Article Type: Research Article
J Name: Modern Phytomorphology
Short name: Modern Phytomorphology
ISSN: ISSN 2226-3063/eISSN 2227-9555
Year: 2023
Volume: 17
Page numbers: 23-36
DOI: 10.5281/zenodo.7939670
(10.5281/zenodo.Year-Volume-PDFNo.)
Short Title: Phytochemical analysis of Unani herbal formulation "Niswani tea"

RESEARCH ARTICLE

Phytochemical analysis of Unani herbal formulation "Niswani tea"

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Received: 23.01.2023, Manuscript No.: mp-23-88996| **Editor Assigned:** 26.01.2023, Pre-QC No. mp-23-88996(PQ) | **Reviewed:** 29.01.2023, QC No. mp-23-88996(Q) | **Revised:** 03.02.2023, Manuscript No. mp-23-88996(R) | Accepted:05.02.2023 | **Published:** 15.02.2023

Abstract

Phytochemicals are bioactive compounds obtained from the plants and are widely applied in the traditional unani herbal medicine. Herbal medicine drugs have been used widely in many countries because of its availability, less cost effectiveness and safer than the synthetic drug. These herbal medicines are used by the local people to cure the various diseases which include the major diseases such as Diabetes Mellitus, Cancer, HIV etc. knowledge of phytochemicals constituents present in the unani herbal drugs will support in treating the disease with better therapeutic efficacy. Realizing this, and effort has been made for the screening of various phytochemicals such as coumarins, anthocyanins, leucoanthocyanins, fatty acids, steroids, saponins, terpenoids, quinones, tannins, phlobatannins, phenolic compounds, flavonoids, alkaloids and determination of ash content are present in Niswani tea, unani herbal medicine. It was concluded that the plants studied were rich in phytochemicals with significant pharmacological and medicinal applications.

Keywords: *Phytochemicals, Unani Herbal Medicine, Drug, Therapeutic, Blood Pressure, Heart care, Capsules, Flavonoid, Saponins, Tannins.*

Introduction

Phytochemicals are bioactive molecules that are also referred to as secondary metabolites that are derived from plants. Primary metabolites and Secondary metabolites are the two types of metabolites generated in plants (Bansode T.S. et al., 2015). Primary metabolites are required for a plant's normal metabolism including growth and development. They may be unnecessary for secondary metabolites generated by plants. These are produced in nearly every part of the plant including the bark, leaves, stem, root, flower, fruits and seeds. Phytochemicals have been utilized as traditional herbal medicine for some years now 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 (Ugochukwu S.C. et al., 2013). These are also frequently utilized in the agricultural sector. Drugs, flavouring agents, perfumes, color, pigments, insecticides 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 [Hussain M.S et al., 2012].

Following are the commercial importance of some phytochemicals

1. Pesticides such as nicotine, pyrethrins, and rotenone are used in small amounts (Balandrin M.F et al., 1985).
2. Tannins are commonly used as an astringent (Ashok P.K et al., 2012).
3. Quinones, such as hypericin, are antibacterial (Tiwari P et al., 2011).
4. Secondary metabolites are used to investigate diverse metabolic processes using pharmacological techniques. Diterpene esters for example, formed from the latices of certain Euphorbia species are potent irritants and co-carcinogens, making them important in chemical carcinogenesis investigations (Balandrin M.F et al., 1985; Hecker E et al. 1977).

Secondary metabolites have aroused a lot of interest in their research production and they're being investigated extensively as a source of medicinal compounds (Gracelin D.H et al., 2013). As a result, evaluating phytochemicals from various different Unani Herbal Medicine is qualitatively screened for phytochemicals from various different Unani Herbal Medicine extracts appeared to be important. In the present study, Unani Herbal medicine is qualitatively screened for phytochemicals using standard chemical tests.

Secondary metabolites such as saponins, tannins and flavonoids have been discovered to have antidiabetic, anti-inflammatory, hepatoprotective, anti-hyperlipidemia, diuretic, and antibacterial properties (Modi D.C et al., 2010). The Unani Herbal Medicine Niswani Tea used in the production of medicines for a number of infectious diseases including chronic ulcers, leucorrhoea, pyorrhea and fungal infections of the skin. It is good for girl's/women care formulated with ashoka and kalongi Berg-e-Heena and Gull-e-Nilofer, it is very effective caffeine free herbal remedies for Heart Care and Blood pressure. The barks, seeds and flowers of the tree are helpful in preparing capsules and tonics to solve various gynecological problems of women. It was found that flavonoid is present abundantly in all species. The objective of the present study was to screen such a phytochemicals analysis (Gul R et al., 2017).

Materials and Methods

Drug Material

The required Niswani Tea Unani Herbal Medicine is a product of Halal Herbal Remedies Hyderabad 500005 Telangana, India.

Preparation of Unani Herbal Medicine Extract (Niswani Tea)

The herbal medicine extract of Niswani tea is prepared using the hot water extraction technique. The powder extract of Niswani Tea was kept in an appropriately labelled plastic bottle. 5gm of powder extract was weighed using an electronic weighing balance, dissolved in a 25 ml of sterile water and then boiled at 50°60° for 30 minutes on water bath. The extract was filtered through Whatman No.1 filter paper and centrifuged the filtrate at 2500 rpm for 15 minutes. For further investigation of phytochemical analyses, the extract was kept in sterile bottle at 4-8°C in refrigerator (Savithramma N et al., 2011)Fig.1(A-E).

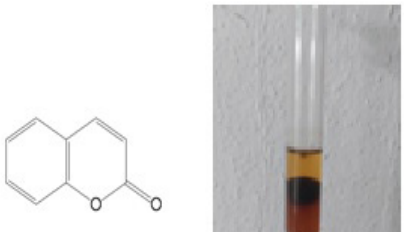

Figure 1: (A)Medicine extract (B) Concentration of extract (C) Filtration (D) Centrifugation (E) Collection of filtrate

Phytochemical analysis

Preliminary qualitative screening for phytochemicals, of Unani herbal medicine Niswani Tea was carried out with the following test methods.

Test for Coumarins

2 ml of extract was treated with 3 ml of 10% NaOH. The formation of yellow colour indicating the presence of coumarins [11]. The presence of lactone is a key structural motif of coumarins which are hydrolysed once attacked by a strong nucleophile like NaoH into water-soluble salt of cis-cinnamic acid derivatives. Acidification of these salt results in a restoration of the original coumarins (Lopez-Castillo N.N et al., 2013) (Fig.2).

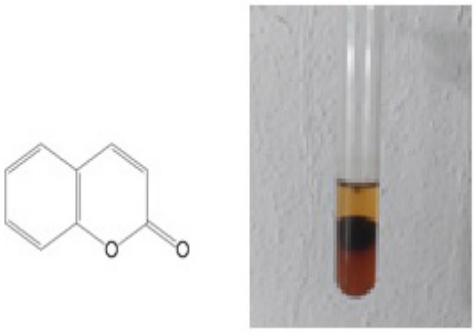

Figure 2: 2H-chromen-2-one

Test for Anthocyanins

2 ml of extract was treated with 2 ml of 2N hydrochloric acid and ammonia was added to it. The appearance of pink-red colour turning blue-violet. This indicates the presence of anthocyanin [11]. Anthocyanin molecules will change their color depending upon the PH of their environment does it may serve as a PH indicator. The anthocyanin turns red to pink in acid (pH 16), (Fig.3) Reddish- purple in neutral solutions (pH 7) and green in alkaline or basic solution (pH 8-14) (Fossen et al., 1998).

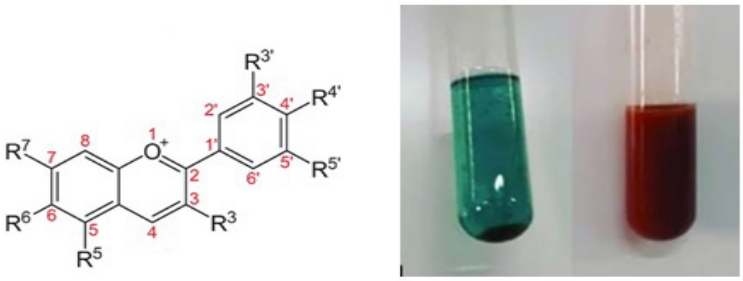

Figure 3: 3-galactoside

Test for Leucoanthocyanins

5 ml of extract was allowed to react with 5 ml of isoamyl alcohol. Appearance of upper layer red in colour indicates the presence of leucoanthocyanins [11]. They have no color of their own, but in acidic environment and at elevated temperature they are converted to colored anthocyanidis. This reaction is completion with condensation to a dimeric leucoanthocyanidi (Daneel F et al., 1999) (Fig.4).

Figure 4: 2-Phenyl-3,4-dihydro-2H-1-benzopyran-3,4-diol

Test for Fatty acids

0.5 ml of extract was added to 5 ml of ether and allowed it to evaporate on filter paper. Then the filter paper was dried and the appearance of transparency on filter paper confirms the presence of fatty acids [11]. The presence of these fatty acids in a considerable amount might serve to recognize the potential pharmacological importance of this plant in disease control (Aziz S et al., 2019) (Fig.5).

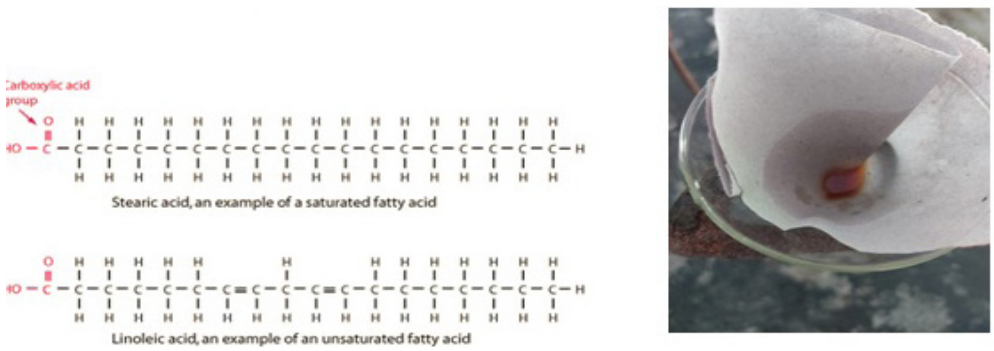

Figure 5 Test for Steroids

Test for Steroids

Libermann-Burchard Test

1 ml of extract was dissolved in 10 ml of chloroform. To this mixture equal volume of concentrated sulfuric acid was added by sides of the test tube. The upper layer becomes red while lower layer of sulfuric acid turns yellow in colour with green fluorescence indicating the presence of steroids (Savithramma N. at al., 2011). Reactions of Steroids with acetic a hydride and sulphuric the

libermann-Burchard Test R. It is then used to classify patient as having mild, classical or severe slows. The Libermann-Burchard or test anhydride testis used for the detection of cholesterol (Cook RP et al., 1961) (Fig.6).

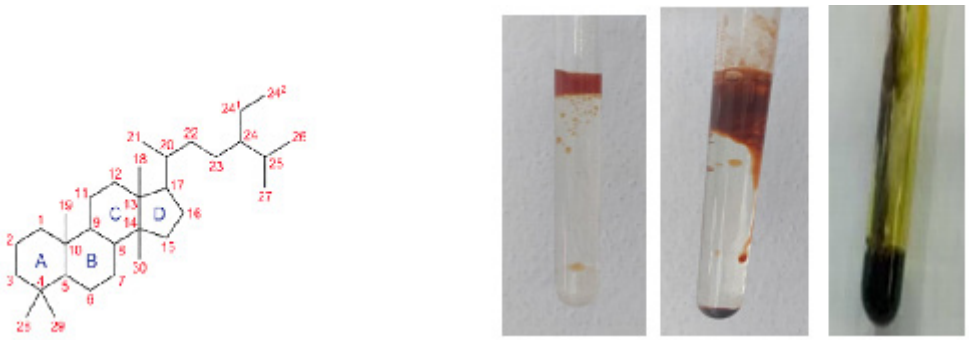

Figure. 6: **24-ethyl-lanostane**

Test for Saponins

Foam test

2 ml of extract was taken in a test tube and 6 ml of distilled water was added to it. The mixture was then shaken vigorously. The persistence of foam was observed that indicates the presence of saponins (Savithramma N. at al., 2011). According to research saponins present in a given plant attribute form a soapy foaming substance when mixed with water persistent foam tests in acidic solutions, as well as blood hemolysis test are done to see the presence of saponins in a given mixture, as well as their capability to generate hemolysis (Hanz W et al., 2017) (Fig.7).

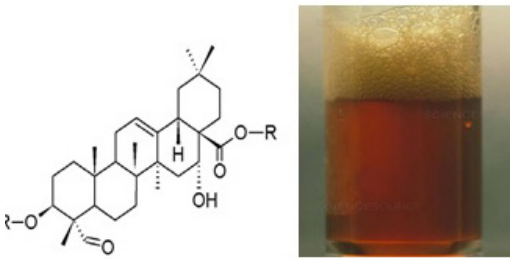

Figure 7: **Triterpene glycosides**

Test for Terpenoids

Salkowski test

2 ml of extract was treated with 2 ml of acetic anhydride. Few drops of concentrated sulfuric acid was then added to this solution and observed the formation of blue, green rings that indicates the presence of terpenoids (Savithramma N et al., 2011). Terpenoids can also be classified according to the number of cyclic structure they contain. The salkowski test can be used to identify the presence of terpenoids. At the least those containing an alcohol functional group, often arise by hydrolysis of carbocationic intermediates produced from geranyl Pyrophosphate (14 December ,2022) (Fig.8).

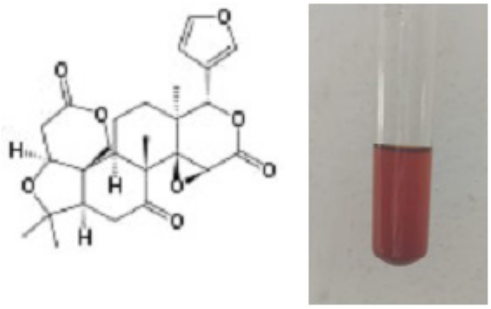

Figure 8: **7,16-Dioxo-7,16-dideoxylimondiol**

Test for Quinones

1 ml of extract was added to the 2 ml of dilute NaOH. Formation of blue green or red coloration confirms the presence of quinones (Soni A et al., 2013).On dilution a red color indophenol is formed which turns to deep blue color sodium salt solution of indophenol on treatment with sodium hydroxide(Test for Phenolic Group., byjus.com) (Fig.9).

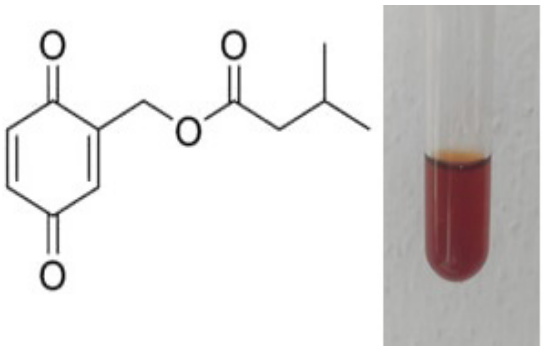

Figure 9: 1,4-benzoquinone or cyclohexadienedione

Test for Tannins

Braymer's test

2 ml of extract was allowed to react with 10% alcoholic ferric chloride solution. Formation of blue or greenish colour of the solution was observed. This was the indication of the presence of the tannins (Soni A. et al., 2013). Hydrolysable tannins give bluish or precipitate and condensed tannins brownish to green ones. If the test is carried on and extract the contents both type of tannins, a blue color is produced which changed to olive green as more ferric chloride is added (Fig.10).

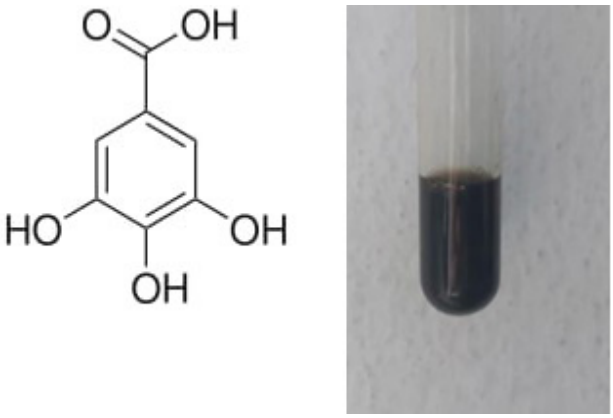

Figure 10: Hydrolyzable tannins

Test for Phlobatannins

Precipitate test

About 2 ml of extract was added to 2 ml of 1% aqueous hydrochloric acid and the mixture was boiled. Deposition of a red precipitate confirmed the presence of phlobatannins (Soni A et al., 2013). Phlobatannis of precipitate test are found to be present in the least concentrations (Nathenial S et al., 2019) (Fig.11).

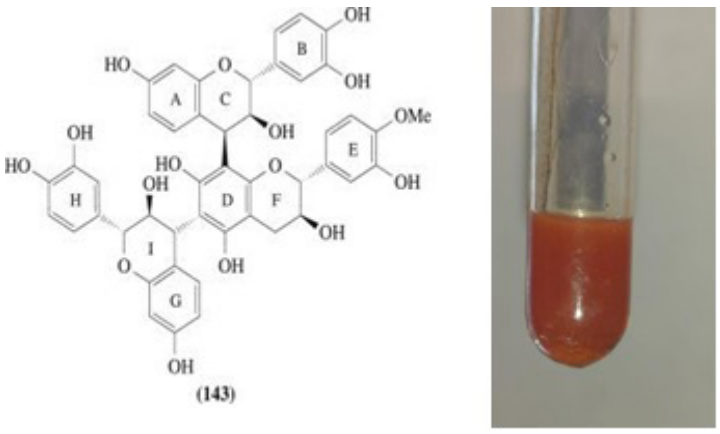

Figure 11: Tetrafucol A

Test for Phenolic Compounds

(Ferric chloride test)

Few drops of the extract were treated with 5% aqueous ferric chloride. Formation of deep blue or black colour indicates the presences of phenolic compounds (Shah P et al., 2014). To detect the presence of a phenol functional group in a given sample. This test is based on the fact that the phenols a coloured complex with neutral ferric chloride solution (Ferric C. ccsu.edu., 2013) (Fig.12).

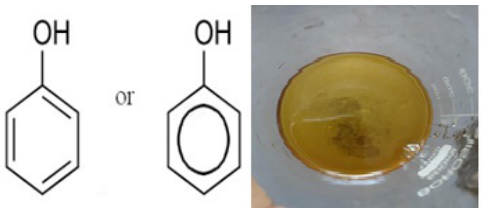

Figure 12: Benzenol

Test for Flavonoids

Alkaline reagent test

2 ml of extract was treated with few drops of 1N sodium hydroxide solution and observed the formation of intense yellow colour. This yellow colour becomes colorless on addition of dilute hydrochloric acid, indicating the presence of flavonoids (Shah P et al., 2014). NaoH is completely ionic containing sodium cations and hydroxide anions. It is a sufficiently strong base which deprotonates phenol entirely. The purpose of 5% NaoH in this test was to deprotonates the polyphenolic molecules contained in flavonoids(Fig.13).

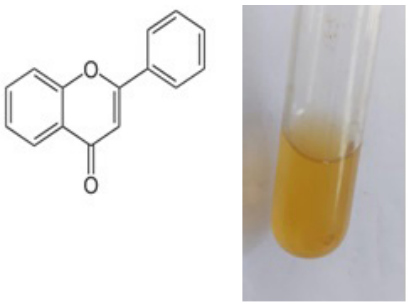

Figure 13: 2-phenyl-1,4-benzopyrone

Test for Alkaloids

Mayer's Test

2 ml of extract was treated with 2 drops of Mayer's reagent. Presence of white creamy precipitate indicates the positive test (Shah P et al., 2014). Most alkaloids are precipitated from neutral or slightly acidic solution by Mayer's reagent (potentiomercuric iodide solution) to give a cream colored precipitate (Mayer's. US Pharmacopeia. ,2012) (Fig.14).

Figure 14: phenethylamine alkaloid

Determination of Ash content

2 g of each plant sample was taken and weighed accurately in a clean silica dish. The dish was first heated over a low burner flame. After that the dish is transferred to a muffle furnace maintained at 300°C-450°C for 15 min. The ash residue obtained was then cooled in desiccator and weighed (Motegaonkar M. B et al., 2012) (Fig.15A-15C). The percentage of total ash content was calculated by the formula:

Total Ash Percent of plant sample (%) = [Weight of dry ash residue (g) ÷ Weight of plant sample (g)] x 100 = 1.57/2 x 100 = 78.5%.

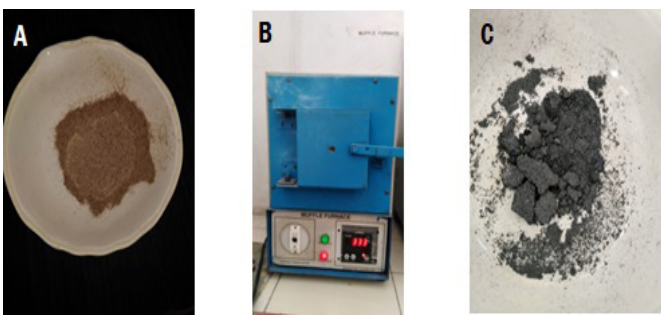

Figure 15: A. Unani herbal Medicine (B) Muffle furnace (C) Ash residue

Determination of Ash Values

Total Ash

3g of the test drug was accurately weighed and incinerated in a crucible dish at a temperature not exceeding 450°C until it was free from carbon. It was then cooled and weighed. The % w/w of ash with reference to the air-dried powder was calculated.

Water Soluble Ash

The total ash was obtained as the above method for preparation of total ash. The ash was boiled with 25 ml of water for 5mins. The insoluble ashes were collected using filter paper. It was then washed with hot water and transferred to the silica crucible. It was then ignited for 15minutes at temperature not exceeding 450°C. For determination of weight of the water soluble ash the silica crucible and residue were weighed until constant weight was attained. The weight of the water soluble ash was determined by subtracting the weight of insoluble ash from the weight of total ash is 1.0 – 0.91 = 0.09(Fig.16A-16C).

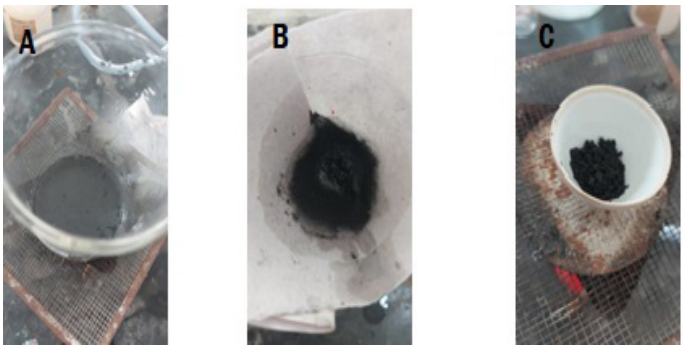

Figure 16: **(A) Boiling ash residue (B) Filtration (C) Collection of filtrate**

Acid insoluble Ash:

The total ash was obtained as the above method for preparation of total ash. The ash was boiled for 5 minutes with 25ml 10% Hcl. The insoluble ashes were collected using filter paper and washed with hot water. It was then transferred to the silica crucible and ignited for 15minutes at temperature not exceeding 4500C. The silica crucible and residue were weighed until constant weight is 1.09 – 0.39 = 0.61 (Fig.17).

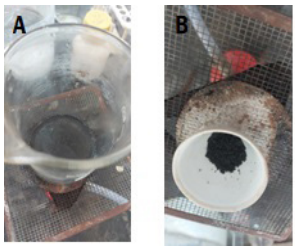

Figure 17: **(A) Boiling ash residue (B) Collection of filtrate**

Determination of Extractive Value

Alcohol Soluble Extractive Value

3g of test drug powder was weighed and macerated with 100ml of ethanol in a closed container for 24 hours. The resulting solution was shaken continuously for 6 hours. It was then allowed to stand and soak for 18 hours(Fig.18A-18C).

The solution was filtered and evaporated of the filtrate in a flat bottomed shallow dish and dried at 105°C. Then the content was cooled and weighed up to 1.34.

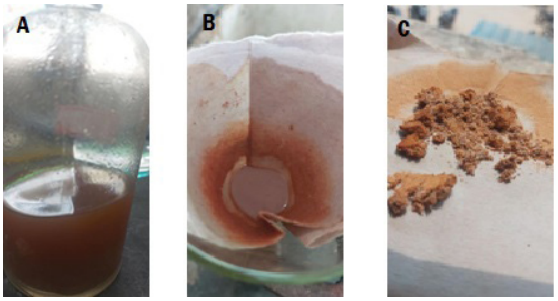

Figure 18: **(A) Concentration of extract (B) Filtration (C) Collection of filtrate**

Water soluble Extractive value:

3g of test drug powder was weighed and macerated with chloroform and water, respectively, at 80°C for 24 hrs. The resulting

solution was shaken continuously for 6 hours and allowed to stand and soak for 24hrs then filtered. The solution from both chloroform and water respectively was filtered and evaporated of the filtrate in a flat bottomed shallow dish. It was dried at 105°C then cooled and weighed up to 0.33 (Fig.19A-19C).

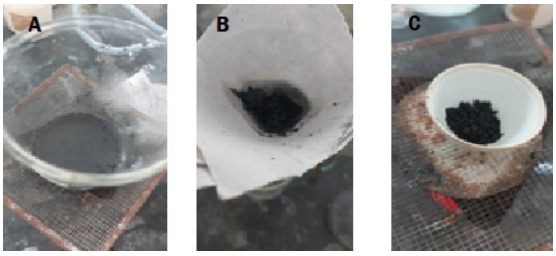

Figure 19: (A) Boiling ash residue (B) Filtration (C) Collection of filtrate

Loss on Drying

The powdered drug was taken and dried in the oven at 100- 105°C to constant weight. The result was noted.

Physical characterization

Solubility: A little of the sample was shaken well with distilled water. A little of the sample was shaken well with Conc. Hcl and Conc. H_2SO_4. Sparingly soluble character indicates the presence of Silicate (Fig.20A-20B).

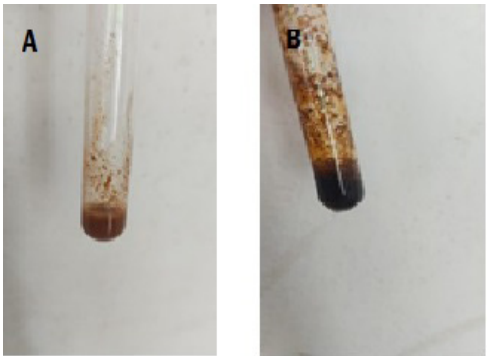

Figure 20. Sample with Conc HCL and Con H_2SO_4.

Action on heat: A small amount of the sample was taken in a dry test tube and heated gently. If there was a strong white fumes evolving it indicates the presence of Carbonate (Fig.21).

Figure 21: Action on heat

Flame test: A small amount of the sample was made into a paste with con. Hcl in a watch glass. It was then introduced into non-luminous part of the Bunsen flame. Appearance of bluish green flame indicates the presence of Copper (Fig.22).

Figure 22: Flame test

Ash Test: A filter paper was soaked into a mixture of sample and cobalt nitrate solution. It was then introduced into the Bunsen flame and ignited. Appearance of yellow colour flame indicates the presence of Sodium (Fig.23).

Figure 23: Ash Test

Bio-chemical analysis

Preliminary Basic and Acidic radical studies

Preparation of extract: 10 g of sample was taken in a 250 ml of clean beaker and 50 ml of distilled water was added to it. Then it was boiled well for about 10 mins. Then it was allowed to cool and filtered in a 100 ml volumetric flask and made up to 100 ml with distilled water. This preparation was used for the qualitative analysis of acidic/ basic radicals and biochemical constituents in it (Fig.24).

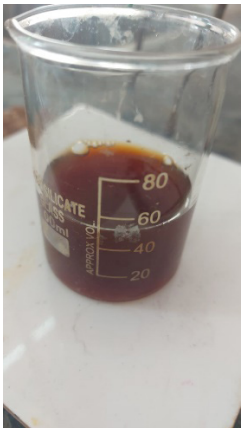

Figure 24: sample was taken in a 250 ml

Test for Basic radicals:

Test for Potassium

To a pinch of the SPC 2 ml of sodium nitrate and 2 ml of cobalt nitrate solution in 30% glacial acetic acid was added and observed for the presence of yellow precipitate(Fig.25).

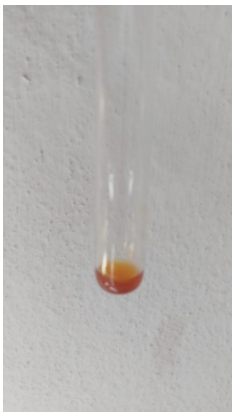

Figure 25: Test for Potassium

Test for Ammonium

To 2ml of SPC extract few ml of Nessler's reagent and excess of sodium hydroxide solution are added for the appearance of

brown colour (Fig.26).

Figure 26: **Test for Ammonium**

Test for Iron (Ferrous)

The SPC extract was treated with Conc. HNO_3 and ammonium thiocyanate and waited for the appearance of blood red colour(Fig.27).

Figure 27: **Test for Iron (Ferrous)**

Test for Zinc

To 2 ml of the SPC extract drops of sodium hydroxide solution was added and observed for white precipitate formation(Fig.28).

Figure 28: **Test for Zinc**

Test for Lead:

To 2 ml of SPC extract 2ml of potassium iodide solution was added and noted for yellow colored precipitate(Fig.29).

Figure 29: **Test for Lead**

Test for Copper:

A pinch of SPC was made into a paste with con. Hcl in a watch glass and introduced into the non-luminous part of the flame and noted for blue colour appearance(Fig.30).

Figure 30: Test for Copper

Test for Mercury:

To 2m1 of the SPC extract sodium hydroxide solution was added and noted for yellow precipitate formation(Fig.31).

Figure 31: Test for Mercury

Test for Sulphate:

To 2 ml of the SPC extract 5% of barium chloride solution was added and observed for the appearance of white precipitate (Fig.32).

Figure 32: Test for Sulphate

Test for Chloride:

The SPC extract was treated with silver nitrate solution and observed for the appearance of white precipitate (Fig.33).

Figure 33: Test for Chloride

Test for Phosphate:

The SPC extract was treated with ammonium molybdate and conc. HNO_3 and observed for the appearance of yellow precipitate(Fig.34).

Figure 34: Test for Phosphate

Test for Carbonate:

The SPC extract was treated with conc. Hcl and observed fourth appearance of effervescence (Fig. 35).

Figure 35: Test for Carbonate

Test for Fluoride & Oxalate:

To 2ml of SPC extract 2ml of dil.acetic acid and 2ml calcium chloride solution was added and heated and watched for cloudy appearance(Fig. 36).

Figure 36: Test for Fluoride & Oxalate

Test for Nitrate:

To 1 gm of the SPC, copper turnings was added and again conc.H_2SO_4 was added, heated and the test tube was tilted vertically down and observed for yellowish red colour(FIG. 37).

Figure 37: Test for Nitrate

RESULTS AND DISCUSSION

The phytochemical constituents and % ash content of unani herbal medicine Niswani Tea were analyzed. It was discovered to have a significant proportion of key for phytochemicals that can be recognized by tests of quality. In our analysis it was cleared that the Niswani Tea is rich in alkaloids, flavonoids, saponins, tannins, fatty acids, quinones, coumarins, leucoanthocyanins, steroids etc., The important thing is that all plant samples contain one common and abundant secondary metabolite, flavonoid. From the literature survey it was found that flavonoids have wide range of biological properties such as anti-inflammatory, antibacterial, antiviral, anti-allergic, cytotoxic antitumor properties. It is used in the treatment of neurodegenerative diseases and has vasodilatory action. It is also reported that flavonoids involved in inhibition of lipid peroxidation, platelet aggregation, capillary permeability and fragility, cyclo-oxygenase and lipoxygenase enzyme activities etc. Flavonoids are also known to inhibit variety of enzymes like hydrolases, hyaluronidases, alkaline phosphatases, arylsulphatases, cAMP phosphodiesterases, lipase, ᵅglucosidase and kinases. According to the literature, it also has been found that quercetin, plays an important role in diabetes. It leads to the regeneration of pancreatic islets and increases insulin release. It also stimulates Ca^{2+} (Sandhar H.K et al., 2011). Uptake from isolated islet cells which is helpful in non-insulin dependent diabetes.

Alkaloids and phenolic compounds along with hypoglycemic (Tiong S. H et al., 2013 ; Maiti D et al., 2012), antidiabetic properties also exhibit anti-inflammatory, antimicrobial and antioxidant effects (Al-Shahwany A. W et al., 2014). Moreover, saponins exhibit various biological activities like it gives a permeability to the cell membrane, helpful in lowering the serum cholesterol levels, it possesses abortifacient properties, it has immunomodulatory property, it has cytotoxic effects on malignant tumor cells and is involved in synergistic enhancement of the toxicity of immunotoxins (Thakur M et al., 2011). Saponins also show antidiabetic property (Zheng T et al., 2012).

Coumarins, a major class of flavonoids, possesses pharmacological properties like antidiabetic, antioxidant, hepato-protective, anticoagulant, antimicrobial, anti-inflammatory, Analgesic, antioxidant, anticancer, antiviral, antimalarial activities etc (Sahoo S. S et al., 2012). Tannins are reported to have a cardio-protective, anti-inflammatory, anticarcinogenic and antimutagenic properties. Tannins are also involved in treatment of noninsulin dependent diabetes mellitus by enhancing the glucose uptake and inhibiting abiogenesis (Kumari M et al., 2012). The most striking feature about quinones is its pharmacological properties that makes it different from other secondary metabolites. It inhibits HIV 1 reverse transcript (Valderrama J. A et al.,2003) and shows antitumor and immunomodulatory activities. It also has antimicrobial, anticancer, antiviral and antibacterial properties (Masih D et al., 2019). The main purpose to calculate the total ash content is to measure the total amount of minerals present in that plant samples. Among the four plants tested for total ash content, Niswani Tea shows highest percentage of the ash i.e. it contains 78.5% of ash indicating high amount of minerals in it. Minerals are used as coenzymes and cofactors in the biochemical processes. Therefore, it is necessary to evaluate the mineral values in the extract.

The results of our investigation proved the presences of various phytochemicals such as coumarins, anthocyanins, leucoanthocyanins, fatty acids, steroids, saponins, terpenoids, quinones, tannins, phlobatannins, phenolic compounds, flavonoids, alkaloids and determination of ash content in the Niswani Tea which may be responsible for pharmacological action.

This medicine is used in the treatment of several health related problems in the production of medicines for a number of infectious diseases, including chronic ulcers, leucorrhoea, pyorrhea and fungal infections of the skin. It is good for girl’s/women care, formulated with ashoka and kalongi Berg-e-Heena and Gull-e-Nilofer, it is very effective caffeine free herbal remedies for Heart Care and Blood pressure.

As herbal medicine is more affordable than conventional medicine, natural healing, strength in immune system, fewer side effects, cost effective, stabilizes hormones, metabolism, easier to obtain than prescription medicine.

Conclusion

The unani herbal medicine researched were determined to be high in phytochemicals are full of pharmacological and therapeutic value. Flavonoid is abundant in almost all plant species investigated in making it the most abundant secondary metabolites. Further research is needed to determine their potential in the biological qualities listed above, such as antidiabetic and anti-tumor properties among others. Saponins and tannins are also present in almost all species studied. It was concluded that the herbal medicine extraction was rich in phytochemicals with significant pharmacological and medicinal applications. Utilizing knowledge of phytochemical constituents present in unani herbal medicine drugs and its consideration in therapeutics can improve the patient care and can contribute further in global requirement of traditional health care facility by Unani herbal Medicine.

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