemical content of plants or mixtures. In this study, two types of plant extracts were tested for phytochemical confirmation; phenol, alkaloid, flavonoid, tannin, saponin, glycoside & protein, some tests showed positive results in aqueous extracts, negative in methanol and some were positive for methanol & not in aqueous.

Keyword: Preliminary, Phytochemical, Qualitative, Non-polar.

Introduction

The phytochemicals are the natural chemical compounds present in plants that are of medicinal importance for various diseases. (Junaid and Patil, 2020) Humans have used plants because of their well-known phytochemicals since the beginning of history. (Rahman Gul et. al. 2017) The medicinal potential (antimicrobial, antidiabetic, anti-cancerous, anti-analgesic, anti-inflammatory, etc.) of plants is due to their bioactive components, which can regulate physiological processes and control human health, (Itoandon et. al. 2012) hence the study of phytoconstituents can help to select the plant for medicinal purposes (Junaid and Patil, 2020).

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Preliminary Phytochemical analysis or screening is the qualitative analysis used for the identification of bioactive constituents, which provides an overview of the class of compounds present in the plant parts (Yahaya et. al. 2014) For the preliminary phytochemical analysis, extracts of plant material are required. Different extraction techniques are known that are in use worldwide for the extraction of phytochemicals from plant material (Firdos and Roopa, 2024).

In a preliminary phytochemical analysis to confirm the presence of phytochemicals in the plant material, the extracts are tested by adding various reagents and chemicals, with heating, cooling, shaking etc. If the reaction gives authenticated results it means the test is positive (Junaid and Patil, 2020).

Automated techniques like, HPTLC (High-Performance Thin Layer Chromatography), HPLC (High-Performance Liquid Chromatography), OPLC (Optimum Performance Laminar Chromatography), and Gas Chromatography, are used at laboratories and institutes for phytochemical analysis (Sahira and Cathrine, 2015).

In the present study, three plants of different family used for the preliminary phytochemical analysis i.e. *Nigella sativa L, Smilax zeylanica L, and Withania somnifera* L (Dunal).

Nigella sativa is known by numerous names, such as black cumin (English), caraway seeds (USA), kalajira (Bangali), Al-Habba Al-Barakahand (Arabic) etc. (Vahita, et al. 2023).

Seeds have been used traditionally in the Middle East as a treatment for various diseases more than 2000 years ago (Fatemeh, et. al. 2014) for lung, kidney, heart, blood circulation, digestive system, and overall health. Black cumin is one of the great reservoirs of phytochemicals like alkaloids, flavonoids, steroids, terpenoids, etc. (Aalaa et. al. 2023).

Smilax zeylanica is commonly known as Chopchini, Sarsaparill, or Kumarika in different languages. It is a potential constituent of Sarsaparilla (Dhanya Shree et. al. 2018) plant steroids like Sarsapogenin and Smilagenin, roots containing Diosgenin, the steroidal saponin glycoside. *Smilax z.* exhibits several bioactivities such as antimicrobial, antioxidant, anti-cancer, anti-analgesic, cytotoxic, antipyretic, antidibetic, etc. (Lakshyajeet, et. al., 2023).

Withania somnifera is widely distributed in warm parts of the world and is commonly used in Ayurveda as a medicine. It is known by various names like Ashwagandha, Indian winter cherry, Indian ginseng, etc. (Bargale et al. 2020). It is a shrub that grows up to 1m in height, flowers are greenish and its fruits are red. Traditionally used as a medicine throughout the world, in-vitro and in-vivo studies reveal that the leaf and root of Ashwagandha show antimalarial and antioxidant activity (Simur, 2018).

India's geographical expanse covers a wide range of ecosystems, including grassland, deserts, coastal, and marine ecosystems. It is among the top 10 worldwide countries for the share of vascular plants, mammals, fishes, reptiles, and amphibians (Asmita and Selvadurai, 2022).

The present study tries to examine the chemical content of three selected plants. For this, preliminary work is done using the preliminary qualitative phytochemical analysis method based on the colour & texture change of the solution.

Materials and Methods

Plant samples and Parts selected for analysis:

1). Withania somnifera L. (Dunal)
- a). Leaf
- b). Stem
- c). Root

2). *Smilax zeylanica* L.
- a). Leaf
- b). Stem
- c). Root

3). Nigella sativa L.
- a). Seeds

Collection of plant material: The plant material of *Withania s.* collected from a college campus of N. E. S. Science College, Nanded, Maharashtra, India during the winter season. *Smilax z.* collected from Nerur, Sindhudurg, Maharashtra, India during the rainy season. The seeds of *Nigella s.* purchase from the local market of Nanded, Maharashtra, India.

Plant Authentication: The three Herbarium samples were identified and authenticated by Dr. A S Dhabe, a Professor and Head of the Department of Botany, Dr Babasaheb Ambedkar Marathwada University, Aurangabad, Maharashtra, India.

Drying and grinding of plant material: The Plant material is clean and washed first and dried under shade. It takes about 20 days for each plant sample to dry completely. After drying, the material is ground into a fine powder with the help of an Electrical mixture.

Extraction: Two types of extracts are prepared Aqueous and Methanolic.

For aqueous extraction: The decoction (Firdos and Roopa, 2024) of material is prepared, powdered material is boiled in water to one-fourth of its original volume and then filtered by filter paper.

For Methanol extraction: The Maceration method of extraction (Firdos & Roopa, 2024) is followed i.e. powdered material is soaked in methanol, after 24 hrs. Filtered by filter paper.

Preliminary phytochemical tests

The extracts were tested for various phytochemicals by following authentic protocols and methods.

Test for Phenol:

Ferric chloride test: Add a few drops of 5% $FeCl_3$ solution to the extract. An appearance of dark green or bluish-black colour indicates the presence of phenol in a sample.

Test for Flavonoid:

Alkaline reagent test: Prepare 2% NaOH solution. In a 2ml of extract add 4ml of 2% NaOH. The formation of an intense yellow colour which then becomes colourless with the addition of diluted acid, for example, HCl, indicates the presence of Flavonoid.

Test for Alkaloid:

Mayer's test: In 2 to 4 ml of filtrate extract add 1 to 2 drops of Mayer's reagent along the side of a test tube, a creamy white/yellow precipitate indicates the presence of Alkaloid.

Test for glycosides:

Aqueous NaOH test: Dissolve the extract by adding 1ml of water then add a few drops of aqueous NaOH solution. A yellow colour shows glycoside presence.

Test for Tannin:

10% NaOH test: Take 0.4ml of sample extract, add 4ml of 10% NaOH and shake well. The formation of Emulsion indicates Hydrolysable tannins.

Test for Saponin:

Foam test: To the 1ml of plant extract add 4ml of water and shake vigorously, if a persistent foam forms for 10 min. it confirms the presence of saponin.

Test for protein and amino acid:

Millon's test: Take 2ml of extract and add a few drops of Millon's reagent, a white precipitate indicates protein and amino acid in a sample.

Xanthoproteic test: plant extract add a few drops of conc. Nitric acid, a yellow colour solution confirms protein and amino acid.

Result and Discussion

The Qualitative phytochemical analysis of Withania somnifera L. (Dunal), Smilax zeylanica L. and Nigella sativa L. reveals the presence of phytochemicals or secondary metabolites in their plant parts. The preliminary analysis is one of the quick and easy methods to evaluate the general idea of the presence and absence of phytochemicals in the plant parts. By adding different chemicals and reagents to the plant extracts and following an authentic protocol, it was very easy to get results about the phytochemical content of the plants.

Table 1: Preliminary qualitative phytochemical analysis of *Nigella s.* seed.

Sr. no.	Phytochemical	Test name	Aqueous NS	Methanol NS
1	Phenol	Ferric chloride test	+	+
2	Flavonoid	Alkaline reagent test	+	+
3	Alkaloid	Mayer's test	+	+
4	Glycoside	Aqueous NaOH test	-	+
5	Tannin	10% NaOH test	+	-
6	Saponin	Foam test	+	-
7	Protein & amino acid	Xanthoproteic test	-	+

Abbreviation: + = Positive, - = Negative, NS= *Nigella* seeds

Table 2: Preliminary qualitative phytochemical analysis of *Smilax z.* leaf, stem & root

Abbreviation: + = Positive, - = Negative,

Sr. no.	Phytochemical	Test name	Aqueous			Methanol		
			SL	SS	SR	SL	SS	SR
1	Phenol	Ferric chloride test	+	-	-	+	+	+
2	Flavonoid	Alkaline reagent test	+	+	+	+	+	+
3	Alkaloid	Mayer's test	-	-	-	-	-	-
4	Glycoside	Aqueous NaOH test	+	+	+	+	+	+
5	Tannin	10% NaOH test	+	+	+	+	-	+
6	Saponin	Foam test	+	+	+	-	-	+
7	Protein & amino acid	Xanthoproteic test	-	+	+	+	+	+

SR= Smilax leaf, SS= Smilax stem, SR= Smilax root

Table 3: Preliminary qualitative phytochemical analysis of *Withania s.* leaf, stem & root.

Sr. no.	Phytochemical	Test name	Aqueous			Methanol		
			WL	WS	WR	WL	WS	WR
1	Phenol	Ferric chloride test	-	-	-	+	+	+
2	Flavonoid	Alkaline reagent test	+	+	+	+	+	+
3	Alkaloid	Mayer's test	+	-	+	+	-	+

4	Glycoside	Aqueous NaOH test	-	-	-	+	-	+
5	Tannin	10% NaOH test	+	-	-	-	-	+
6	Saponin	Foam test	+	+	+	-	-	-
7	Protein & amino acid	Xanthoproteic test	-	-	-	+	+	+

Abbreviation: + = Positive, - = Negative
WL=Withania leaf, WS=Withania stem, WR= Withania root

This phytochemical analysis of Nigella sativa L. aqueous extract indicates positive results for phenol, flavonoid, alkaloid, tannin & saponin. Negative for glycosides & Protein. The methanolic extract of seeds shows positive for phenol, flavonoid, alkaloid, glycosides & protein. But negative for tannin & saponin as shown in Table no. 1

The Smilax zeylanica L. aqueous solution giving positive results for Tannin, Flavonoid, Saponin & Glycoside & methanolic extract shows all positive results for phenol, flavonoid & protein but glycoside & tannin for leaf only. Negative results for alkaloid & saponin, as shown in the Table no. 2

Withania somnifera L. (Dunal) in aqueous extract shows the presence of flavonoid and saponin in all three parts, alkaloid in the leaf and root, Tannin in the leaf & showing the absence of Protein, Glycosides & Phenol in the plant. On another side, the Methanolic extract shows more positive results i.e. presence of phenol, flavonoid, & protein in the parts but Alkaloid & glycoside only in the leaf & root, and tannin in the root. Saponin is not found in the root of Withanias.

Conclusion

The preliminary qualitative phytochemical analysis is conducted mainly to detect the presence and absence of phytochemicals or secondary metabolites in plant parts. In this phytochemical analysis of three plants; *Withania somnifera* L. (Dunal), *Smilax zeylanica* L. and *Nigella sativa* L. the general idea about the phytochemical content is gained. Two types of extracts are studied during the testing; aqueous and methanolic. It is seen that the results of polar & non-polar extracts show variations, the methanolic extract shows maximum positive results than the aqueous. All three plants show good phytochemical content in future these plants will be test for further Antimicrobial, Anti-cancer activity etc. If we talk about the quality of plants, it is seen that parts of *Smilax z.* show maximum positive results compared to the other two. No doubt every plant has its own properties & qualities.

It has been studied that; plant chemicals for their solubility require a solvent of that type (Haq et. al. 2020). For example, a non-polar chemical cannot mix or be soluble in polar solvents and vice-versa. And except this, there are so many varieties of secondary metabolites that have some uniqueness in their structural, molecular and chemical properties (Baan and Md. Nazmul , 2022). So it is very

unattainable to detect the exact quality of phytochemicals present in the samples by preliminary analysis. The preliminary phytochemical analysis gives us a general idea about the phytochemicals of these plants for complete detection we have to move toward sophisticated instrumental analysis.

Fig. 1: *Smilax zeylanica* leaf

Fig 2:*Withania somnifera* leaf

Fig 3: Boiling of NS powder

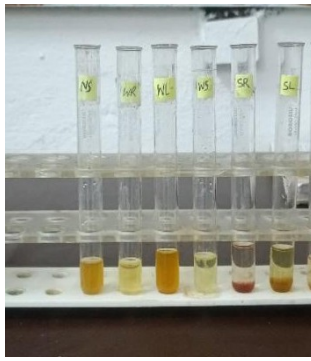

Fig 4: Phytochemical analysis of plant extracts

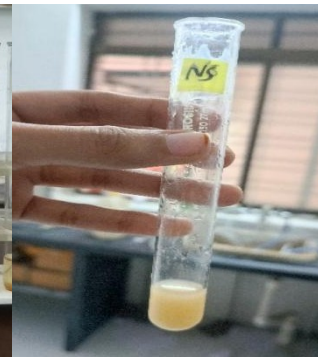

Fig 5: Test for saponin NS in aqueous

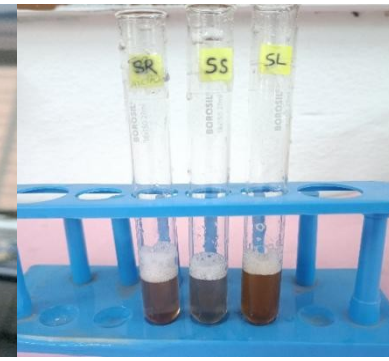

Fig 6: Test for saponin in aqueous *Smilax* after 10min.

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