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PRELIMINARY PHYTOCHEMICAL SCREENING OF MEDICINALLY IMPORTANT PLANTS

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Abstract : Since ancient times the medicinal plants have utilized by the humans in order to treat various illness due to their possible pharmacological activities, which include antioxidant, antimicrobial, anti-inflammatory, antineoplastic, analgesics, anti-diabetic, anti-hypertensive and other activities. Some of the significant phytochemicals with varied biological activities are alkaloids, flavonoids, phenolics, tannins, saponins, steroids, glycosides, terpenes, and so on. The discovery of phytochemicals can forecast a plant's pharmacological action. *Phyllanthus emblica, Swietenia macrophylla, and Withania coagulans* are the medicinal plants that are clinically utilized for treatment for disorders like diabetes. Phytochemicals are now found using a variety of contemporary methods, but traditional qualitative assays are still used for preliminary phytochemical screening of plants.

Keywords: Preliminary phytochemical screening, *Phyllanthus emblica, Swietenia macrophylla, Withania coagulans*

I. INTRODUCTION

Since the beginning of time, nature has provided us with therapeutic agents. It is impossible to overstate the value of herbs in the treatment of human illnesses. It is obvious that the plant kingdom contains an endless supply of active substances; India is one of the countries that produce the most therapeutic herbs. Utilizing medicinal plants in the medical system, i.e. Siddha, Unani, and Ayurveda are well known. Over 6000 plants are employed in India's folk, herbal, and traditional medicine system, and India has formally acknowledged close to 3000 species for their medical usefulness [1] (Kalaiselvi, V., et al., 2016). As per the World Health Organization (WHO, 1978), around 80% of the globe's indigenous populace largely depends on medicinal plants as their alternative source of health care [2] (Madike, L.N., et al., 2017). Phytochemicals are bio-active substances derived from plants and are regarded as secondary metabolites. They are produced naturally in all plant parts, including the bark, leaves, stem, roots, flowers, fruits, and seeds, therefore any portion of the plant could have active ingredients [3] (Jyothiprabha, V. and Venkatachalam, P., 2016). Phytochemicals are the biologically active compounds that have a variety of chemical structures and curative or disease-preventive capabilities. The richest bioreservoirs of different phytochemicals are found in medicinal plants that are used to treat various illnesses and disorders. The phytochemical components of plants establish their therapeutic qualities. Alkaloids, flavonoids, phenols, tannins, saponins, steroids, glycosides, terpenes, are significant phytochemicals found in diverse regions of plants. Especially, alkaloids, terpenoids, steroids, and phenolics compounds are the main classes of phytochemicals found in nature, which is a unique source of structures with a high phytochemical diversity [4] (Shaikh, J.R. and Patil, M.K., 2020). The richness of these secondary metabolites are responsible for the medicinal properties of plant such as anti-oxidant, anti-diabetic, anti-cancer, and anti-inflammatory (Anand, S.P. and Deborah, S., 2017).

Phyllanthus emblica (Euphorbiaceae) popularly known as Indian gooseberry or Amla. Its plant is found in the regions of India, China, and Pakistan. The fruit is rich source of ascorbic acid (vitamin C) due it is rich in antioxidant property (Gul et al., 2022). The fruit of this tree contains a variety of nutraceuticals such as calcium, vitamin C, lysine, minerals, methionine, nicotinic acid, phosphorus, riboflavin, and tryptophane which are believed to have immune-boosting properties against a variety of diseases. It's also widely used in traditional medicinal system of India “Ayurveda” (Gantait et al., 2021). *Swietenia macrophylla* (Meliaceae) commonly known as sky fruit. *Swietenia macrophylla* can be found in countries like Brazil, Bolivia, Mexico, India, Peru, and other Central American countries. Macrophylla trees typically grow to heights of more than 30 metres, with a straight stem and a cylindrical trunk spanning 100 to 200 centimetres (Telrandhe et al., 2022). The tree is of economic importance and its fruits has long been employed to cure a variety of illnesses, including

hypertension, elevated blood pressure, and diabetes. According to scientific studies, the unrefined extract from *S. macrophylla* seeds has antibacterial, antidiarrheal, anti-malaria, antioxidant, anti-diabetic, anti-hepatitis, anti-inflammatory, anti-mutagenic, antitumor and antinociceptive properties (Telrandhe et al., 2022). *Withania coagulans* L. Dunal (Solanaceae) commonly known as Indian cheese maker or paneer ka phool as its berries are used as a base for the clotting of milk due to the coagulating enzyme. It is widely distributed in the drier parts of India, Pakistan, Iran and Afganistan. The berries are globose shaped and are 6-8 mm in diameter (Khattak et al., 2021). Due to the presence of various secondary metabolites the plant has number of medicinal properties like anti-cancer, anti-diabetic, anti-inflammatory, neuroprotective, hepatoprotective, antiinflammatory, antitumor, immuno-suppressive and wound healing activity (Khan et al., 2021). It also has anti-oxidant property (Peerzade et al., 2018).

Hence, the present study aims the extraction of phytoconstituents from *Phyllanthus emblica* fruits, *Swietenia macrophylla* seeds, *Withania coagunas* berries using ethanol solvent by soxhlet method and investigate the preliminary phytochemical present in the extracts

II. MATERIALS AND METHODS

2.1 Extraction of different plant extract using sozhlet method

Swietenia macrophylla seeds were collected from the Botanical Garden of the Karnatak University, Dharwad, Karnataka, India, in February. *Phyllanthus emblica* fruits and *Withania coagulans* berries were obtained from the local market of Dharwad, Karnataka, India. They were washed thoroughly with double distilled water, chopped into thin slices, freeze-dried, and powdered. The individual powder (25g) was subjected to extraction process using 300 ml of 70% ethanol at 50 ◦C for 12 h in Soxhlet apparatus and dried in a vacuum rotary evaporator at 40 ◦C for 4 h. The 1 g of dried extract was dissolved in 10 ml of ethanol and made up the volume of 100 ml using distilled water to get 1% stock solution and stored in an air-tight container in the refrigerator at 4 ◦C for the further studies.

2.2 Preliminary phytochemical screening

By using the techniques described in this study, biochemical tests were carried out to determine if the phytochemicals alkaloids, flavonoids, saponins, steroids, and tannins were present in the *S. macrophylla* seed ethanol extract (Parekh and Chanda, 2007; Gul *et al*., 2017; Kumari *et al*., 2017; Alqethami and Aldhebiani, 2021).

2.2.1 Tests for Alkaloids:

2.2.1.1 Wagner's Test: To 2mL of extract, a few drops of Wagner's reagent are added. The presence of alkaloids was indicated by the reddish-brown precipitate.

2.2.1.2 Dragendorff's Test: To 2 mL of extract add a few drops of Dragendorff's reagent. The presence of alkaloids was denoted by the formation of orange brown precipitate.

2.2.2 Detection of glycosides

2.2.2.1 Kellar – Killani test: To 2 mL of extract 1 mL of glacial acetic acid, 1 mL of $FeCl_3$ and 1 mL of H_2SO_4 was added. The presence of glycosides was indicated by an orange-brown colour.

2.2.2.2 Molisch's test: To 1mL of extract and 2 drops of Molisch's reagent and 2 ml of Conc.H2SO4 along the sides of the test tube. The presence of glycosides was denoted by the formation of a purple or violet ring at the junction of 2 layers.

2.2.3 Test for Flavonoids:

2.2.3.1 Pews test: Zinc powder was added to 2 ml extract, followed by dropwise addition of conc.HCl. The formation of purple reagents or cherry colours indicates the presence of flavonoids.

2.2.3.2 Shinoda test: Filtrate extract (2 ml) and a few fragments of magnesium metal is added into the test tube followed by drop-wise addition of Conc. HCl. The presence of flavonoids was indicated by the formation of a magenta colour.

2.2.4 Test for Phenol

2.2.4.1 Ferric chloride test: To 2ml of extract the four drops of alcoholic ferric chloride ($FeCl_3$) solution was added. Change in this solution as bluish-black confirms the presence of phenolic compounds.

2.2.4.2 Ellagic acid test: Extract (2ml) added to a few drops of 5% glacial acetic acid and 5% of sodium nitrate solution. The contents turned muddy or brown precipitates indicating the presence of phenols.

2.2.5 Detection of saponins

2.2.5.1 Foam test: To 2mL of extract 5 mL of distilled water was mixed with vigorous shaking. The presence of stable froth denotes the presence of saponins.

2.2.6 Detection of Terpenoids

2.2.6.1 Salkowski's test: Chloroform (2ml) and 2ml of Conc. H_2SO_4 added 2ml of extract shake well. Deep reddish brown color indicates the presence of terpenoids.

2.2.7 Test for Triterpinoids:

2.2.7.1 Liebermann-Burchard Test: Chloroform (2ml) mixed into 2 ml of extract, 2ml acetic anhydride and two drops of Conc. H_2SO_4 carefully. The presence of sterols was denoted by the appearance of reddish violet colour.

2.2.8 Test for Tannins:

2.2.8.1 Gelatin test: Gelatin dissolves in warm water, and this solution is added to the extract. The presence of tannins was confirmed by the appearance of white precipitation.

2.2.8.2 Lead acetate test: Few drops of 10% lead acetate were added to 2ml of extract. The presence of tannins was confirmed by the appearance of white precipitation.

2.2.9 Tests for Oils and Fats:

2.2.9.1 Spot Test: A few drops of extract were pressed between two filter papers. The appearance if oil stain on the paper signifies the presence of oils and fats.

III. RESULTS AND DISCUSSION

In order to identify the presence or absence of various secondary metabolites the preliminary phytochemical screening of extract of *Phyllanthus emblica* fruits, *Swietenia macrophylla* seeds, and *Withania coagulans* berries ethanolic extract was performed using various chemical assays. Table 1 summarizes the result of various phytoconstituents of ethanolic extracts of *P. emblica*, *S. macrophylla*, and *W. coagulans*. Evaluation of chemical components of fruit extract of *P. emblica* showed high content of flavonoids, phenols moderate concentration of saponins, terpinoids, tannins, steroids and low levels of alkaloids and glycosides, whereas, *S. macrophylla* seed revealed the presence of alkaloids, terpenoids, triterpenoids, and oils and fats in high concentration, saponins are present in moderate concentration, and flavonoids, phenols, and tannins in low concentrations and *W. coagulans* berries extract showed presence of alkaloids, glycosidase, steroids, and oil and fats in high concentrations flavonoids, phenols, terpenoids, and tannins in low concentrations. Plant-derived substances have recently become of great interest owing to their versatile applications. Medicinal plants are the richest bio resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Doss, 2009). Medicinal plants are enriched with primary and secondary plant metabolites such as alkaloids, phenols, flavonoids, glycosides, saponins, terpenoids, and sterols are responsible for the various biological activities like anti-diabetic, anti-cancer, anti-oxidant, anti-inflammatory and other biological activity (Suntar, 2020). Previously, Hossen *et al*., (2014) found that fruit extract of *P. emblica* extracted in 80% ethanol by cold extraction process showed the presence of flavonoids, alkaloids, tannin, steroids, reducing sugar and gum. Milanda *et al*., (2017) Fruit extract of *P. emblica* extracted in 70% ethanol by maceration process showed the presence of alkaloids, flavonoids, polyphenols, tannins, quinines, monoterpenes and sesquiterpenes, and saponins. The previous study demonstrates the phytoconstituents extracted from *S. macrophylla* leaves using different solvents like hexane, chloroform, ethyl acetate, acetone, and ethanol had shown the presence of secondary metabolites like flavonoids, alkaloids, steroids, terpenes, glycosides, saponins and tannins (Ushie *et al*., 2016). Durai *et al*., 2016, reported the methanolic extraction of *S. macrophylla* leaf, seed, and central fruit axis showed the presence of various secondary metabolites like significant amount of components such as alkaloids, terpenoids, and carbohydrates were found in both leaf extract and seed extract. Whereas, leaf extract lacks fixed oil and flavonoids. *W. coagulans* methanol extract showed presence of carbohydrates, alkaloids, glycosides steroids, oils in high concentrations, and phytosterols, saponins, tannins in low concentrations whereas in aqueous extract showed presence of glycosides, alkaloids, steroids in concentration. (Mathur *et al*., 2011). Peerzade *et al*., (2018) *W. coagulans* methanol extract showed presence of alkaloids, glycosides, reducing sugar, flavanoids, phenols, tannins, and phytosterols. Plants containing these secondary metabolites are responsible for the medicinal property of plant such as anti-cancer, anti-inflammatory, anti-diabetic, and anti-oxidant.

Table 1: Phytochemical Screening in different plant extract

Sl. No.	Secondary Metabolites	Phytoconstituent Tests	*Phyllanthus emblicus*	*Swietenia macrophylla*	*Withania coagulans*
1	Alkaloids	Wagner's Test	+	+++	+++
		Dragendorff's Test	+	+++	+++
2	Glycosides	Kellar – Killani test	+	_	+++
		Molisch's test	+	_	+++
3	Flavonoids	Pews test	+++	+	+
		Shinoda test	+++	+	+
4	Phenols	Ferric chloride test	+++	+	+
		Ellagic acid test	+++	+	+
5	Saponins	Foam test	++	++	_

6	Terpenoids	Salkowski's test	++	+++	+
7	Steroids	Liebermann-Burchard Test	++	++	+++
8	Tannins	Gelatin test	++	+	+
		Lead acetate test	++	+	+
9	Oils and Fats	Spot Test	++	+++	+++

IV. CONCLUSION

The phytochemical analysis is critical for evaluating a plant's potential medicinal properties as well as determining the active principles accountable for the plant's recognized biological activities. Thus, the existence of different phytochemical substances in *Phyllanthus emblica* fruit extract, *Swietenia macrophhylla* seed extract, and *Withania coagulans* berries extract supports the plants' therapeutic uses. Furthermore, it serves as a foundation for focused compound separation and more exact studies. The sort of liquid used in the extraction of a phytochemical from plant material is crucial. Additionally, the test used for phytochemical analysis reveals whether or not a phytochemical is present in the material. As a consequence, for more accurate findings, two or more different tests should be done. Following toxicology testing, the current research findings could provide future understanding of this plant to be helpful in medicinal sectors for human wellbeing.

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