

## Phytochemical evaluation of Fever Five (Muntingia Calabura), A Unani polyherbal medicine.

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### ABSTRACT:

Phytochemicals are plant-derived bioactive compounds that are widely used in traditional Unani herbal medicine. Herbal medicine is also known as botanical medicine. Herbalism, herbology, phytotherapy is the oldest form of health care known to mankind. Plant parts such as leaves, bark, flowers, roots, fruits, and seeds are used to make these herbs. Plant-based drugs are widely available, less expensive, safe, and efficient with few side effects. The various Phytochemicals present in fever five were investigated using standard procedures, our study confirms the presence of phytochemicals such as Tannins, Saponins, Flavonoids, Diterpenes, Triterpenes and carbohydrates. The results are listed in table 1. In the present work we have also investigated the different types of minerals present in this medicine. This medicine is found to be rich in carbonate, nitrate, phosphate and ammonium ions. The result of mineral analysis is given in table 2. Results for the analysis of some phytochemicals such as phenolic compounds, phytosterols, proteins and phenols are found to be negative, which confirms the absence of the mentioned phytochemicals. Our analysis of this unani medicine further shows strong evidence for the absence of metal ions like copper, aluminium, zinc and magnesium. The acid radicals such as sulphate, phosphate, carbonate, fluoride and oxalate are not found in this medicine whereas the nitrate ion is present in this fever five unani medicine. This herbal medicine fever five is rich in phytochemicals, biochemicals and minerals which shows its medicinal significance.

**KEYWORDS:** Phytochemicals, Phytochemical Screening, Cold fever, Solubility, Fever Five extract.

### I. INTRODUCTION:

The creation of herbal-based medications is just one of the many uses for which natural resources can be put to use. Herbal medicine is also called phytomedicine or phytotherapy. The World Health Organization (WHO) recognizes that herbalism is common to cultures of the world and

actively promotes the development of what it calls traditional medicine. Often referred to as secondary metabolites, phytochemicals are bioactive compounds that are obtained from plants. Natural components used in traditional medicine have been taken by many people all over the world for a long time. Moreover, 85% of the constituents in traditional medicines are plant extracts, which are used by 80% of the people in impoverished nations worldwide as a health supplement. Modern medicine makes use of many plant-derived compounds as the basis for evidence-based pharmaceutical drugs. FEVER FIVE is one of the plant-based unani medicines used in the treatment of cold and high fever. Meanwhile, the Peruvians have used the flowers and stem as an antiseptic to treat prostate gland swelling, headaches, and fever. Several studies have shown that extracts of fever five had a variety of tested activities, including antioxidant, anticancer, antidiabetic, antibacterial, and anti-inflammatory properties [13]. Recognizing the importance of herbal medicine in the treatment of various diseases, we have chosen FEVER FIVE, a unani medicine, for the analysis of its various phytochemical constituents and minerals.

### II. MATERIALS AND METHODS:

#### PREPARATION OF EXTRACT:

The extract of fever five is prepared using the hot water extraction technique. 5 gm of fever five powder was weighed using an electronic weighing machine and dissolved in 150 ml of distilled water, and boiled for 3 hours on a water bath. The extract was filtered using Whatman no. 1 filter paper. The extracted filtered solution was kept in a sterile bottle in a refrigerator for future use in analysis.

#### PHYTOCHEMICAL ANALYSIS:

The phytochemical analysis of the extract provides broad insights into the chemical constituents found in the crude drug. The phytochemical tests were carried out using the methods shown.

**Test for Alkaloids:**

A small portion of solvent free extracts were stirred separately with few drops of dil. hydrochloric acid and filtered & tested carefully with various alkaloid reagents.

**Mayer's Reagent:**

Addition of Mayer's reagent shows the absence of cream precipitate which indicated the absence of alkaloid

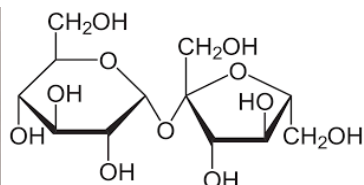
(1.35 HgCl<sub>2</sub>, 5g of KI<sub>2</sub>)

**Wagner's Reagent:**

No reddish-brown precipitate is found when Wagner's reagent is added to the extract. Wagner's reagent indicates the absence of alkaloids (2.5g I, 12.5g KI)

**Test of Carbohydrates:****A) Molisch's test:**

In a test tube, 1ml of herbal extract was treated with 2-3 drops of 1% alcoholic alpha naphthol, followed by 2ml of H<sub>2</sub>SO<sub>4</sub>. Carbohydrates were detected by the appearance of a violet ring between the two layers.

**Test for reducing sugars****A) Benedict's test:**

1ml of herbal extract was treated with 2-3 drops of Benedict's reagent and treated strongly. No orange-red precipitate indicates absence of reducing sugars.

**B) Fehling's test:**

1ml of herbal extract was treated with equal volume of Fehling's solution A and B and heated gently. No orange-red precipitate indicates the absence of reducing sugars.

**Test for Glycosides:**

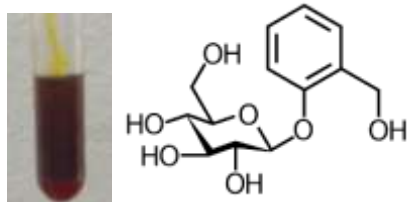
The extract was hydrolyzed with dil. HCl and subjected to test for glycosides.

**A) Modified Bontrager's test:**

1ml of ferric chloride solution was added to the hydrolysed extract and immersed in boiling water for about 5 minutes. The mixture was cooled before being extracted with equal parts of benzene. The benzene layer was separated and treated with ammonia solution using a separating funnel. In the ammoniacal layer, no pink color is formed. This confirms the absence of Anthranol glycoside.

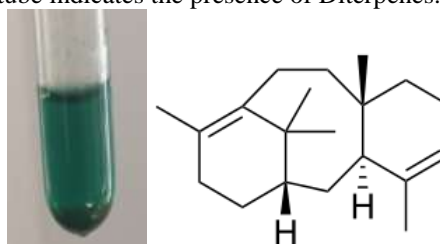
**B) Legal's test:**

The hydrolysed extract was treated with 1ml of sodium nitroprusside in pyridine and 1ml of sodium hydroxide. The formation of blood red color, indicates the presence of cardiac glycosides.



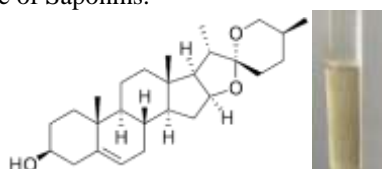
**Test for Diterpenes:  
Copper acetate test:**

1ml of the fever five 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.



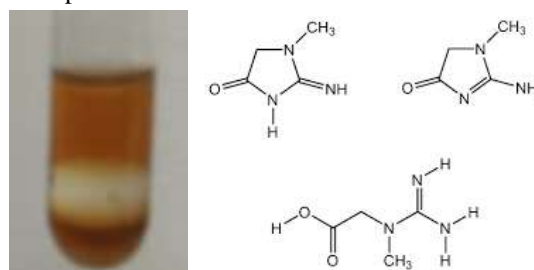
**Test for Saponins:**

In a test tube, 2ml of drug was mixed with 5ml of distilled water. The test tube was vigorously shaken. The formation of foam indicates the presence of Saponins.



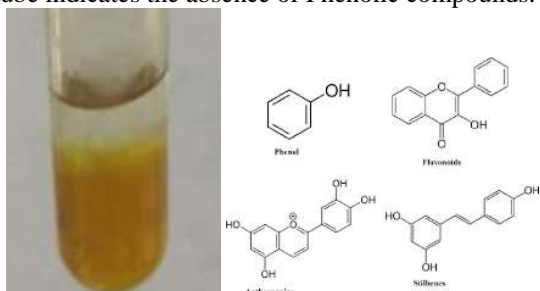
**Test for Triterpenes:  
Salkowski's test:**

1ml of 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 shows the presence of Triterpenes.



**Test for Phenolic Compounds:**

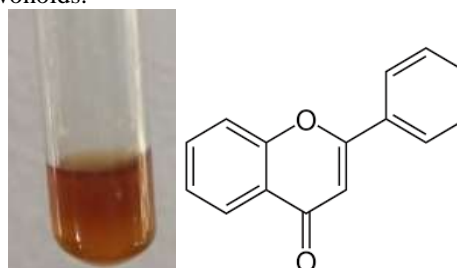
0.5ml filtrated Extract of fever five was treated with 1ml alcoholic ferric chloride in a test tube. No appearance of bluish green color solution in the test tube indicates the absence of Phenolic compounds.



**Test For Flavonoids:**

**A) Alkaline reagent test:**

To 1ml of fever five extract, 1ml of 10% sodium hydroxide solution was added. Formation of dark yellow color confirms the presence of Flavonoids.



**Test for phytosterols:**

**Ferric chloride –acetic acid test:**

1ml of fever five extract was treated with 1ml of chloroform, followed by 2ml of ferric chloride-acetic acid reagent and 1ml of concentrated sulphuric acid. No Appearance of reddish pink color, confirms the absence of phytosterols



**b) Lead Acetate test:**

To 1ml of fever five extract 3-4 drops of 10% lead acetate solution was added. Formation of yellow precipitate indicates the presence of flavonoids.



**c) Ferric chloride test:**

To 1ml of Herbal extract 3-4 drops of Ferric chloride solution was added. Formation of dark green color solution Indicates the presence 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 the absence of proteins.



**B) Millon's test:**

To 0.5ml of herbal extract, 2.5ml of millon's reagent was added. Formation of white precipitate indicates presence of proteins.



**Test for amino acids**

**C) Biuret test:**

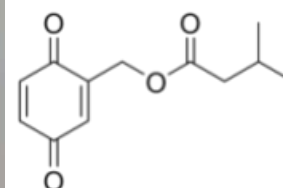
0.5ml of extract, 2.5ml of dilute biuret reagent was added. No Appearance of brick red precipitate indicates the absence of free amino acids.



**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.



**Test for basic radicals:**

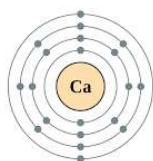
**1) Test for Potassium:**

To a pinch of extract 2ml of sodium nitrate & 2ml of cobalt nitrate solution in 30% glacial acetic acid was added and observed the presence of yellow precipitate.

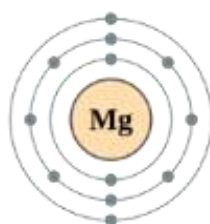


**2) Test for Calcium:**

To 2ml of herbal extract, 2ml of 4% ammonium oxide solution was added. No appearance of white precipitate confirms the absence of calcium ions in fever five.

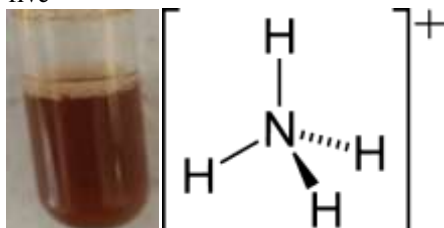


**3) Test for Magnesium:** To 2ml of Herbal extract, drops of sodium hydroxide solution were added. No appearance of white precipitate indicates absence of magnesium, in fever five.



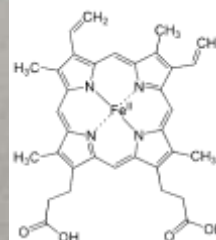
**4) Test for Ammonium:**

To 2ml of herbal extract, few ml of Nessler's reagent and excess of sodium hydroxide solution are added for appearance of grey color. This confirms the presence of ammonium ions in fever five



**5) Test for Iron:**

The herbal extract was treated with Conc. HNO<sub>3</sub> and ammonium thiocyanate. Appearance of blood red color, confirms the presence of Iron.



**6) Test for Zinc:**

To 2ml of herbal extract, drops of sodium hydroxide solution were added. No appearance of white precipitate formation, indicates the absence of Zn<sup>2+</sup> in this drug.

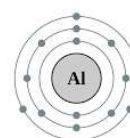


**7) Test for Aluminium:**

To 2ml of Herbal extract, drops of sodium hydroxide solution were added. No formation of white precipitate shows the absence of Al<sup>3+</sup>.

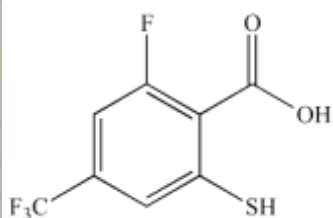


13: Aluminium 2,8,3



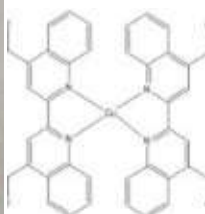
**8) Test for Lead:**

To 2ml of fever five, 2ml of potassium iodide solution was added. Formation of yellow colored precipitate, indicates the presence of Pb<sup>2+</sup>.



### 9) Test for Copper:

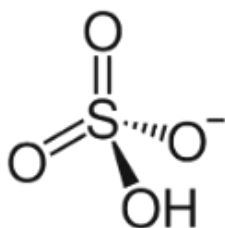
- A pinch of fever five was made into a paste with conc. HCl in a watch glass and introduced into the non luminous part of the flame. No appearance of blue color flame, confirms the absence of Cu<sup>2+</sup>
- To 2ml of Herbal extract excess of ammonia solution was added and no appearance of green colored precipitate confirms the absence of copper<sup>2+</sup> ions in fever five.



### Test for acid radicals:

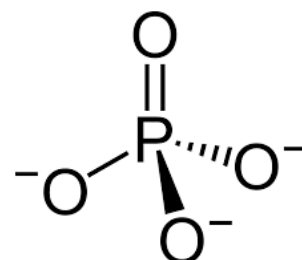
#### 1. Test for sulphate:

A 5% barium chloride solution was added to 2ml of the medicinal extract and no white precipitate was observed. Thus the sulphate ions are not found in this unani medicine.



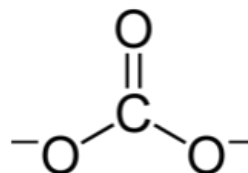
#### 2. Test for Phosphate:

The herbal medicinal extract was treated with ammonium molybdate and conc. HNO<sub>3</sub>, stand for an hour. The no appearance of yellow precipitate indicates absence of phosphate.



### 3. Test for Carbonate:

The extract of fever five was treated with concentrated HCl, and no effervescence was observed, hence Carbonate is absent.



### 4. Test for Fluoride & oxalate:

To 2ml of fever five extract 2ml of dil. acetic acid and 2ml of calcium chloride solution was added and heated. No cloudy appearance was seen. Thus, this unani formulation is free of fluoride & oxalate ions.



### 5. Test for Nitrate:

To 1gm of the fever five, copper turnings were added followed by the addition of conc. This is further heated and the test tube heated and the test tube was tilted vertically down and black color solution with dense whitefumes were observed which, indicates the presence of nitrates.



### III. RESULT AND DISCUSSION:

**Table-1: Phytochemicals & Bio-chemical analysis tests:  
Phytochemicals and biochemicals present in fever five**

S.No	PHYTOCHEMICALS	TEST CONDUCTED	OBSERVATION	RESULT
1.	Carbohydrates	a. Molisch test.	Appearance of violet ring between 2 layers.	Present
		b. Benedict's test.	No Appearance of orange red precipitate.	Absent
		c. Fehling's test.	No appearance of precipitate.	Absent
2.	Glycosides	a. Modified Bontrager's test.	No pink color formation in ammonical layer.	Absent
		b. Legal's test	Blood red color solution.	Present
3.	Saponins	Froth test	Presence of foam.	Present
4.	Phenolic Compounds	Alcoholic Ferric Chloride test.	No appearance of bluish green color.	Absent
5.	Phytosterols	Ferric chloride – acetic acid	No appearance of Reddish pink color.	Absent
6.	Diterpenes	Copper Acetate test	Appearance of Emerald Green color.	Present
7.	Triterpenes	Salkowski's test	Golden yellow color solution.	Present
8.	Flavonoids	a. Alkaline reagent test	Dark yellow color solution.	Present
		b. Lead Acetate test	Formation of yellow precipitate.	Present
		c. Ferric chloride test	appearance of dark green color.	Present
9.	Proteins	Xanthoproteic test	No Formation of yellow precipitate.	Absent
		Million's test	Formation of white precipitate.	Present
		Biuret test	No formation of brick red precipitate.	Absent
10.	Quinones	Sodium Hydroxide test	Appearance of Red orange color.	Present

**Table-2 Test for Acid & Basic radicals:**

11.	BASIC RADICALS:	Test for Potassium	Presence of yellow precipitate.	Present
12.		Test for Calcium	No change in solution.	Absent
13.		Test for Magnesium	No Appearance of white precipitate.	Absent
14.		Test for Ammonium	Appearance of brown color.	Present
15.		Test for Iron	Appearance of blood red color.	Present
16.		Test for Zinc	No white precipitate	Absent

			formation.	
17.		Test for Aluminum	No Appearance of white precipitate.	Absent
18.		Test for Lead	Formation of yellow precipitate.	Present
19.		Test for Copper	No appearance of blue color.	Absent
22.	ACID RADICALS:	Test for Sulphate	No Appearance of white precipitate.	Absent
23		Test for Chloride	No appearance of white precipitate.	Absent
24		Test for Phosphate	No Appearance of yellow precipitate.	Absent
25		Test for Carbonate	No Appearance of effervescence.	Absent
26		Test for Fluoride and oxalate	No Cloudy appearance.	Absent
27		Test for Nitrate	No Appearance of yellowish red color.	Present

The results of phytochemical and biochemical analysis of fever five a unani herbal medicine are reported in the tables. The phytochemical constituents of fever five were analysed and the results were reported in table 1 and table 2. Fever five was discovered to have a significant proportion of secondary metabolites. Our studies showed that fever five is rich in alkaloids, flavonoids, saponins, diterpenes, triterpenes, carbohydrates, quinones and proteins. The results of our study further prove the presence of various basic radicals and acidic radicals in fever five, with these phytochemicals and minerals may be responsible for its pharmacological actions the herbal medicine fever five is cost effective with lesser side effects.

#### IV. CONCLUSIONS:

The present investigation deals with the evaluation of phytochemicals present in Fever five, which reflects its pharmacological & therapeutic action. The result obtained from the study has confirmed the validity of Fever Five, a unani herbal medicine. The studies confirms the presence of Glycosides, Tannins, Saponins, Carbohydrate etc. It was concluded that the aqueous extract of the herbal medicine has significant pharmacological & medicinal applications.

The preliminary phytochemical screening can be further subjected for the isolation of therapeutically active substances with medicinal importance.

Our present study indicates the presence of carbohydrates & basic radicals such as

Potassium, Ammonium, Iron, Lead and absence of acid radicals listed in the above table 2.

Our study finding support the use of fever five, a unani herbal medicine in the treatment of high temperature, cold fever, without any side effects as it is free of toxic elements. Thus, the preliminary analysis of "FEVER FIVE" drug will give fingerprint to clinical studies.

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