Phytochemical Screening Of Leaf Extract Of Aerva Lanata Collected From Agricultural Lands Of India

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Abstract - **Medicinal plants possess biologically active components called phytochemicals which are administered for curing of various human ailments and also take part in significant role in healing. Phytochemicals are of two categories i.e., primary and secondary constituents. Primary constituents include proteins, chlorophyll, sugar and amino acids. Secondary constituents contain alkaloids and terpenoids. Medicinal plants have antifungal, antibacterial and anti-inflammation activities because of these phytochemicals. The aqueous extract of leaf samples of the medicinal plant Aerva lanata are collected from agricultural lands of S.Kota. This extract is used for the phytochemical analysis to find out the phytochemical constituents in the plants with an objective to check the presence or absence of the phytochemical constituents in the selected plant. The results of the phytochemical analysis of the plant leaf extract shows that the terpenoids, tannins, reducing sugar, flavonoids and alkaloids are found to be present in afore mentioned medicinal plant. The phytochemical analysis of the plants is very much essential commercially and has huge attention in pharmaceutical industries for the fabrication of the novel drugs for treating various diseases. It is supposed that the key phytochemical properties identified by this study will be very helpful in the treatment of various diseases.**

keywords - **Phytochemicals, Aerva lanata, alkaloids, flavonoids, terpenoids, FTIR, tannins.**

1.1 INTRODUCTION

Phytochemistry is the part of chemistry that deals with the chemical processes involved in plant life. Phytochemicals are naturally occurring and biologically active chemical substances present in plants. Proteins, chlorophyll and regular sugars are the primary constituents and alkaloids, terpenoids, phyto sterols, flavonoids, glycosides, tannins and phenolic compounds are being the secondary constituents [1]. Phytochemicals guard plant cells from pollution, drought, stress and pathogenic attack [2]. Phytochemicals are synthesized in almost all parts of the plant like leaves, root, bark, stem, root, fruits, flower, seeds etc. [3, 4]. Phytochemicals are responsible for the colour and organoleptic properties of plant. Recent research shows that phytochemicals play a vital role in protecting humans against diseases. To extricate these compounds from plants phytochemical screening is inevitable. Phytochemical screening deals with the extraction, screening, and identification of the bioactive substances found in plants [5, 6].

Aerva lanata is a woody perennial shrub belonging to the family Amaranthaceae which is commonly found in the lands and fields of India (**Figure 1.1**). It is a good source of phytochemicals terpenoids, flavonoids, alkaloids, phenolic compounds, glycosides, gums, tannins, terpenes, carbohydrates and aminoacids. Thus, it plays inherent role to cure human diseases [7]. It is used as antiurolithiatic and diuretic drug in indian Ayurveda with the name of Pashanabeda (which means stone dissolving) for urinary disorders [8]. Studies on *Aerva lanata* proved that betulin and quercetin are the two compounds isolated from the plant have inhibitory property on enzyme activity which is responsible for kidney stone formation [9, 10]. There are other constituents such as ferulic acid, syringic acid, narcissin and feruloyltyramine (**Figure 1.2**) which are isolated from methanoic extract of *Aerva lanata* which are responsible for its antibacterial, antioxidant, anti asthmatic and anthelmintic activities [11]. This plant is also used traditionally for arresting hemorrhage during pregnancy, as an anti inflammatory, to treat nasal bleeding, scorpion sting . The amount of phytochemicals varies from species to species and plant to plant, based on the age and different ecological and climatic factors [12]. This chapter reports the preparation of *Aerva lanata* leaf extract and its phytochemical screening.

Figure 1.1: *Aerva lanata* plant

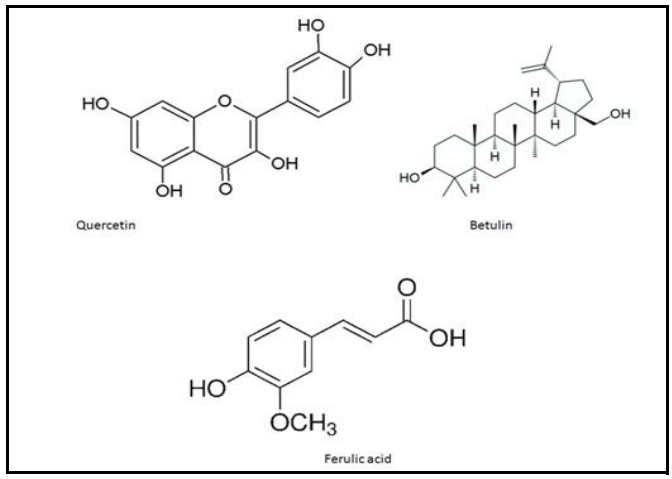

Figure 1.2: Chemical constituents identified in *Aerva lanata* plant

Aerva lanata is a good source of phytochemicals terpenoids, flavonoids, alkaloids [13], phenolic compounds, glycosides [14], gums, tannins, steroids, carbohydrates [15] and essential oil. Thus, it plays inherent role to cure human diseases [6]. Literature shows that like other plants *Aerva lanata* is wealthy sources of secondary metabolites and have antibacterial [16], antifungal [17], antioxidant [18], cytotoxic [19], anti-HIV [20], anti tumour [21], anti diabetic [22, 23] and anticancer [24] activities. This chapter reports the phytochemical screening of *Aerva lanata*.

1.2 MATERIALS AND METHODS

1.2.1 Chemicals required

The chemicals required for the phytochemical screening of the leaf extract are Mayers reagent (potassium mercuric iodide), Hager's reagent, Molisch's reagent, Benedict's reagent, Fehling's reagent, Schiffs reagent sodium nitroprusside, NaOH, ferric Chloride, benzene, H_2SO_4, chloroform, lead acetate, gelatin, HNO_3, acetic anhydride, ferric chloride, Ninhydrin reagent, copper acetate, sodium bicarbonate, hydrochloric acid, litmus papers, 2,4-DNP, Tollens reagent, iodine solution and deionized water.

1.2.2 Collection of *Aerva lanata* leaves

Fresh leaves of *Aerva lanata* plant are collected from agricultural fields located at S. Kota mandal in Vizianagaram district, Andhra Pradesh, India (**Figure 1.3**). 100 g of leaves are weighed and thoroughly cleaned with running tap water to eliminate debris on surface of leaves followed by deionized water to remove other contaminants from leaves and dried up under shade for nine days i.e., until the weight of the dried leaves remains constant. These leaves are sliced into tiny pieces and made homogenized powder by using home blender. The obtained powder is stored in an air tight container for further usage.

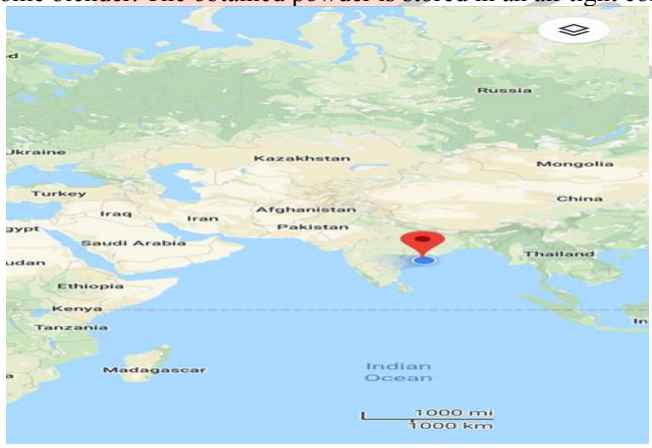

Figure 1.3: Map showing plant collection site in India

1.2.3 Preparation of leaf Extract

200 mL deionized water is taken in 500 mL beaker to this 10 g stored powder weighed and added. The contents in the beaker boiled for 30 minutes with occasional stirring with glass rod and then cooled to acquire room temperature. The cooled leaf broth is filtered 2 times with Whatman No.1 filter paper and reserved in refrigerator at 4ºC. This is taken as leaf extract throughout the experiment. **Figure 1.4** shows image of *Aerva lanata* leaf extract prepared.

Figure 1.4: Image of *Aerva lanata* leaf extract

1.3 PHYTOCHEMICAL SCREENING TESTS

Aqueous extract of *Aerva lanata* is screened to various phytochemical tests. Standard methods are used for phytochemical screening [25].

1.3.1 Test for Alkaloids

a) Mayers Test

To 5 mL of 1% HCl, 5 mL of leaf extract is added boiled in a water bath and then filtered. 2 mL of the filtrate is treated with two drops of Mayer's reagent. Formation of yellow precipitate indicates the presence of Alkaloids.

b) Hager's Test

To 5 mL of 1% HCl, 5 mL of leaf extract is added boiled in a water bath and then filtered. 2 mL of above filtrate is treated with 2 drops of Hager's reagent. Formation of yellow precipitate shows the presence of alkaloids.

c) Wagner's Test

To 5mL of 1% HCl, 5 mL of leaf extract is added boiled in a water bath and then filtered. 2 mL of above filtrate is treated with two drops of Wagener's reagent. Formation of brown colour precipitate indicates the presence of alkaloids in leaf extract.

1.3.2 Test for Carbohydrates

a) Molisch's Test

To 2 mL algal extract 5mL of distilled water is added and filtered. To the 2 mL of filtrate 2 drops of Molisch's reagent (alcoholic solution of α-naphthol solution) is added followed by the addition of concentrated sulphuric acid along the walls of the test tube. Formation of violet ring indicates the presence of carbohydrates.

b)Benedict's Test

To 2 mL algal extract 5mL of distilled water is added and filtered. To the 2 mL of filtrate 2 drops of Benedict's reagent is added and heated gently for two minutes. Formation of red precipitate indicates the presence of carbohydrates (reducing sugars).

c) Fehling's Test

To 2 mL algal extract 5mL of distilled water is added and filtered. To the 2 mL of filtrate 1mL of each Fehling solution A and B is added and boiled in a water bath for 2 min. Formation of brown precipitate indicates the presence of carbohydrates (reducing sugars).

1.3.3 Test for Glycosides

a) Legal's Test

5 mL of extract is treated with 4mL of pyridine contained 2 mL of sodium nitroprusside solution. This is neutralized with 10% NaOH. Appearance of pink colour shows the existence of glycosides.

b) Modified Borntrager's Test

5 mL of extract is treated with 2 mL of $FeCl_3$ solution and immersed in boiling water for about five minutes. The mixture is cooled and extracted with equal volumes of benzene. The benzene layer is separated and treated with ammonia solution. Formation of rose-pink colour indicates the presence of anthranol glycosides.

c) Keller-kilani test

Another test to confirm the presence of glycoside is carried out by mixing the crude extract (2 mL) with glacial acetic acid (2 mL) containing 1-2 drops of 2% $FeCl_3$ solution. The mixture is then transferred into another test tube already containing 2 mL concentrated H_2SO_4. Appearance of brown ring at the interphase confirms the presence of cardiac glycosides.

1.3.4 Test for Saponins

a) Foam Test

To 5 mL of crude extract 20 mL of distilled water is added and this solution shaken vigorously in a 100 mL conical flask for 15min. Persistent foaming on shaking confirms the presence of saponins.

1.3.5Test for Steroids and Phytosterols

a) Libermann-Burchard's Test

5mL of extract is treated with chloroform and filtered. The filtrate is treated with few drops of acetic anhydride, boiled and cooled. Conc. H_2SO_4 is added. Formation of reddish brown colour indicates the presence of steroid ring.

b) Salkowski's Test

5mL of extract is treated with chloroform and filtered. The filtrate is treated with few drops of conc. H_2SO_4, shaken and allowed standing. Appearance of golden yellow colour indicates the presence of steroids.

1.3.6Test for Phenolic compounds

a) Ferric Chloride Test

The extract is dissolved in 5mL of distilled water and 2-4 drops of 5% $FeCl_3$solution is added. Formation of deep green colour specifies the presence of phenolic compounds.

b) Lead Acetate Test

2mL of 5% lead acetate solution is added to the extract solution. Formation of yellow precipitate indicates presence of phenolic compounds.

1.3.7Test for Tannins

a) Gelatin Test

To 1% gelatin solution containing 10% NaCl 5mL diluted algal extract is added. The formation of white precipitate indicates the presence of tannins.

1.3.8 Test for Flavonoids

a) Alkaline Reagent Test

5mL of extract is treated with few drops of sodium hydroxide solution. Formation of an intense yellow colour, which becomes colourless on addition of dilute acid connotes the presence of flavonoids.

1.3.9 Test for proteins

The extract is treated with few drops of Conc. HNO_3. Formation of yellow colour suggests the presence of proteins.

1.3.10 Test for Amino acids

a) Ninhydrin Test

5mL of extract is diluted by the addition of 15mL of distilled water. To the extract, 0.25% w/v Ninhydrin reagent is added and boiled for a few minutes. Formation of blue colour denotes the presence of amino acids.

1.3.11Test for Diterpenes

a) Copper acetate Test

Extract is dissolved in water and treated with 2-4 drops of copper acetate solution. Formation of emerald green colour confirms the presence of diterpenes.

b) Crude extract (2 mL) is dissolved in chloroform (2 mL) and then evaporated to dryness. Concentrated H_2SO_4 (2 mL) is added to the resulting solid and heated for 2 minutes. The appearance of greyish colouration indicates the presence of terpenoids.

1.4 Detection of functional groups present in the *Aerva lanata* leaf extract by FTIR analysis

The FTIR spectrum is used to identify the functional groups of the active components based on its peak values in the region of IR radiation [26].

The strong intense peaks observed between 3200 cm^{-1} to 3950^{-1} may be due to N-H, O-H stretching of 1^0 amines and polyhydroxy groups present in the extract (**Figure 1.5**). The strong absorption at 1643 cm^{-1}indicates the presence of C=O group of amides. This result gives the evidence about the high protein content of the extract. The small peak at 2892 cm^{-1} may be due to C-H symmetrical stretching of methylene groups. The peak position at 1519 cm^{-1} may be due to the C=C stretch of aromatic ring. The peak at 598 cm^{-1} is denoting the presence of C-Cl group.

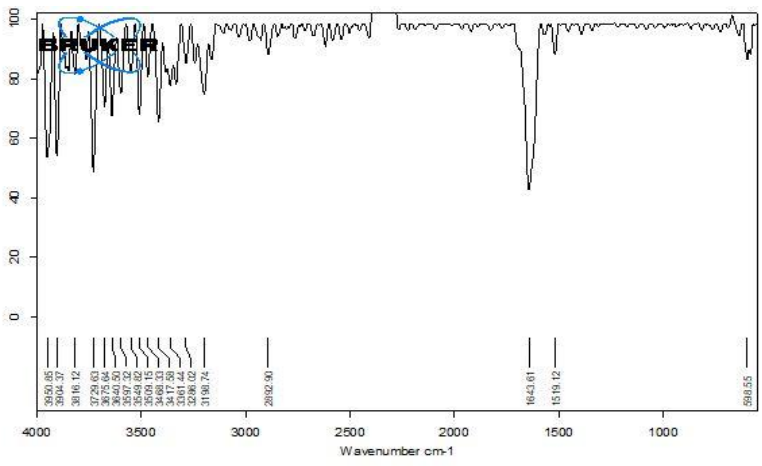

Fig. 1.5: FTIR spectrum of *Aerva lanata* leaf extract

3.5 CONCLUSIONS

The phytochemical screening and FTIR spectroscopic analysis of *Aerva lanata* leaf extract confirm the presence of alkaloids, carbohydrates, glycosides, saponins, phytosterols, phenolic compounds, tannins, flavonoids, proteins, amino acids and terpenes. These are the plant secondary metabolites present in the leaf extract. The important phytochemical properties recognized by this study will be very useful in the development of new drugs for the treatment of various diseases of mankind.

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