PHYTOCHEMICAL EXAMINATION OF PLANTS

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Abstract : Natural products obtained from the plants have a wide range of medicinal values. The pure chemical components separated from the plant extract provides unlimited opportunities for new drug discoveries as they have unmatched chemical diversity. Due to the increasing therapeutic use of natural products interest in edible plants has grown throughout the world. Chemical synthesis of the natural product is also carried out to learn a variety of biological activities. The focus of the work is on methodologies that include extraction, separation and identification of natural products. The objective of the proposed paper is to study extraction, isolation, and identification of compounds present in plant.

The work was carried out by extraction of different medicinal plant's leaves, flowers and fruit in three different solvent systems and then the identification tests of the chemical constituents were done. Leaves of Aloe vera, Fragaria × ananassa (Strawberry Fruit) and Butea monosperma flower and were analysed. Different techniques such as chromatography i.e. Column chromatography and Thin Layer Chromatography (TLC) as well as spectroscopic techniques were used.

Key words : Column Chromatography, Thin layer chromatography, UV-Vis

I.Introduction

The natural product chemistry usually begins from the separation and isolation of a single pure compound from such similarly related ingredients. Natural products offer more drug like features to the molecules from combinatorial chemistry in terms of functional groups, chirality and structural complexity. The amount of energetic ingredients are fairly low in it which turns out to be crucial to be isolated and identified. Plant tissues always contain several classes of compounds with markedly different structures. Isolation of the different natural product from the target plant begins with the extraction with solvents. The extraction of natural products from plants, marine and microbial sources is done by using organic solvents. Many of the products such as peptides, tannins, saponins, etc are water soluble and remain in aqueous phase whereas some alkaloids are extracted with organic solvents like Chloroform , Ethyl acetate , etc. the extract is concentrated which is further subjected to column of Silica gel for separation and purification followed by structure elucidation by MP and spectroscopic properties.

a. ALOE VERA PLANT:

Aloe vera has been referred as a *miraculous* plant used by the mankind since centuries, for treatment of mainly skin diseases but also for disorders like constipation, stomach diseases, hair loss, renal disorders and much more. From the ecological nature of the plant it was originated from Africa and the history states its uses since 6000 years.

Aloe vera has triangular, fleshy leaves with serrated edges, yellow tubular flowers and fruits containing numerous seeds. The leaf is mainly composed of three layers with different chemical composition and thus showing different pharmacological activities.

Outer thick layer: The outer green layer is called rind.

Middle layer : The middle layer contains the latex which is bitter in taste. The latex is the bittering agent and is rich in anthraquinone.

Inner layer Gel: The inner layer contains gel called pulp. It is clear, mucilaginous gel arising from parenchymatous cells. It contains 99% water, glucomannans, amino acids, lipids, sterols, vitamins, etc.

Aloe vera leaves contain a diverse array of 75 compounds including anthraquinones and their glycosides, carbohydrates, proteins, glycosides, amino acid, saponins etc.

Fig.1: Aloe vera leaves

Medicinal Uses :

Used as an Analgesic, antibacterial, Antifungal, antiviral, Wound healing and anti-inflammatory, Antiseptic, have cleansing property, Antioxidants, etc.

b. *FRAGARIA* × *ANANASSA*(STRAWBERRY FRUIT) :

Fragaria × *ananassa* are a widely grown species of the genus Fragaria, collectively known as the strawberries, which are cultivated worldwide for their fruit. The fruit is widely used for its aroma, bright red color, juicy texture, and sweetness. It is consumed in large quantities, either fresh or as preserved foods like jam, juice, pies, milkshakes, and chocolates. Artificial strawberry flavorings and aromas are also widely used in products such as candy, soap, lip gloss, perfume, and many others.

Many berry fruits contain micronutrients such as minerals, vitamin C and folic acid, which are essential for health. However berries may provide additional health benefits because they also contain high levels of a diverse range of phytochemicals/phytonutrients consisting predominantly of phenolic type molecules. Phenolic compounds contain aromatic ring bearing hydroxyl group and can range from simple monomeric molecules to very large oligomers. They frequently occur naturally in berry fruits in their glycosylated forms, which make them more water-soluble although the higher molecular weight oligomers are more insoluble. Berry fruits are reported to contain a wide variety of phenolics including hydroxybenzoic and hydroxycinnamic acid derivatives, anthocyanins, flavonols, condensed tannins etc.

Fig.2: Strawberry fruits

Medicinal Uses:

Studies conducted indicate that berry phenolics have a wide range of biological properties such as anti-cancer, antioxidant, anti-inflammatory, and cell regulatory effects. Among commonly and popularly consumed small and soft berry fruits, strawberries are widely known for their potential health benefits due to their high fiber, potassium, vitamin C and folate contents.

c. BUTEA MONOSPERMA :

Butea monosperma belongs to a family of fabaceae native to tropical southeastern Asia and a popularly ornamental tree grown around the world. It is a deciduous tree growing to 12–15 m tall, with a crooked in mature tree. The leaves are pinnate and the flowers are bright orange in color, and produced in racemes up to 15 cm long which appear only in spring. Butea monosperma flowers also known as *"Tesu", "Kesu"* and *"Kesudo"* in different parts of the country.

Fig.3: Butea monosperma flowers

Medicinal Uses :

Butea monosperma is used in distinct parts of the world for the remedies of stomatitis, sores and skin problems, constipation, ringworm, insomnia, dysentery, muscular pains, liver disorders, ulcer, tumor, fever, gonorrhea, diabetic, inflammation, fungal infection, piles, urinary disorder, etc.

II.Research Methodology : The initial analysis is done by conducting phytochemical tests on the different solvent extracts of the plant for the detection of the chemical constituents present in it. To study the Ethyl acetate extract in detail by using column chromatography and TLC followed by elucidation of structure of the chemical compounds present on the basis of the physical properties viz. melting points and other spectroscopic techniques.

III.Materials and method:

- **Plant Material :**

Aloe vera leaves were collected from Shilpin Nursery, Vasan of Navsari district, Gujarat. The species collected was Barbados Aloe.

Fresh Strawberry fruits were purchased from local market of Vadodara.

Dried Butea monosperma flowers were collected from Gandevi, of Navsari district, Gujarat.

They were identified and authenticated by Parul Institute of Agriculture, Vadodara, India.

- **Preparation of plant material :**

The solvents used for extraction were **Ethyl acetate, Methanol and Water.**

Aloe vera leaveswere collected and washed twice with water. The leaves were then sliced in tiny pieces along with the rind. 100 g of pieces were packed in soxhlet apparatus and extraction was carried out with 250ml of different solvents along with few tiny pieces of porcelain and temperature was maintained at 60º C for 24 hours.

Strawberry fruits were collected and washed twice with water and were chopped into fine slices. 100 g of pieces were packed in soxhlet apparatus and extraction was carried out with 250ml of different solvents along with few tiny pieces of porcelain and temperature was maintained at 60º C for 24 hours.

Dried Butea monosperma flowers were cleaned and powdered.15g of powdered material was packed in soxhlet apparatus and extraction was carried out by 250ml of different solvents along with few tiny pieces of porcelain and temperature was maintained at 60º C for 24 hours.

IV.Procedure :

Tests conducted on the solventextracts for Phytochemical analysis of the components:

i. **SHINODA TEST**: Yellow coloration is observed when few drops of NaOH was added to 1 ml extract. On further addition of few drops of dilute HCl it turns colourless. This indicates presence of **FLAVANOIDS.**

ii. **WAGNERS TEST**: A fraction of extract was treated with 5 drops of Wagner's Reagent (1.2 g of iodine and 2g of KI in 100ml of water) and observed for the formation of brown precipitate or coloration. This indicates the presence of **ALKALOIDS.**

iii. **PHENOL TEST** : A fraction of extract was treated with 5% ferric chloride and observed for the formation of deep blue or black precipitates or coloration. The change in colour indicates the presence of **PHENOL.**

iv. **MOLISCH TEST** : Few drops of Molisch reagent (alpha napthol + ethanol) was added to extract. This was followed by the addition of concentrated sulphuric acid. The mixture was allowed to stand for a few minutes. Formation of red or dull violet colour at interphase of two layers indicates the presence of **CARBOHYDRATES**.

v. **QUINONES TEST :** A small amount of extract was treated with concentrated HCl and observed. Formation of yellow precipitates or coloration indicates the presence of **QUINONES.**

vi. **SAPONIN TEST** : A fraction of the extract was taken and filtered with filter paper in a test tube. Five ml of water was added to the test tube and was vigorously shaken. Appearance of froth on the top of the solution indicates the presence of **SAPONINS.**

vii. **GLYCOSIDE TEST** :A fraction of the extract was taken and 10% Lead acetate was added to it. Appearance of yellow or white precipitates indicates the presence of **GLYCOSIDES.**

viii. **TANNIN TEST:** 2-4 ml of the extract was taken and treated with 10% alcoholic ferric chloride. Appearance of blue or green precipitates indicates the presence of **TANNINS**.

ix. **AMINO ACID TEST:** 2-4ml of extract was treated with 2-4 drops of Ninhydrine and then kept in water bath. Appearance of pink colour indicates the presence of **AMINO ACIDS**.

Column chromatography (CC):

Column chromatography is a technique used to isolate chemical compounds from a mixture. It is a method used to separate substances based on differential adsorption of compounds to the adsorbent; compounds move through the column at different rates which allows them to get separated into fractions. This technique is widely applicable because it can be used with a wide range of solvents according to their polarities.

Experimental procedure:

- The packing of column is done by wet method. The extract of Ethyl acetate was concentrated and slurry was made with silica gel (60-120 mesh) and eluent petroleum ether and then kept in water bath for few minutes. The column was prepared by packing the column with cotton at the bottom. The stationary phase silica gel was used by making its slurry with petroleum ether. The silica gel slurry is added first and then the slurry of the extract was added and packed with cotton at the top.

- Petroleum ether is used as the first solvent according to its least polarity. The solvents selected and eluted in the following order (for Aloe vera leaves extract):

Petroleum ether < Benzene <Ethyl acetate < Acetone

- Pure petroleum was used as the first solvent followed by mixture of 90% petroleum ether – 10% Benzene. Successive mixtures containing 20%, 30%, 40% followed till 100% Benzene, Ethyl acetate and acetone respectively complete the transition of the polarity.

For Strawberry fruit extract solvents selected were

Petroleum ether < Benzene < Ethyl acetate < Methanol< Water

For Butea monosperma flower extract solvents selected were in following order

Petroleum ether <Benzene <Ethyl acetate <Acetone <Methanol<Water

- The fractions were collected from the column and then distilled. The concentrated samples were collected in the test tube and then TLC for components were done.

- The samples were again concentrated in the water bath and then crystallized with solvents. Melting point of the obtained pure compounds was determined in the laboratory.

- During the column chromatography, chemical compounds were obtained from the different fractions of the plant extracts. They were collected, purified & recrystallized by solvent Ethanol and then further analysis like determination of MP were done.

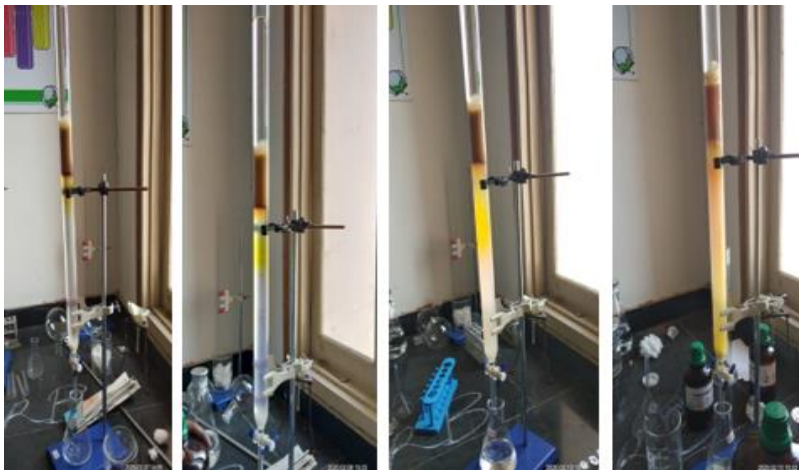

Fig.4 : Column of Aloe vera leaves extract

Fig.5 : Column of Strawberry fruits extract

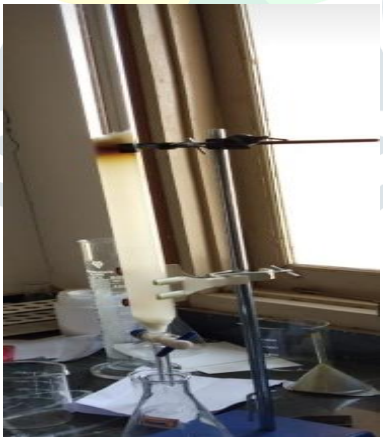

Fig.6 : Column of Butea monosperma flowers extract

V.Result :

Table No. 1: Test results of the Ethyl acetate extract

S.N.	Tests conducted	Aloe vera leaves	Strawberry fruit	Butea monosperma flower
1.	**Shinoda test**	+	+	-
2.	**Wagners test**	-	-	-
3.	**Phenol test**	-	+	-

4.	**Molisch test**	+	+	+
5.	**Quinones test**	+	+	+
6.	**Saponin test**	-	-	-
7.	**Glycoside test**	+	-	-
8.	**Tannin test**	-	-	-
9.	**Amino acid test**	-	-	-

Table No. 2 : : Test results of the Methanol Extract

S.N.	Tests conducted	Aloe vera leaves	Strawberry fruit	Butea monosperma flower
1.	**Shinoda test**	-	-	+
2.	**Wagners test**	-	-	+
3.	**Phenol test**	-	+	+
4.	**Molisch test**	+	-	+
5.	**Quinones test**	-	+	-
6.	**Saponin test**	+	+	+
7.	**Glycoside test**	-	-	+
8.	**Tannin test**	-	+	-
9.	**Amino acid test**	-	+	+

Table No. 3 : : Test results of the Water Extract

S.N.	Tests conducted	Aloe vera leaves	Strawberry fruit	Butea monosperma flower
1.	**Shinoda test**	-	-	-
2.	**Wagners test**	-	-	+
3.	**Phenol test**	-	-	-
4.	**Molisch test**	+	+	+
5.	**Quinones test**	-	-	+
6.	**Saponin test**	+	+	+
7.	**Glycoside test**	+	-	-
8.	**Tannin test**	+	-	+
9.	**Amino acid test**	-	-	-

- During the CC of the Ethyl acetate extract of the plants following chemical compounds were extracted.
- From the column of Aloe vera leaves extract, Salicyclic acid and Anthraquinone were isolated.
- From the column of Fragaria × ananassa(Strawberry Fruit) extract, Gallic acid and Cinnamic acid were isolated.
- From the column of Butea monosperma flower extract, Stearic acid was isolated.

Fig. 7 : Salicylic acid

Fig.8 : Anthraquinone

Fig. 9: Gallic acid

Fig.10: Cinnamic acid

VI.Conclusion

- During the phytochemical examination of the Ethyl acetate extract of Aloe vera, Flavonoids, Carbohydrate, Quinones and Glycoside were detected while Alkaloid, Phenol, Saponins, Tannins and Amino acids were found to be absent. The tests on Methanol extract of Aloe vera depicts the presence of Carbohydrate and Saponins. Water extract of Aloe vera on testing shows the presence of Carbohydrate, Saponins, Tannins and Glycosides. Salicylic acid and Anthraquinone were separated by column chromatography and their structure was established by comparing with the original sample.

- During the phytochemical examination of the Ethyl acetate extract of Strawberry, Flavonoids, Carbohydrate, Quinones and Phenol were detected while Alkaloid, Glycosides, Saponins, Tannins and Amino acids were found to be absent. The tests on Methanol extract of Strawberry depicts the presence of Phenol, Amino acid, Quinones, Tannins and Saponins. The tests on Water extract of Strawberry depicts the presence of Carbohydrates and Saponins. Gallic acid and Cinnamic acid were separated by column chromatography and their structure was established by comparing with the original sample.

- During the phytochemical examination of the Ethyl acetate extract of Butea monosperma flower Carbohydrate and Quinones were detected while Alkaloid, Glycoside, Phenol, Saponins, Flavonoids, Tannins and Amino acids were found to be absent. The tests on Methanol extract of Butea monosperma flower depicts the presence of Carbohydrate, Glycoside, Amino acids Alkaloids, Flavanoids, Saponins and Phenol. The tests on Water extract of Butea monosperma flower depicts the presence of Carbohydrate, Saponins, Quinones, Tannins and Alkaloid. Stearic acid was separated by column chromatography and its structure was established by comparing with the original sample.

VII.Acknowledgement:

Authors would like to convey their sincere thanks to the management of Parul University for allowing to carry out the research work.

Authors would like to thank to HOD, Chemistry for her constant encouragement.

Authors would like to convey their gratitude to the principal of PIAS for his efforts and encouragement.

VIII.References :

1. Aloe vera , their chemical composition and applications, A review, International Journal of Biology and Medical Research 2008 by Sharrif Moghaddasi M, Sandeep Kumar Verma.

2. Aloe vera Short Review, Indian Journal of Dermatology 2008 by Amar Surjuhe, Resham Vasani, D G Saple.

3. The medicinal plant components and application, Journal of Medicinal Plant Studies 2018; 6(3): 89-95 by Mini Tiwari and Dr Manish Upadhyay.

4. Identification of bio-active components in leaf extracts of Aloe vera, Journal of Medicinal Plants Research 2015 by Jyoti Vastrad, Giridhar Goudar, Shameembanu A. Byadgi, Rajkumari Dhanlaxmi Devi and Rajashri Kotur.

5. Phytochemical Constituents of Aloe vera and their multifunctional properties: A comprehensive review, International Journal of Pharmaceutical Sciences and Research by Sanjit Kumar Kar and Tushar Kanti Bera.

6. Bioactive compounds and medicinal properties of Aloe vera Leaves, Journal of Plant Sciences 2014; 2(3) : 102-107 by Bawankar Raksha, Singh Pooja and Subramaniam Babu

7. Ph D thesis entitled Synthetic and Natural studies of some plant polyohenolic, 1978 by Ashish Nagar.

8. Phytochemical constituents and antibacterial efficacy of the flowers of Peltophorum Pterocarpum (DC.) Baker Ex. Heyne, Asian Pacific Journal of Tropical Medicine. 2011; 10:735-738 by Sukumaran A, Kiruba C, Mahesh F, Nisha F, Miller P, Ben O.

9. Exploration of phytochemical potential on flower of Butea monosperma, Journal of Pharmacognosy and Phytochemistry 2019; 8(3); 2038-2085 by Milan Hait, Sagar Kumar Behera, Amit Kumar Chaturvedi and M. M. Vaishav.

10.Phytochemical screening and Extraction. Int. Pharma. Sciencia. 2011; 1:98-106 by Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H.

11.Indian medicinal plants, Allahabad, India. 2nd edition. 1935; 2:785-788 by Kirtikar KR, Basu BD.

12.Bioaccessibility of micronutrients in fresh and frozen strawberry fruits grown under elevated carbon dioxide and temperature, Food Chemistry ,2020 by Himali Balasooriya, Kithsiri Dasanayake, Said Ajlouni.

13.Nutritional and Nutraceutical Quality of Strawberries in Relation to Harvest Time and Crop Conditions, Journal of Agricultural and Food Chemistry 2014, by Ikram Akhatou and Ángeles Fernández-Recamales.

14.Promising Health Benefits of the Strawberry: A Focus on Clinical Studies, Journal of Agricultural and Food Chemistry 2016, by Sadia Afrin, Massimiliano Gasparrini, Tamara Y. Forbes-Hernandez, Patricia Reboredo-Rodriguez, Bruno Mezzetti, Alfonso Varela-López, Francesca Giampieri, and Maurizio Battino .

15.Indian Medicinal Plants Reprint ,2008 by Khare CP.