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An evaluation of phytochemical constituents of sweet flag (*Acorus calamus*), a Unani herbal medicine

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Abstract

Phytochemicals are bioactive compounds obtained from plants and are widely applied in the traditional Unani herbal Medicine. Herbal medicine is a practice that includes herbs, herbal material, and preparations that contain parts of plants or combinations thereof as active ingredients. These herbs are derived from plant parts such as leaves, bark, flowers, roots, fruits, and seeds. The importance of plants is well known to us. The plant kingdom is a treasure house of potential drugs and in the recent years there has been an increasing awareness about the importance of medicinal plants. Drugs from the plants are easily available, less expensive, safe, and efficient and rarely have side effects. The plants which have been selected for medicinal use over thousands of years constitute the most obvious choice of examining the current search for therapeutically effective new drugs such as anticancer drugs, antimicrobial drugs, antihepatotoxic compounds. According to World Health Organization (WHO), Medicinal plants would be the best source to obtain variety of drugs. About 80% of individuals from developed countries use traditional medicines, which have compounds derived from medicinal plants. The various phytochemicals found in sweet flag, Unani medicine such as Tannins, Saponins, Flavonoids, diterpenes and minerals such as Carbonate, Nitrate, Phosphate, Ammonium listed in table 1, 2 and 3. It was concluded that the herbal medicine SWEET FLAG extraction is rich in Phytochemicals and biochemical & minerals with medicinal properties.

Keywords: Phytochemicals, minerals, drug, nerve repair, nerve pain, saponins, tannins, solubility, Unani medicine

Introduction

The physio-chemical properties of a compound are the intrinsic physical and chemical characteristics of a substance. These include appearance, boiling point, water solubility, density, and flammability etc [1]. About 80% of individuals from developed countries use traditional medicines, which have compounds derived from medicinal plants [2]. Phytochemicals are bioactive molecules which are also referred to as secondary metabolites that are derived from plants. Primary metabolites and Secondary metabolites are the two types of metabolites generated by plants [3]. Primary metabolites are necessary for a plant's normal metabolism, including growth and development. Secondary metabolites produced by plants may have little need for them. These may be found in nearly every part of the plant, including the bark, leaves, stem, root, flower, fruits, seeds, and so on. Phytochemicals have been utilized as traditional herbal medicines for numerous years all over the world. As a result, both the pharmaceutical industry and researchers place a higher focus on phytochemical research. These phytochemicals which are found in many plant sections, are also employed by indigenous peoples to treat various ailments [4]. These are also frequently utilized in the agricultural sector. Drugs. Flavoring agents, perfumes, dyes, pigments, pesticides, and food additives all rely on secondary metabolites for their synthesis. Many medicines generated from secondary metabolites are simply synthetic alterations or duplicates of these naturally occurring compounds [5]. Phytochemicals have plenty of applications such as Tannins and resins adhesives are used to prepare plywood [6]. The alkaloids are used in the regulation of microbial and schizonticide activity and as pharmaceuticals [7]. Many of the phenolic molecules are also effective antioxidants and free radicals, scavengers, especially flavonoids [8]. Saponins have demonstrated numerous pharmacological properties. Some saponins have antitumor, pesticidal, molluscicide, spermicidal, sedative, expectorant and analgesic properties [9].

Like all groups of terpenes, diterpenes have demonstrated a range of pharmacological properties including analgesic, antibacterial, antifungal, anti-inflammatory, antineoplastic and antiprotozoal activities [10]. Realizing the importance of herbal medicine in the treatment of various diseases we selected “SWEET FLAG” a Unani medicine for the analysis of various phytochemical constituents present in it Sweet flag is mainly used in medicine. Oil is used to cure gastritis. In the form of infusion, it is a carminative and possesses emetic and anti-spasmodic properties. It is used in the perfumery industry [12].



Fig 1: Sweet flag

Sweet flag (*Acorus calamus*) is a commonly known drug in traditional system of medicine. It is a tall perennial wetland monocot plant from the Acoraceae family. The scented leaves and rhizomes of sweet flag have been traditionally used as a medicine and the dried and powdered rhizome has a spicy flavor and is used as a substitute for ginger, cinnamon, and nutmeg for its odor [13].

The rhizomes are considered to possess antispasmodic, carminative, anthelmintic, aromatic, expectorant, nauseate, nervine, sedative, stimulant properties and used for the treatment of epilepsy, mental ailments, chronic diarrhea, dysentery, abdominal pain [14]. The interpretation of the compounds found in the drug are Carbohydrates, play an important role in storage of glucose. Carbohydrates play an

important role in homeostasis of glucose and fatty acids in liver [15]. Phytosterol are plant sterols, Phytosterols have anti-inflammatory effect, Phytosterols reduce oxidative stress. Various bioactivities of Phenolic compounds are responsible for their chemo preventive properties. Phytosterols have an antioxidant property [16]. Suppress the inflammatory response. The Triterpenes are the best immuno-modulator and have antioxidant property. Anti-microbial activity. Anti-bacterial agent [17]. Flavonoids are the most important group of polyphenol compounds in plants. Flavonoids are a group of plants metabolites which provide health benefits through cell signaling pathways and antioxidant effects. Flavonoids can exert their Antioxidant activity by scavenging the free radicals, by chelating metal ions or by inhibiting enzymatic systems responsible for free radical generation. Flavonoids are immuno-modulator [18].

Materials and Methods

Preparation of extract

The extract of the following herbal medicine is prepared by using the Hot water Extraction technique. The extract filtered solution was kept in a Labelled plastic bottle. 10gm of the herbal drug powder extract was weighed in an electronic weighing machine, dissolved the powdered extract in 100 ml of distilled water and boiled it for 3 hours on water bath. The extract was filtered through Whatman No.1 Filter paper. For further use of the solution for analyses, the extract was kept in sterile bottle in a refrigerator [11].

Phytochemical analysis

The phytochemical screening of the extract gives general ideas regarding the nature of chemical constituents present in the crude drug. The phytochemical tests were done as the methods illustrated.

Test of Carbohydrates and reducing sugars

a) Molisch’s test:

1ml Herbal extract was treated with 2-3 drops of 1% alcoholic alpha naphthol and 2ml of H2SO4 was added in a test tube.

The appearance of violet ring between the two layers indicated presence of carbohydrates.

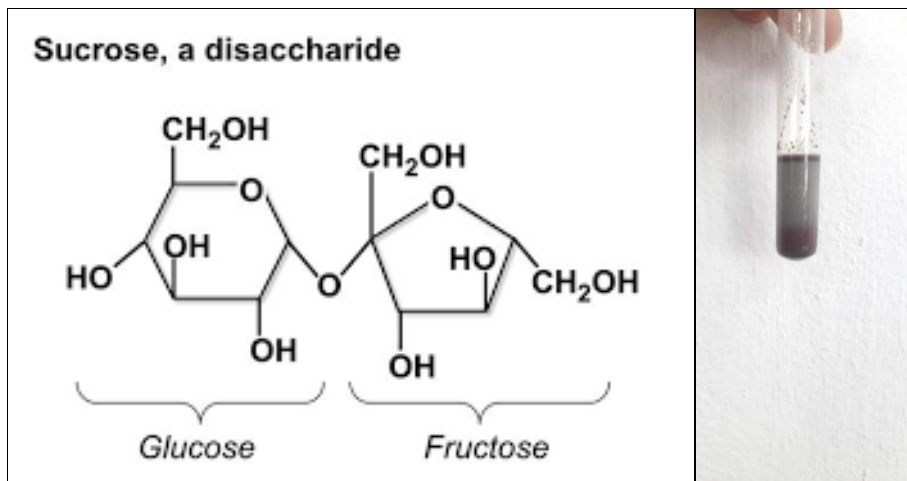


Fig 2: Molisch test

a) Benedict's test:

1ml Herbal extract was treated with 2-3 drops of Benedicts reagent was added and treated strongly.



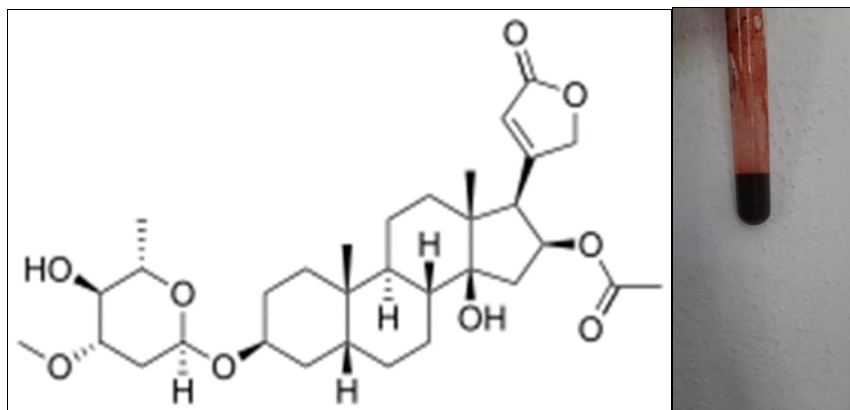
The appearance of Orange- red precipitate indicates presence of reducing sugars.

Test for Glycosides:

The extract was hydrolyzed with dil. HCl and subjected to test for glycosides.

a) Legal's test:

1ml hydrolysate extract was treated with 1ml of sodium nitroprusside in pyridine and 1ml of sodium hydroxide. Presence of blood red color.



Indicates presence of cardiac glycosides.

Test for saponins:

2ml of Drug extract was taken in a Test tube and it was mixed with 5ml of distilled water. The test tube was shaken

vigorously. Afterwards, the presence of Foam was noted, and this indicates the presence of Saponins.

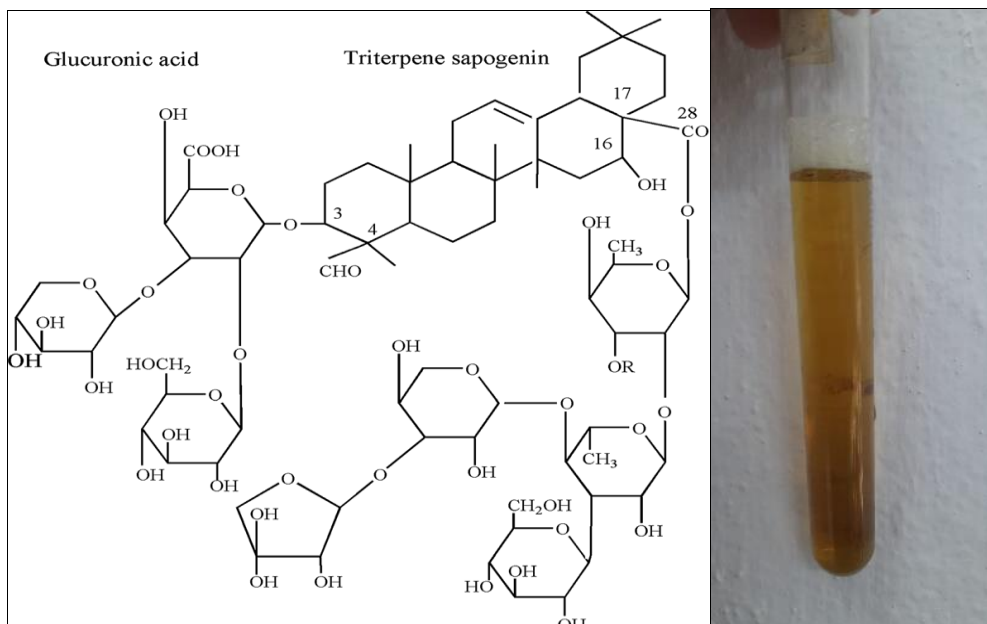


Fig 3: Saponins

Test for phenolic compounds:

0.5ml filtrate Extract was treated with 1ml alcoholic Ferric

Chloride in a Test tube. No appearance of bluish green color solution in the test tube indicates absence of Phenolic

compounds.

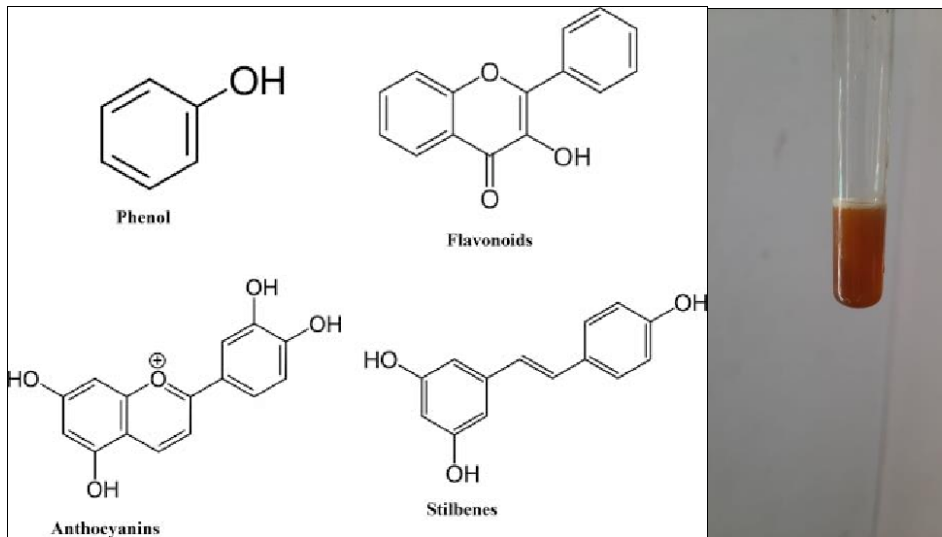


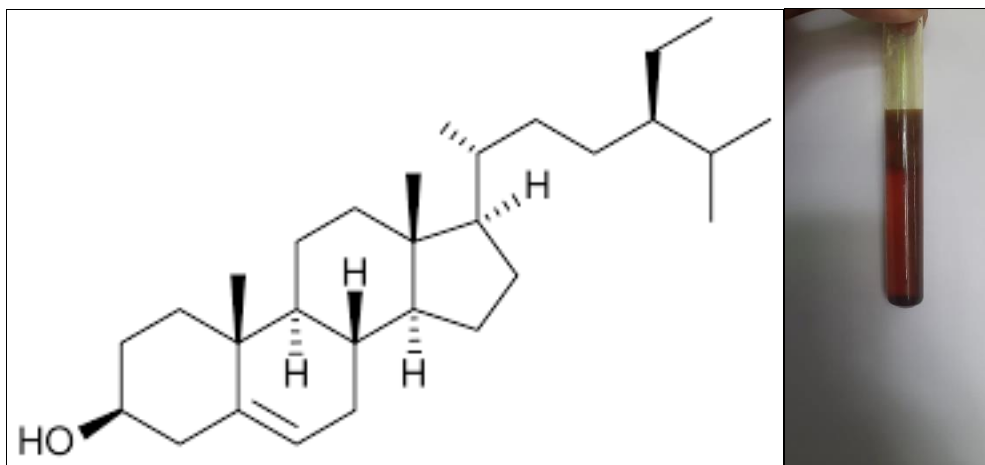
Fig 4: Phenolic compounds

Test for phytosterols:

Ferric chloride- acetic acid test:

1ml herbal extract was treated with 1ml of chloroform and

then 2ml of ferric chloride- acetic acid reagent was added followed by 1ml of concentrated sulphuric acid. Appearance of reddish pink color in test tube.



Indicates presence of phytosterols.

Test for diterpenes

Copper acetate test

1 ml of the extract solution was added in a Test tube and

Few Drops of 10% Copper Acetate solution. The formation of Emerald Green Color in a Test Tube indicates the presence of Diterpenes.

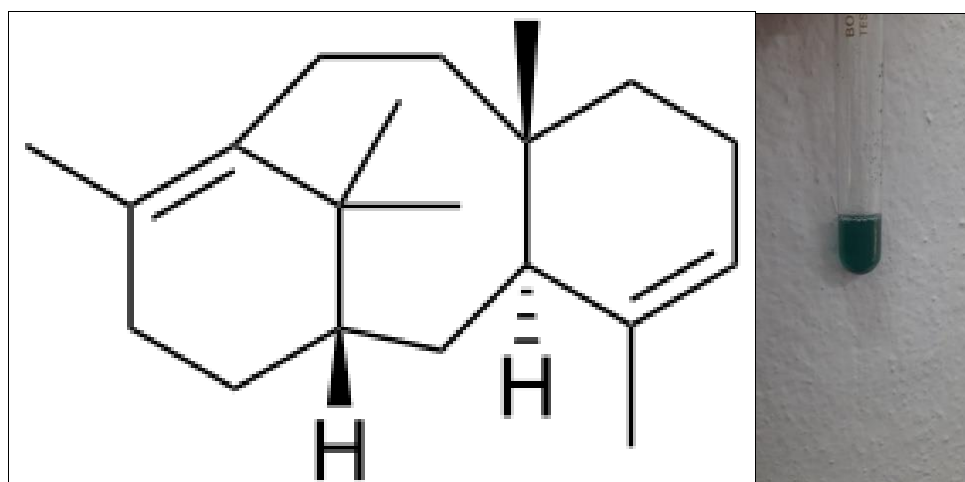


Fig 5: Diterpenes

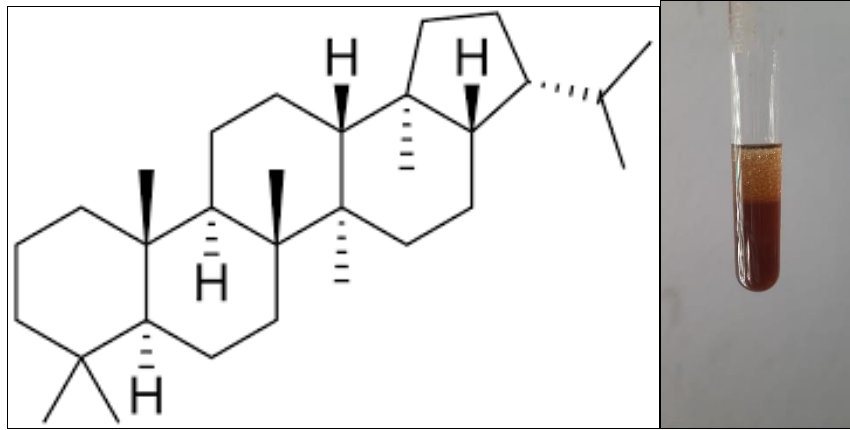
Test for triterpenes

Salkowski's test

1ml of herbal extract was treated with 1ml of chloroform

followed by 1ml of concentrated sulphuric acid, shaken and allowed to stand.

The appearance of golden yellow color.



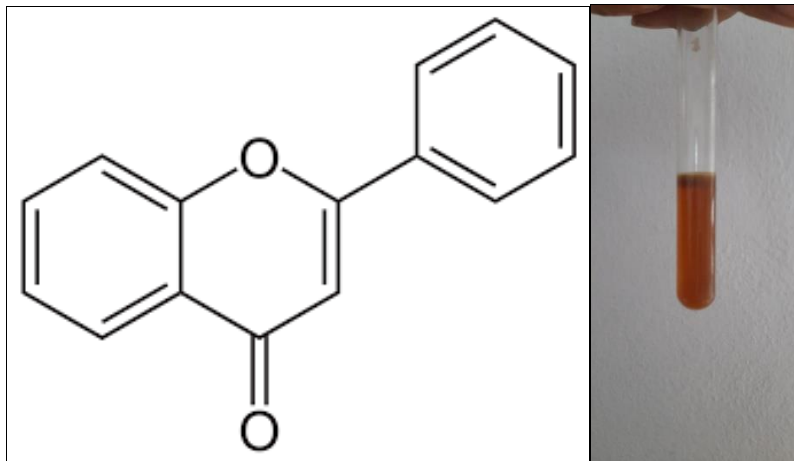
Indicates presence of triterpenes.

Test for flavonoids

a) Alkaline reagent test

To 1ml of extract, 1ml of 10% sodium hydroxide solution

was added. Formation of dark yellow color.



Indicates the presence of flavonoids.

b) Ferric chloride test:

To 1ml of herbal extract 3-4 drops of ferric chloride solution was added.

No formation of dark green color solution. Indicates absence of flavonoids.

Test for proteins and free amino acids

a) Xanthoproteic test:

To 1ml of extract, 3-4 drops of concentrated nitric acid was added. No formation of yellow precipitate. Indicates absence of proteins.

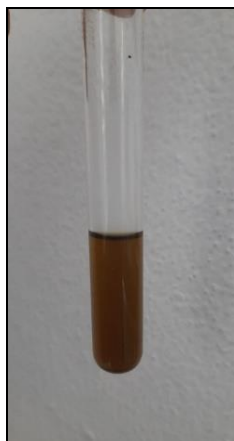


Fig 6: Flavonoids



Fig 7: Xanthoproteic test

b) Million's test

To 0.5ml of herbal extract, 2.5ml of Million's reagent was

added. No formation of white precipitate. Indicates absence of proteins.



Fig 8: Million's test

c) Biuret test:

0.5ml of extract, 2.5 ml of dilute Biuret reagent was added.

Appearance of brick red precipitate. Indicates absence of proteins or free amino acids.



Fig 9: Biuret test

Test for quinones:

Sodium hydroxide test:

To 0.5ml of herbal extract, 1ml of sodium hydroxide was

added. appearance of red orange color. Indicates presence of quinones.

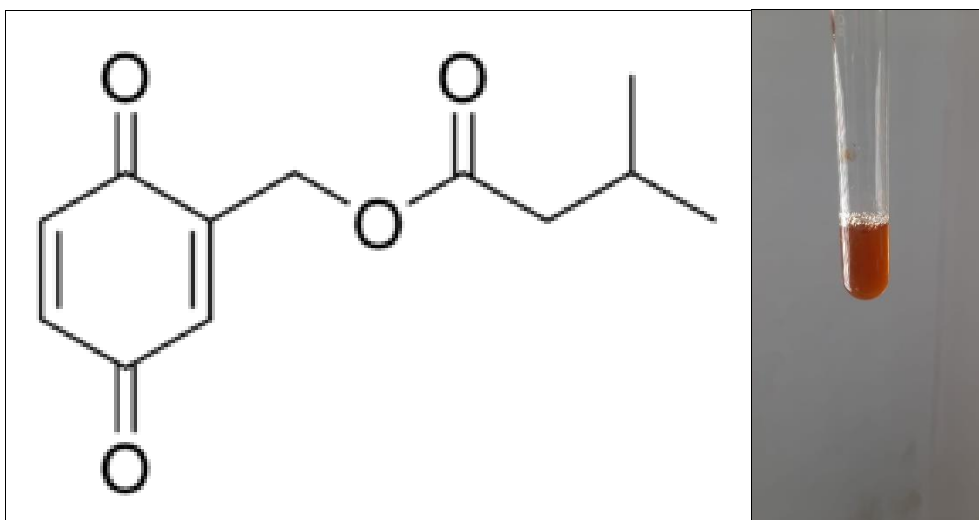


Fig 10: Quinones

Test for basic radicals:

1) Test for Calcium:

To 2ml of herbal extract, 2ml of 4% ammonium oxide

solution was added.

No appearance of white precipitate was observed. Thus, absence of calcium.



Fig 11: Calcium

Test for Magnesium:

2) To 2ml of herbal extract, drops of sodium hydroxide

solution was added. White precipitate was not observed indicates the absence of magnesium.



Fig 12: Magnesium

3) Test for Ammonium

To 2ml of herbal extract few ml of Nessler's reagent and

excess of sodium hydroxide solution are added for the appearance of grey color. Indicates presence of ammonium.

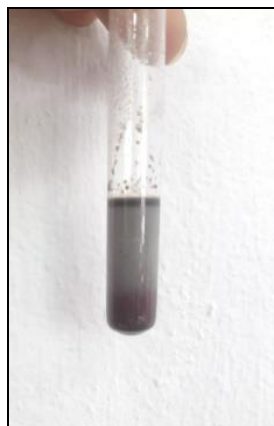


Fig 13: Ammonium

4) Test for Sodium

Hydrochloric acid was added with a pinch of sweet flag, made as paste and introduced into the blue flame of Bunsen burner. Intense yellow color flame was not observed, indicates absence of sodium.

5) Test for Iron

The herbal extract was treated with Conc. HNO₃ and ammonium thiocyanate. Appearance of blood red color, indicates presence of iron.

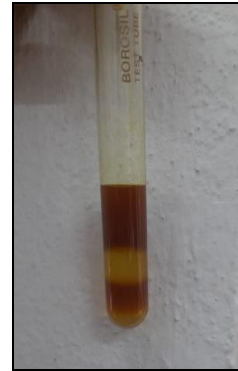


Fig 16: Lead

6) Test for Zinc:

To 2 ml of the Herbal extract drops of sodium hydroxide solution was added. White precipitate was not formed, indicates absence of zinc.



Fig 14: Zinc

7) Test for Aluminum

To the 2ml of the herbal extract sodium hydroxide was added in drops. Appearance of white precipitate formation, indicates presence of Aluminum.



Fig 15: Aluminum

8) Test for Lead

To 2 ml of SPC extract 2ml of potassium iodide solution was added. No formation of yellow color precipitate was not observed, indicates absence of lead .

9) Test for Copper

- a. A pinch of SWEET FLAG was made into a paste with con. HCl in a watch glass and introduced into the non-luminous part of the flame. Blue color flame was not observed.

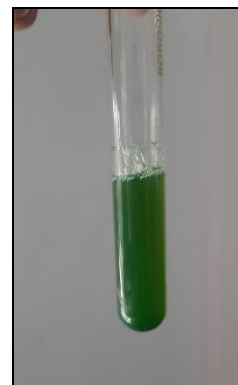


Fig 17: Copper

- b. To 2 ml of herbal extract excess of ammonia solution was added and appearance of green colored precipitate. Indicates absence of copper.

Test for Mercury

To 2ml of the herbal extract sodium hydroxide solution was added and yellow precipitate was not formed, indicates absence of Mercury.



Fig 18: Mercury

10) Test for Arsenic

To 2 ml of the herbal extract 2ml of sodium hydroxide solution was added and no precipitate formation was noted. Indicates absence of arsenic.



Fig 19: Arsenic

The herbal extract was treated with ammonium molybdate and conc. HNO₃, stand for an hour. The no appearance of yellow precipitate indicates absence of phosphate.



Fig 21: Phosphate

Test for acid radicals

1. Test for Sulphate

To 2 ml of the herbal extract 5% of barium chloride solution was added, no precipitate was observed, indicates absence of Sulphate.



Fig 20: Sulphate

2. Test for Carbonate

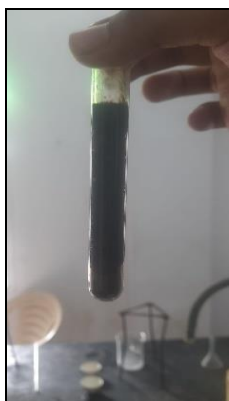
The herbal extract was treated with concentrated HCl and observed, appearance of effervescence, indicates presence of carbonates.



Test for Phosphate

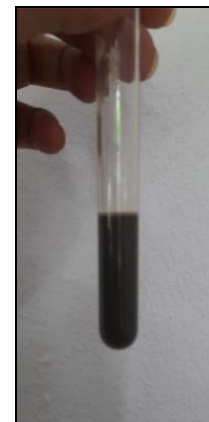
3. Test for Fluoride & Oxalate

To 2ml of herbal extract 2ml of dil.acetic acid and 2ml calcium chloride solution was added and heated. No Cloudy appearance was not seen, indicates absence of flavonoids.



4. Test for Nitrate

To 1 gm of the sweet flag , copper turnings was added and again conc.H₂SO₄ was added, heated and the test tube was tilted vertically down and black color solution with dense white fumes were observed. Indicates absence of nitrates.



Result and Discussion

Phytochemicals are natural bioactive compounds, found in plants and fibers, which act as a defense system against diseases and more accurately protect against diseases. The phytochemical analysis reveals that the presence of carbohydrates, Saponins, phytosterols, diterpenes, Triterpenes, Flavonoids and Quinones. Listed in table 1.

Table 1: Phytochemicals screening tests

S. No	Phytochemicals	Test conducted	Observation	Result
1.	Carbohydrates	a. Molisch test	Appearance of violet ring between 2 layers.	Positive
		b. Benedicts test	Appearance of orange red precipitate.	Positive
2.	Glycosides	Legal's test	Blood red color solution.	Positive
3.	Saponins	Froth test	Presence of foam	Positive
4.	Phenolic compounds	Alcoholic ferric chloride test	No appearance of bluish green color.	Negative
5.	Phytosterols	Ferric chloride- acetic acid test	Reddish pink color solution	Positive
6.	Diterpenes	Copper acetate test	Emerald green color solution	Positive
7.	Triterpenes	Salkowski's test	Golden yellow color solution	Positive
8.	Flavonoids	Alkaline reagent test	Dark yellow color solution	Positive
		Xanthoproteic test	No formation of yellow precipitate.	Negative
		Millon's test	No formation of white precipitate	Negative
9.	Proteins	Biuret test	No formation of brick red precipitate	Negative
		Sodium hydroxide	Red orange color solution	Positive

The sample contains ammonium, Iron, Aluminum, Carbonate. These trace quantities of minerals play an important role in the functioning of various enzymes in biological systems and have immunomodulatory functions

and thus influence the susceptibility to the course and the outcome of a variety of viral infections. Listed in table 2 and 3.

Table 2: Results of basic radicals studies

S. No	Parameter	Observation	Result
1.	Test for Calcium	No change in solution	Negative
2.	Test for Magnesium	Pale green color solution	Negative
3.	Test for Ammonium	Appearance of grey color	Positive
4.	Test for Sodium	No intense yellow color flame	Negative
5.	Test for Iron	Appearance of blood red color	Positive
6.	Test for Zinc	No precipitate formation	Negative
7.	Test for Aluminum	Appearance of white precipitate	Positive
8.	Test for Lead	No precipitate formation	Negative
9.	Test for Copper	Green color precipitate	Negative
10.	Test for Mercury	No formation of precipitate	Negative
11.	Test for Arsenic	Formation of brown precipitate.	Negative

Table 3: Results of acid radicals studies

S. No	Parameter	Observation	Result
1.	Test for Sulphate	No formation of white precipitate	Negative
2.	Test for Phosphate	No formation of yellow precipitate	Negative
3.	Test for Carbonate	Appearance of effervescence	Positive
4.	Test for Fluoride and Oxalate	No cloudy appearance	Negative
5.	Test for Nitrate	Black color solution with dense fumes	Negative

Many Phytochemicals such as Tannins, Flavonoids, Alkaloids, Saponins, Phenols and inorganic constituents such as Nitrate, Ammonium, Phosphate, Chloride ions were found. This Medicine is Used for the treatment of Nerve pain, and it also has other Neuropathic functions. This drug also helps in strengthening nerves.

Conclusion:

Thus, the preliminary analysis of "SWEET FLAG" drug will give fingerprint to clinical studies. The present investigation deals with the evolution of phytochemicals present in SWEET FLAG, which reflects its pharmacological and therapeutic actions. Tannins, Flavonoids, Phenols are good antioxidants with anti-diarrheal activity and prevent Oxidative stress related disorders. Our study finding support the use of SWEET FLAG; a unani herbal medicine in the treatment of nerve related disorders without producing any side effects as it is free from toxic elements such as Arsenic, copper and Mercury. Our present study indicates the presence of Tannins, Saponins, Flavonoids, diterpenes, carbonates, Phosphate etc.

Conflict of Interest

Not available

Financial Support

Not available

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