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# Phytochemical analysis of unani herbal formulation "Mazher-Ul-Ajaib"

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**Abstract**
Phytochemicals are bioactive compounds obtained from the plants and are widely applied in the traditional unani herbal medicine. Herbal drugs have been used widely in many countries because of its availability, less cost effectiveness and safer than the synthetic drugs. These Herbal Medicines are used to cure the various diseases which include the major diseases such as Diabetes Mellitus, Cancer and HIV etc. knowledge of phytochemical constituents present in unani herbal drugs will support in treating the disease with better therapeutic efficacy [10]. Realising this, an 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 Mazher-Ul-Ajaib, unani herbal medicine. Our studies revealed that extract of Mazher-Ul-Ajaib is rich in phytochemicals with significant pharmacological and medicinal applications.

**Keywords:** Phytochemicals, unani medicine, drug, therapeutic, PCOD, irregular menstruation, migraine & headache, flavonoid, saponins, tannins, steroids, physic–chemical analysis, phytochemical analysis

## 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 by plants [1]. 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 utilised 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 [2]. These are also frequently utilised in the agricultural sector. Drugs, flavouring 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 [3].

### Following are the commercial importance of some phytochemicals
Nicotine, pyrethrins, and rotenone are employed as pesticides in small amounts [4]. Tannins are used as astringents in most cases [5]. Quinones, such as hypericin have antibacterial properties [6]. Pharmacological tools are utilised to examine various metabolic processes using secondary metabolites. Diterpene esters produced from the latices of certain Euphorbia species are considered as a powerful irritants and co-carcinogens and making them significant in chemical carcinogenesis studies [4, 7].

The growing commercial relevance of secondary metabolites has acquired a great interest in analysis as well as production of these natural products and is extensively investigated as a source of medicinal agents [8]. As a result, evaluating 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-hyperlipidemic, diuretic and antibacterial effects [9].

The unani herbal medicine Mazher-Ul-Ajaib which is selected for phytochemical screening in our study is used to cure a variety of infectious illnesses including Polycystic Ovary Syndrome (PCOD), Irregular Menstruation, Glaucoma & all Eye Diseases, Vision Improvement, Thyroid, Migraine & Headache, Carpal Tunnel Syndrome, Pilonidal Cyst, Plantar Fasciitis & Heel Spur, Treating Bell's Palsy/CVA (Faalij).
Herbal drugs have been used widely in many countries because of its availability, less cost effectiveness and safer than the synthetic drugs.
Then the Phytochemical screening, Biochemical and Physicochemical parameters of Mazher-Ul-Ajaib, a unani formulation have been carried out in the present work.

## Materials and Methods

### Drug Material

The required Mazher-Ul-Ajaib Unani Herbal Medicine is a product of Halal Herbal Remedies Hyderabad 500005 Telangana, India.

### Preparation of Unani Herbal Medicine Extract (Mazher-Ul-Ajaib):

The herbal medicine extract of Mazher-Ul-Ajaib is prepared using the hot water extraction technique. The powder extract of Mazher-Ul-Ajaib 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 °C 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 [11].

**Fig 1:** Medicine extract

**Fig 2:** Concentration of extract

**Fig 3:** Filtration


**Fig 4:** Centrifugation


**Fig 5:** Collection of filtrate

### Phytochemical analysis

Preliminary qualitative screening for phytochemicals, of unani herbal medicine Mazher-Ul-Ajaib 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 hydrolyzes once attacked by a strong nucleophile like NaOH into water-soluble salts of cis-cinnamic acid derivatives. Acidification of these salts results in a restoration of the original coumarins [12].

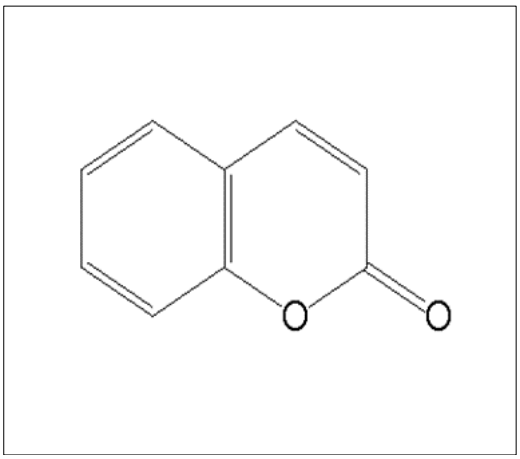

**Fig 6**

### 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 which then turns to blue-violet indicates the presence of anthocyanins [11]. Anthocyanin molecules will change their colour depending upon the pH of their environment thus it may serve as a pH indicator. The anthocyanin turns red-pink

in acids (pH 1-6), reddish-purple in neutral solutions (pH 7) and green in alkaline or basic solutions (pH 8-14) [13].

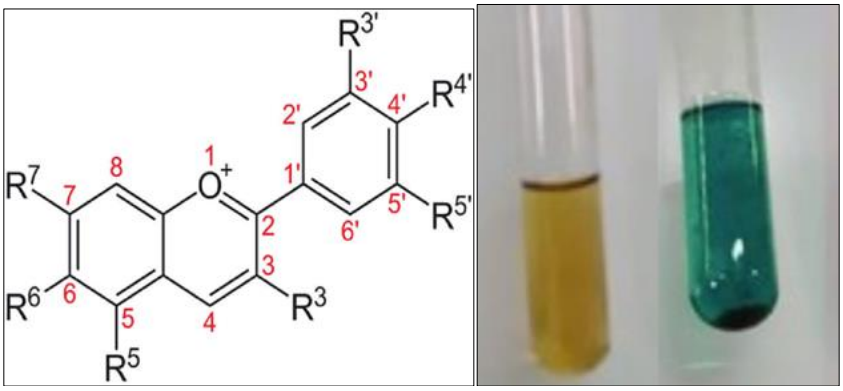


**Fig 7**

***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 colour of their own, but in acidic environments and at elevated temperatures they are converted to colored anthocyanidins. This reaction is in competition with the condensation to a dimeric leucoanthocyanidin [14].

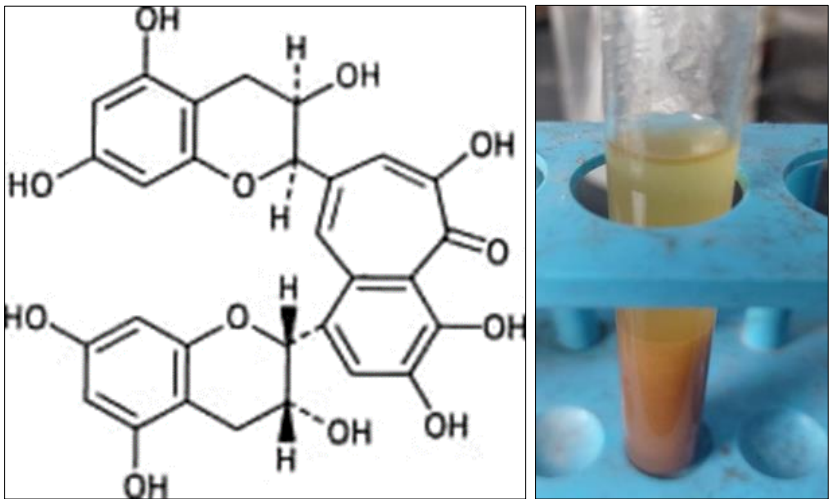


**Fig 8**

**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 herbal medicine in disease control [15].


**Fig 9**

**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 [11]. Reactions of steroids with sulphuric acid, the Libermann-Burchard test

reaction. It is then used to classify patients as having mild, classical or severe. The Libermann-Burchard is used for the detection of cholesterol [16].

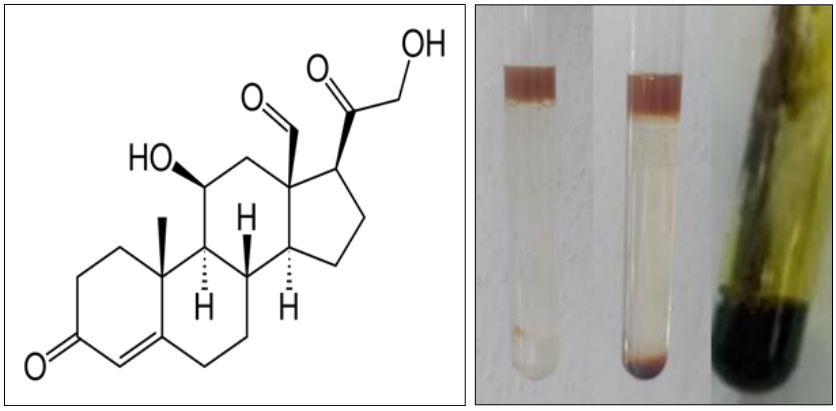


**Fig 10**

**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 which indicates the presence of saponins [11]. Persistent foam tests in acidic solutions, as well as blood haemolysis tests are done to see the presence of saponins in a given mixture, as well as their capability to generate haemolysis [17].

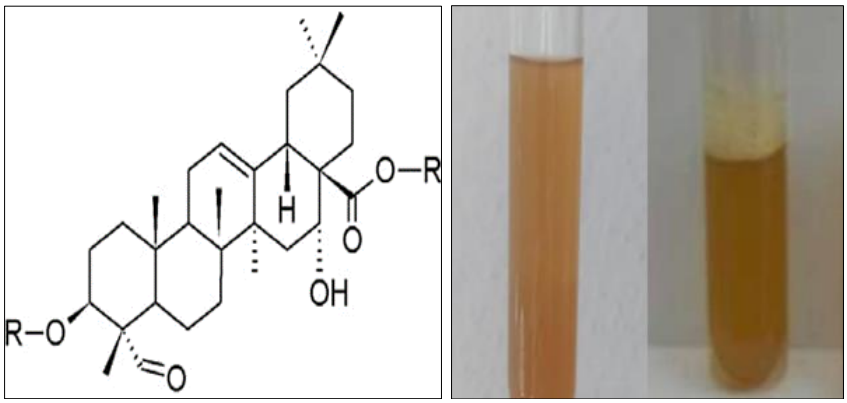


**Fig 11**

**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 [11]. Terpenoids can also be classified according to the number of cyclic structures they contain. The Salkowski test can be used to identify the presence of terpenoids**.** Terpenoids, at least those containing an alcohol functional group, often arise by hydrolysis of carbocationic intermediates produced from geranyl pyrophosphate [18].

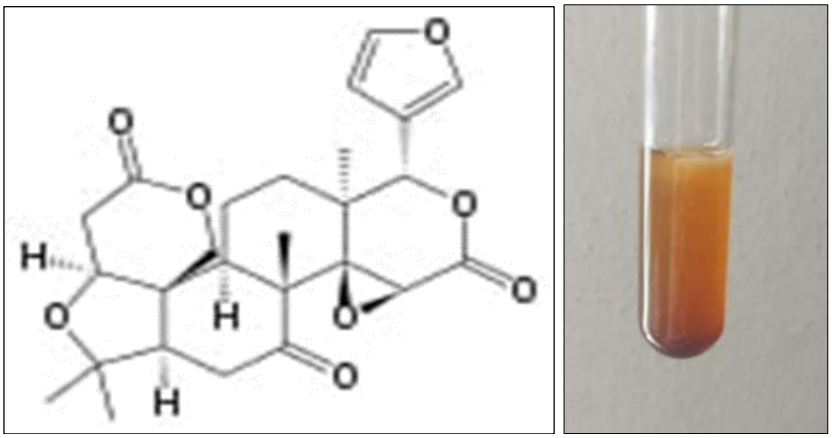


**Fig 12**

**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 [19]. On dilution a red colour indophenol is formed which turns to deep blue colour sodium salt solution of indophenol on treatment with sodium hydroxide [20].

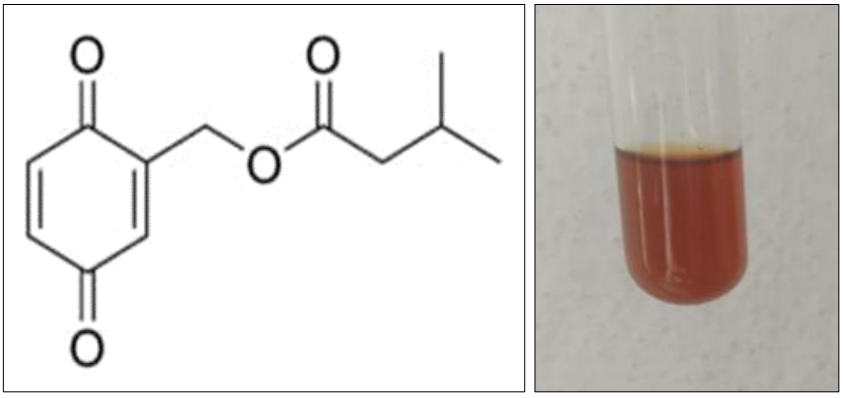

**Fig 13**

**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 tannins [19]. Hydrolysable tannins (galitannins and ellagitannins) give bluish-black colour or precipitate and condensed tannins brownish-green ones. If the test is carried on an extract produced a blue colour which changes to olive-green as more ferric chloride is added.

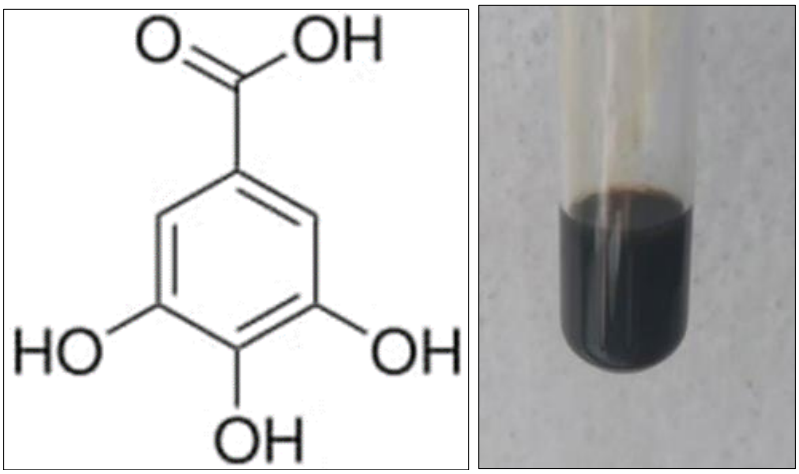

**Fig 14**

**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 [19]. Phlobatannins of precipitate test are found to be present in least concentrations [21].

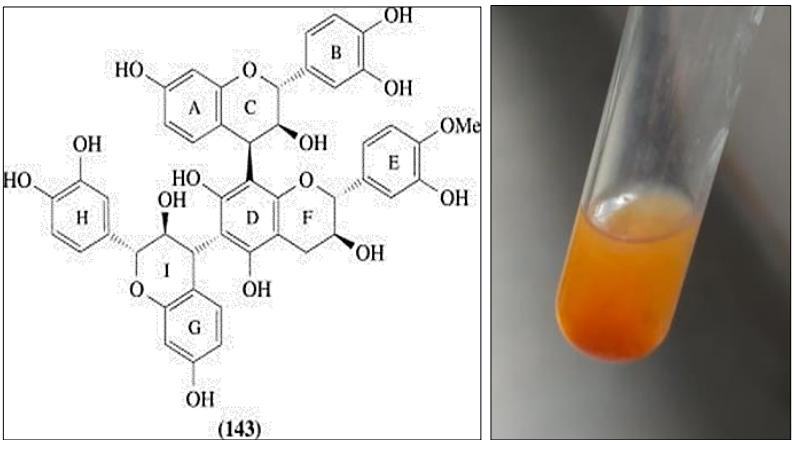


**Fig 15**

**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 [22]. To detect the presence of a phenol functional group in a given sample**.** This test is based on the fact that the phenols give a colored complex with neutral ferric chloride solution [23].

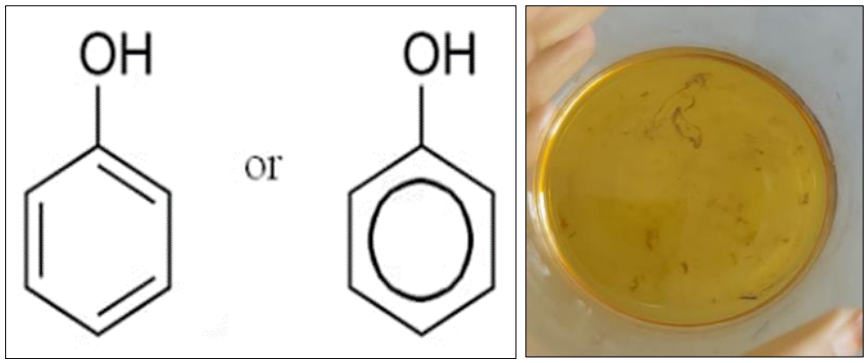


**Fig 16**

**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 colourless on addition of dilute hydrochloric acid, indicating the presence of flavonoids [22]. NaOH is completely ionic containing sodium cations and hydroxide anions. It is a sufficiently strong base which deprotonates phenol entirely. The purpose of NaOH in this test was to deprotonate the polyphenolic molecules contained in flavonoids [24].

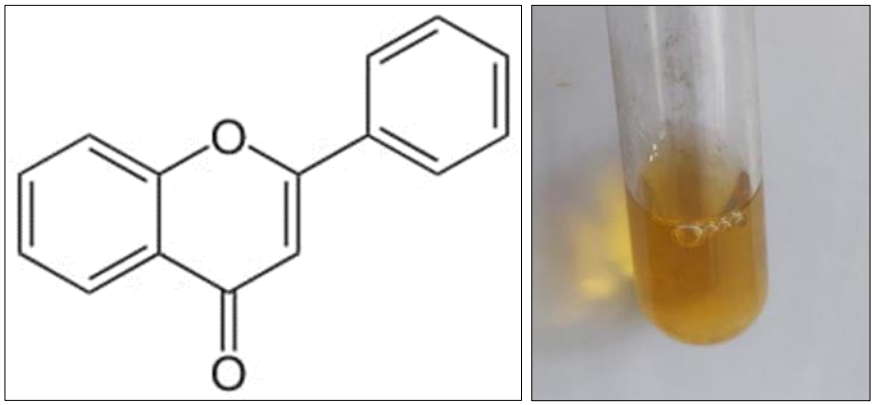


**Fig 17**

**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 [22]. Most alkaloids are precipitated from neutral or slightly acidic solution by Mayer's reagent (potassiomercuric iodide solution) to give a cream coloured precipitate [25].

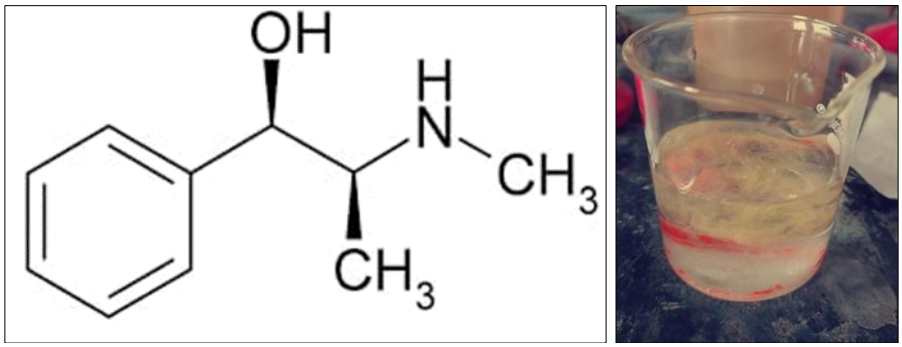


**Fig 18**

**Determination of Ash content**
2 g of Unani Herbal Medicine 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 $300^0$C-$450^0$C for 20 minutes. The ash residue obtained was then cooled in desiccator and weighed [26]. The percentage of total ash content was calculated by the formula as follows:

Total Ash Percent of plant sample (%) = [Weight of dry ash residue (g) ÷ Weight of plant sample (g)] x 100 = [1 / 2] x 100 = 50%.

**Fig 19:** Unani herbal Medicine

**Fig 20:** Muffle furnance

**Fig 21:** Ash residue

**Physicochemical analysis**

Physicochemical studies of the trial drug have been done.

**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 450ºC 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 25ml 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 ºC. 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. 1.57 - 0.31 = 1.26.

**Fig 22:** Boiling ash residue

**Fig 23:** Filtration

**Fig 24:** 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 5minutes 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 450 ºC. The silica crucible and residue were weighed until constant weight was attained. 1.57 – 0.71 = 0.86.



**Fig 25:** Boiling ash residue **Fig 26:** Filtration **Fig 27:** 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.

The solution was filtered and evaporated of the filtrate in a flat-bottomed shallow dish and dried at 105ºC. Then the content was cooled and weighed upto 1.22g


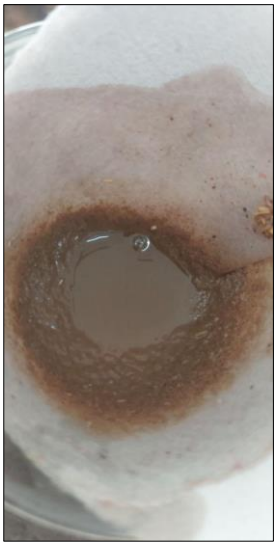


**Fig 28:** Concentration of extract **Fig 29:** Filtration **Fig 30:** Collection of filtrate

**Water soluble Extractive value**

3g of test drug powder was weighed and macerated with chloroform and water, respectively, at 80 ºC 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 ºC then cooled and weighed upto 1.10 g.



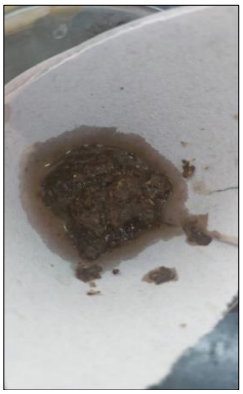


**Fig 31:** Concentraation of extract **Fig 32:** Bolied – 80 °C **Fig 33:** Filtration **Fig 34:** Collection of filtrate

**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 con Hcl and Con $H2SO_4$. Sparingly soluble character indicates the presence of Silicate.

**Fig 35**

**Fig 36**

**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 fume evolving it indicates the presence of Carbonate.

**Fig 37**

**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.

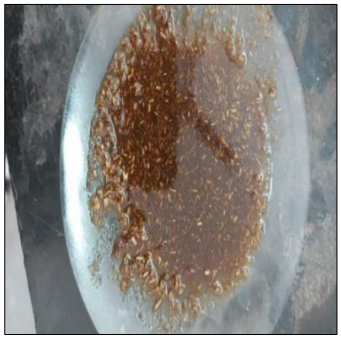

**Fig 38**

**Fig 39**

**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 40**

**Bio-chemical analysis**
**Preliminary Basic and Acidic radical studies** [8]
**Preparation of extract**
10g 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.

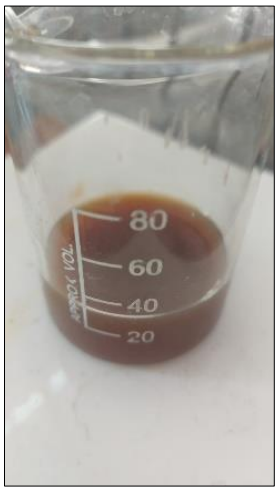


**Fig 41**

**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 42**

**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.

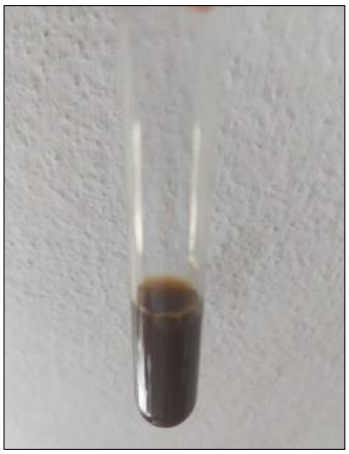

**Fig 43**

**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 44**

**Test for Zinc**

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

**Fig 45**

**Test for Lead:**

To 2 ml of *SPC* extract 2ml of potassium iodide solution was added and noted for yellow coloured precipitate**.**

**Fig 46**

**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 47**

**Test for Mercury:**

To 2m1 of the *SPC* extract sodium hydroxide solution was added and noted for yellow precipitate formation.

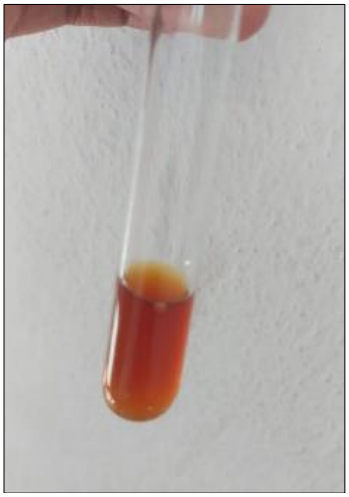

**Fig 48**

**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 49**

**Test for Chloride**
The *SPC* extract was treated with silver nitrate solution and observed for the appearance of white precipitate.

**Fig 50**

**Test for Phosphate:**
The *SPC* extract was treated with ammonium molybdate and conc. $HNO_3$ and observed for the appearance of yellow precipitate.

**Fig 51**

**Test for Carbonate**
The *SPC* extract was treated with conc.Hcl and observed fourth appearance of effervescence.

**Fig 52**

**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 53**

**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 54**

## Results and Discussion

The phytochemical constituents and % ash content of unani herbal medicine Mazher-Ul-Ajaib were analysed. It was discovered to have a significant proportion of key for phytochemicals that can be recognised by tests of quality. In our analysis it was cleared that the Mazher-Ul-Ajaib is rich in alkaloids, flavonoids, saponins, tannins, fatty acids, quinones, coumarins, leucoanthocyanins, steroids etc., According to a literature review flavonoid were discovered to have a wide range of biological activities including anti-inflammatory, antibacterial, antiviral, anti-allergic, cytotoxic and anticancer properties. It has a vasodilatory function and is used to treat neurological disorders. Flavonoids have also been linked to lipid peroxidation, platelet aggregation, capillary permeability and fragility, cyclo-oxygenase, lipoxygenase enzyme activity and other processes. Hydrolases, hyaluronidases, alkaline phosphatases, arylsulphatases, cAMP phosphodiesterases, lipase, -glucosidase and kinases are all known to be inhibited by flavonoids. According to research, quercetin also has a role in diabetes. It promotes pancreatic islets and increases insulin secretion. It also promotes the uptake of Ca2+ by isolated islet cells, which is beneficial in non-insulin-dependent diabetes [27].

Alkaloids and phenolic compounds have anti-inflammatory, antibacterial, and antioxidant [30] capabilities in addition to hypoglycaemic [28, 29] and antidiabetic qualities. Saponins also have a variety of biological activities such as increasing cell membrane permeability, lowering serum cholesterol levels, abortifacient properties, immunomodulatory properties, cytotoxic effects on malignant tumour cells and being involved in the synergistic enhancement of the toxicity of immunotoxins [31]. Saponins have anti-diabetic properties [32]. Coumarins, a type of flavonoids have pharmacological effects such as anti-diabetic, antioxidant, hepatoprotective, anticoagulant, antibacterial, anti-inflammatory, analgesic, antioxidant, anticancer, antiviral and antimalarial [33].

Tannins have been shown to be cardio protective, anti-inflammatory, anti- carcinogenic and anti-mutagenic. There are also used to treat non-insulin-dependent diabetes mellitus by increasing glucose absorption and decreasing abiogenesis [34]. Quinones has pharmacological activities which set them apart from other secondary metabolites. It has anticancer, immunomodulatory properties [35] as well as inhibiting HIV 1 reverse transcriptase. Antimicrobial, anticancer, antiviral and antibacterial activities are also present in it [36].

Calculating the total ash content, Mazher-Ul-Ajaib shows % of the ash i.e. it content 50% of ash indicating high amount of minerals in it. Minerals are used as coenzymes and cofactors in the biochemical processes.

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 Mazher-Ul-Ajaib which may be responsible for pharmacological action.

This medicine is used in the treatment of several health related problems such as Polycystic Ovary Syndrome (PCOD), Irregular Menstruation, Glaucoma & all Eye Diseases, Vision Improvement, Thyroid, Migraine & Headache, Carpal Tunnel Syndrome, Pilonidal Cyst, Plantar Fasciitis & Heel Spur, Treating Bell's Palsy/CVA (Faalij).

As herbal medicine are 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.

Through this method Organoleptic characters, pH, Ash (%), Acid Insoluble ash (%), Water soluble ash (%), Loss on drying (%), Solubility, Action on heat, Flame test, Ash test and biochemical compound shows the presence or absence of this constituents. The Phytochemical screening of the extract gives an idea nature of chemical substances in it.

## 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-tumour properties among others. Saponins and tannins are also present in almost all species studied. It was concluded that the herbal medicine extraction were 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.

## References

1. https://www.researchgate.net/publication/281690290_Phytochemical_analysis_of_some_selected_Indian_medicinal_plants
2. Ugochukwu SC, Arukwe UI, Onuoha I. Preliminary phytochemical screening of different solvent extracts of stem bark and roots of Dennetia tripetala G. Baker. Asian J Plant Sci. Res. 2013;3(3):10-13.
3. Hussain MS, Fareed S, Ansari S, Rahman MR, Ahmad IZ, Saeed M. Current approaches toward production of secondary plant metabolites. J Pharm Bioallied Sci. 2012;4(1):10-20.
4. Balandrin MF, Klocke JA, Wurtele ES, Bollinger WH. Natural plant chemicals: Sources of industrial and medicinal materials. Sci. 1985;228(4704):1154-60.
5. Ashok PK, Upadhyaya K. Tannins are Astringent. J Pharm. Phyto. 2012;1(3):45-50.

6. Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical screening and Extraction: A Review. Int. pharma. Sci. 2011;1(1):98-106.
7. Hecker E, Kinghorn AD. in Toxic Plants, A.D. Kinghorn, Ed., Pure Appl. Chem. 1977;49:1243.
8. Gracelin DHS, Britto AJD, Kumar PBJR. Qualitative and quantitative analysis of phytochemicals in five *Pteris* species. Int J Pharm Pharm Sci. 2013;5(1):105-107.
9. Modi DC, Patel JK, Shah BN, Nayak BS. Pharmacognostic studies of the seed of *Syzygium cumini* linn. Int. J Pharm. Sci. 2010;1(1):20-26.
10. (PDF) Phytochemical Constituents in Unani drugs and its consideration in therapeutics (researchgate.net)
11. Savithramma N, Linga Rao M, Suhrulatha D. Screening of Medicinal Plants for Secondary Metabolites. Middle-East J Sci. Res. 2011;8(3):579-584.
12. Effect of the sodium hydroxide concentration. | Download Scientific Diagram (researchgate.net)
13. Anthocyanin acid base indicator - Google Search
14. How are leucoanthocyanidins converted to colored compounds? - Bing
15. Analysis of fatty acid and determination of total protein and phytochemical content of Cassia sophera Linn leaf, stem, flower, and seed | Beni-Suef University Journal of Basic and Applied Sciences | Full Text (springeropen.com)
16. Liebermann Burchard Test PDF (epsscentral.info)
17. Test for Saponins | Hunker
18. Salkowski test is used for terpenoids why ? – Bing
19. Soni A, Sosa S. Phytochemical Analysis and Free Radical Scavenging Potential of Herbal and Medicinal Plant Extracts. J Pharma. Phytochem. 2013;2(4):22-29.
20. Test for Phenolic Group - Chemistry Practicals Class 12 (byjus.com)
21. 8-5-294-941.pdf (phytojournal.com)
22. Shah P, Modi HA, Shukla MD, Lahiri SK. Preliminary Phytochemical Analysis and Antibacterial Activity of Ganoderma lucidum collected from Dang District of Gujarat, India. Int. J Curr. Microbiol. App. Sci. 2014;3(3):246-255.
23. Test for Phenolic Group Practical Experiment - Theory, Procedure and Observation (vedantu.com)
24. Tests For Flavonoids | PDF | Sodium Hydroxide | Alkene (scribd.com)
25. Mayer's reagent – Wikipedia
26. Motegaonkar MB, Salunke SD. The Ash and Iron Content of Common Vegetable Grown in Latur District, India. Res. J Recent. Sci. 2012;1(4):60-63.
27. Sandhar HK, Kumar B, Prasher S, Tiwari P, Salhan M, Sharma P. A Review of Phytochemistry and Pharmacology of Flavonoids. Int. Pharma. Sci. 2011;1(1):25-41.
28. Tiong SH, Looi CY, Hazni H, Arya A, Paydar M, Wong WF, *et al*. Antidiabetic and Antioxidant Properties of Alkaloids from *Catharanthus roseus* (L.) G. Don. Mol. 2013;18(8):9770-9784.
29. Maiti D, Majumdar M. Impact of bioprocessing on phenolic content and antioxidant activity of soy seed to improve hypoglycemic functionality. Asian J. Plant Sci. Res. 2012;2(2):102-109.
30. Al-Shahwany AW. Alkaloids and Phenolic Compound Activity of *Piper Nigrum* against Some Human Pathogenic Bacteria. Biomed. Biotech. 2014;2(1):20-28.
31. Thakur M, Melzig MF, Fuchs H, Weng A. Chemistry and pharmacological of saponins: special focus on cyctotoxic properties. Botanics: Targets and Therapy. 2011;1:19-29.
32. Zheng T, Shu G, Yang Z, Mo S, Zhao Y, Mei Z. Antidiabetic effect of total saponins from *Entada phaseoloides* (L.) Merr. in type 2 diabetic rats. J Ethnopharmacol. 2012;139(3):814-821.
33. Sahoo SS, Shukla S, Nandy S, Sahoo HB. Synthesis of novel coumarin derivatives and its biological evaluations. Euro. J. Exp. Bio. 2012;2(4):899-908.
34. Kumari M, Jain S. Tannins: An Antinutrient with Positive Effect to Manage Diabetes. Res. J Recent Sci. 2012;1(12):70-73.
35. Valderrama JA, Benites J, Cortes M, Pessoa-Mahana H, Prina E, Fournet A. Studies on Quinones. Part 38: synthesis and leishmanicidal activity of sesquiterpene 1, 4-Quinones. Bioorg. Med. Chem. 3. 2003;11(22):4713-4718.
36. http://www.phytojournal.com (journal of pharmacognosy and phytochemical)