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EFFECT OF SOLVENT EXTRACTION ON PHYTOCHEMICAL ANALYSIS OF *LEUCAS STELLIGERA* AND *POGOSTEMON BENGHALENSIS*

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ABSTRACT

Plants possess of structurally and functionally diverse chemical which prepares them strategies to prevent enemies, pathogens and some environmental compounds called as secondary metabolites. Phytochemicals are biologically active and important sources of medicine. *Leucas stelligera* and *Pogostemon benghalensis* are easily available aromatic plant species belongs to the family Lamiaceae. These plants show medicinal and antioxidant properties. In the present study, preliminary phytochemical screening shows the presence of alkaloid, glycoside, flavonoid, phenol, tannin, and terpenoids considerably in different five solvents i.e. Petroleum ether, chloroform, acetone, ethanol and water extract of powdered leaf material. Most the phytochemicals showed a presence in ethanolic extract followed by water extract.

KEYWORDS: Phytochemicals, preliminary, Lamiaceae, organic solvents.

INTRODUCTION

Phytochemicals are produced under some abiotic stresses and pathogenic attacks, hence helpful to the survival of plants. A huge number of such phytochemicals and their biosynthetic pathways have been discovered in the plant kingdom.[1] Such metabolic chemicals as “secondary metabolites” which include alkaloids, phenols, flavonoids, coumarins, gums, tannins, terpenoids, terpenes, polysaccharides, and glycosides according to Oku.[2] Phytochemicals are easily accessible, effective, safe and competent than synthetic drugs as medicine hence popular in society as a drug.[3] In ancient China and India are using medicinal herbs for a long time, they contains prescriptions of countless plant-derived

medicines.[4,5] phytochemical screening one can detect the various important compounds from herbs which may be useful as a modern drug for curing various diseases.[6]

Lamiaceae (mint family) is considered the sixth largest family of the angiosperm group and contains many medicinally, culturally, and economically significant species used as food, medicine, and cosmetics.[7] The family contains aromatic plant with 220 genera and 3,500 species have a wide distribution and used in conventional medicine from ancient time in.[8] In vitro studies have proven their bactericidal activities against foodborne pathogens from essential Oils of Lamiaceae Family members.[8] It also shows Anti-inflammatory, antidiarrheal, analgesic, antioxidant, antimicrobial and insecticidal activities. Species of Lamiaceae family has medicinal, perfumery, culinary, and ornamental properties, and shows the presence of aromatic essential oil, tannins, saponins, and organic acids. The oil is used for its soothing effects in aromatherapy; these plants have sedative, diuretic, tonic, antispasmodic, antifungal, antimicrobial, anti-inflammatory, and antiseptic properties.[9] *L. stelligera* is a commonly found the wild plant of the Lamiaceae family, a plant used orally in females as an emmenagogue.[10] Leaf paste of *P. benghalensis* applied on skin burning, Its essential oil has antifungal properties, leaf juice administered for fever. [11-13]

In the present study, preliminary phytochemical analysis of leaf of four plants has been done in the different organic extracts to know the best result for further research. As those are medicinal plants phytochemical investigation may create an opportunity for further study of the plants with a suitable solvent.

MATERIALS AND METHODS

Plant material - Selected four Plants *Leucas stelligera* Wall. ex Benth. and *Pogostemon benghalensis* (Burm.f.) Kuntze, were freshly collected from different localities of Ahmednagar district. Plant material was identified by a taxonomist Identified by Botanical Survey of India (BSI).

Preparation of extract - Freshly collected leaf material was washed under tap water and shade dried for 8 days, then powdered fine through a grinder. Powdered material extracted in five different solvents according to polarity i.e. Petroleum ether, chloroform, acetone, ethanol, and water by soxhlet apparatus. Extract dried and used for analysis.

Phytochemical screening - Crude extract of petroleum ether, chloroform, acetone, ethanol, and water subjected to Phytochemical screening using different tests for identification of phytochemicals, alkaloid flavonoid, tannin, phenols, carbohydrate, cardiac glycoside, protein, amino acid, terpenoids, saponins, coumarins by Harborne, (1973) method.[17]

1. Test for carbohydrates

Molisch's test

3-5 drops of Molisch's reagent was added in 1 ml of plant extract each. Followed by addition of 1ml concentrated H_2SO_4. Formation of a violet ring indicates the presence of carbohydrates.

2. Test for Alkaloids

Wagner's test

In 1 ml of each plant extract was acidified with 1.5% v/v of HCl and then 3-5 drop of Wagner's reagent was added. Formation of reddish brown precipitation confirmed the presence of alkaloids.

3. Test for Flavonoids

Alkaline reagent test

1 ml sample of each extract was treated with 3-5 drops of 20% NaOH formation of yellow colour was observed, which become colorless on addition of 0.5 ml dilute HCl indicating the presence of flavonoids.

4. Test for Phenols

Ferric chloride test

1 ml of each extract was treated with 5-6 drop of $FeCl_3$ solution. Formation of deep blue colour developed, indicated presence of phenol.

5. Test for Tannins

a) Braymer's test: 1 ml of 10% alcoholic chloride added in 1 ml sample, the formation of greenish color occurred.

6. Test for Glycosides

Keller Kelliani test

1 ml of each extract was treated with 1 ml of glacial acetic acid and 2-3 drops of $FeCl_3$ solution, followed by the addition of 0.5 ml of concentrated H_2SO_4. Formation of brown ring at the junction of two liquids indicates the presence of glycosides.

7. Test for Terpenoids

Salkowski test

1 ml of each plant extract was dissolved in chloroform and 3-5 drops of concentrated H_2SO_4 added to it, reddish brown precipitation found at inner face indicated the presence of terpenoids.

8. Test for Saponins

Foam test

1 ml of each sample was mixed with 5 ml of water taken in the test tube and shaken vigorously, foam seen for 10-15 minute, indicated the presence of saponins.

9. Test for Amino acids and Proteins

In 1 ml of each extract, 2-3 drop of ninhydrin solution was added and kept for boiling in waterbath for 1-2 minutes, formation of purple color seen, suggested presence of amino acids and protein.

10. Test for Steroids

Salkowski test

In 1ml of each plant extract was dissolved in chloroform and 3-5 drops of concentrated H_2SO_4 acid were added to it. Red color appeared indicated the presence of steroids.

11. Test for Quinones

In 1 ml of each extract 0.5 ml of concentrated HCL was added, it showed yellow precipitation indicating presence of quinones.

12. Test for Resins

1 ml of each plant extract and 5 ml water was added in it then solution become turbid, indicated presence of resins

13. Test for Coumarins

1.5 ml of 10% NaOH added in 1 ml of each sample, yellow colour observed, indicates presence of coumarin.

RESULTS

Table 1: Phytochemical analysis of *Leucas stelligera* leaves.

Test	PE	CH	AC	ET	AQ
Carbohydrates	-	+	-	+	+
Alkaloids	+	+	+	+	+
Flavonoids	-	+	+	+	+
Phenols	-	-	-	+	+
Tannins	-	+	-	+	+
Glycosides	+	+	+	+	+
Terpenoids	+	+	-	+	-
Saponins	+	-	-	+	+
Amino acids and proteins	+	-	-	+	+
Steroids	-	-	-	-	-
Quinones	-	-	+	+	-
Resins	-	-	-	-	-
Coumarins	-	+	+	+	-

Abbreviations: PE-petroleum ether, CH-Chloroform, AC-Acetone, ET-Ethanol, AQ-Water, + Present, - Absent

Table 2: Phytochemical analysis of *Pogostemon benghalensis* leaves.

Test	PE	CH	AC	ET	AQ
Carbohydrates	+	+	-	+	+
Alkaloids	+	+	+	+	+
Flavonoids	-	-	+	+	+
Phenols	-	-	+	+	+
Tannins	-	-	-	+	+
Glycosides	+	+	+	+	+
Terpenoids	+	+	-	+	+
Saponins	+	+	+	+	+
Amino acid and proteins	-	-	-	-	+
Steroids	-	-	-	-	-
Quinones	-	-	+	-	+
Resins	+	+	+	+	+
Coumarins	+	+	+	+	+

Abbreviations: PE-petroleum ether, CH-Chloroform, AC-Acetone, ET-Ethanol, AQ-Water, + Present, - Absent

It was observed that among different solvents; ethanol was found to be most suitable solvent, marking the presence maximum phytochemicals (carbohydrate, alkaloids, phenolic

compounds, glycosides, terpenoids, saponins, quinones, proteins and coumarins). This was followed by water (absence of resins, steroids, terpenoids, quinones and coumarins). However in acetone extract, phenolic compound, terpenoids, saponins and resins were found to be absent. Petroleum ether has been proved as a weak solvent in present case as only terpenoids, glycosides and saponins were recorded. However, chloroform has better than petroleum ether which having two more phytochemicals (**Table 1)**. In *P. benghalensis* aqueous extract showed presence of all phytochemicals under consideration except of steroids. This was followed by ethanol extract of the plant material showcasing absence of only 3 phytochemicals viz. protein, steroid and quinones. Similarly, acetonic extract also showed a rich profile as alkaloid, flavonoids, phenolic compounds, glycosides, saponins, quinones, resins and coumarins. Chloroform and petroleum ether however, showed low solubility with absence of major compounds like, flavonoid and phenolic along with protein, steroids and quinones (**Table 2).**

DISCUSSION

Geethika and Sunoj Kumar (2017) reported that, flavonoids, phenol, terpenoids, saponins, carbohydrates, protein and glycosides were found in aqueous extract, whereas absence of alkaloids. Terpenoids, carbohydrates, glycosides, flavonoids found to be present in ethanolic extract and absence of phenolic compounds. In chloroform extract, terpenoids, carbohydrate, glycosides and saponins were recorded. Terpenoids, carbohydrates, glycosides, flavonoids were found to be present in ethanolic extract. Preliminary phytochemical screening showed the presence of flavonoids, alkaloids, saponins, phenolic compounds, terpenoids, and steroids in both ethanolic and aqueous extract (Patel *et al*., 2014) in *P. benghalensis.* With essential oil, it comprises an astringent resin, an alkaloids and a yellow varnish with slightly bitter taste. Similarly, Thakur and Sidhu, (2014) recorded carbohydrates, flavonoids, glycosides, steroids and terpenoids in both ethanolic and aqueous extract, whereas resin and saponins were found to be absent in ethanolic extract and phenol in water. Caryophyllene-9-B-10-olide and sesqui terpene lactone has been isolated from the whole plant (Shigwan *et al.,* 2013).

The choice of extraction solvent is most important aspect in solid-liquid extraction process. Hence safety of solvent and solubility of target compound should consider when choosing the solvent or solvent system for extraction of phytochemicals. Apart from that solubility of the target compound is also important factor in in extraction (Seidel, 2012; Tiwari *et al*., 2013).

CONCLUSION

Among the solvents ethanol shows the highest solubility phytochemicals from selected plants followed by water which almost solubilize all the studied phytochemicals. Acetone has intermediate solubility of phytochemicals whereas petroleum ether and chloroform have less solubility comparatively.

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