



## Physicochemical and Phytochemical Investigation of the roots of *Grewia Microcos* linn.

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### ABSTRACT

The present study was undertaken for the development of physicochemical and phytochemical parameters of the roots of *Grewia microcos* Linn. belonging to the family Tiliaceae. In Hindi it is known as Shiral and Konkani as Chivra or aasale. Physicochemical and phytochemical investigation confirms the purity and authenticity of the roots of *Grewia microcos* using standard methods. Physicochemical studies of the crude powder of the roots revealed the moisture content as 7.5%w/w, Swelling Index as 1.3 cm, Foaming Index as 454.44, Alcohol soluble extractive as 0.244%w/w, Water soluble extractive as 0.232%w/w, Ether soluble extractive 0.04% w/w. Total Ash as 5% w/w, Water soluble ash as 2.15%w/w and Acid Insoluble ash as 2%w/w. Fluorescence analysis is a tool to determine the chemical nature of the crude drug which is reported in short wavelength, Long wavelength and day light. The preliminary phytochemical screening of the ethanolic extract of the roots revealed the presence of alkaloids, carbohydrates, tannins, saponins, triterpenoids and steroids. All these methods will help in laying down the pharmacopoeial standards in determining the quality and purity of the roots of *Grewia microcos*.

**Keywords:** *Grewia microcos*, Tiliaceae, Physicochemical, Fluorescence analysis.

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## INTRODUCTION

*Grewia microcos* is a small semi-deciduous tree, sometimes shrubby up to 50 feet high and 5 feet girth, belonging to the family Tiliaceae<sup>1</sup>.



Figure 1: The plant of *Microcos paniculata*

The plant is also known as *Microcos paniculata*<sup>2</sup>. It is found in North eastern parts of India, Western Ghats and Andaman islands. Flowers are in terminal panicles and auxiliary towards the apex, is the distinguishing character of this plant<sup>3</sup>. Boiled leaves along with turmeric and shell of snail are taken for the treatment of Jaundice. Traditionally it is used to improve digestion and is also for used for cold, hepatitis, diarrhea, heat stroke, dyspepsia, typhoid fever, syphilitic ulceration of the mouth, small pox, eczema and itches<sup>4,5</sup>.

Literature survey revealed that, the stem bark of *Grewia microcos* contained a new alkaloid, N-methyl-6- $\beta$ -(deca-1',3',5'-trienyl)-3- $\beta$ -methoxy-2-methylpiperidine which showed good insecticidal activity against *Aedes aegypti* second instar larve<sup>6,7</sup>. Two new piperidine alkaloids microcosamine A and B were isolated from the leaves, showed significant larvicidal activity against *Culex quinquefasiatus*<sup>8</sup>. A new triterpene named methyl -3-o-p-hydroxy-cinnamoyloxy-2- $\alpha$ , 23-dihydroxyolean-12-en-28-oate, epicatechin, 3-trans-feruloyl masilinic acid and masilinic acid were identified from the stem bark<sup>9</sup>. Analgesic and cytotoxic activity of leaves extract were also reported<sup>10</sup>.

From the extensive literature survey, it was revealed that no substantial work is carried out on the roots of the plant. Hence, an effort is made to investigate both physicochemical and preliminary phytochemical screening of the roots of *Grewia microcos*.

## MATERIALS AND METHOD

### Collection of the Plant Material

The roots of *Grewia microcos* were collected from the hills of Dhavali, Ponda –Goa during the

month of October 2012. It was authenticated by Prof. G.I. Hukkeri, Associate Professor, Department of Botany, Dhempe College of Arts and Science, Miramar-Goa<sup>3</sup>. The roots were washed thoroughly to remove the adhered soil and dried in shade. It was then powdered and used for physicochemical study, fluorescence analysis and also for the preparation of ethanolic extract.

### **Preparation of Ethanolic extract**

The powdered roots (500gms) were exhaustively extracted by maceration with ethanol for three days. After three days, the ethanolic layer was decanted. The process was repeated thrice. The solvent from the total extract was distilled off and the concentrate was evaporated using Rotary vacuum evaporator (Superfit) to a syrupy consistency and then evaporated to dryness (49gms)<sup>11</sup>.

## **PHYSICOCHEMICAL INVESTIGATION**

### **Determination of Ash Values<sup>12</sup>**

#### **Total Ash**

Accurately weighed 2g of the powdered material into a previously, ignited and tarred silica crucible. The material was then spread in an even layer in the crucible, ignited by gradually increasing the heat to 500-600<sup>0</sup>C until free from carbon, cooled in a desiccator and weighed. The percentage of ash (TA) was calculated with reference to the air dried drug.

#### **Acid Insoluble Ash**

To the crucible containing the total ash, 25 ml of hydrochloric acid (approx. 70g/l) TS was added, covered with a watch glass and boiled gently for 5min. The watch glass was rinsed with 5ml of hot water, which was then added to the crucible. The insoluble matter was collected on an ashless filter paper and washed with hot water until the filtrate is neutral. The filter paper containing the insoluble matter was then transferred to the original crucible, dried on hot plate and ignited to constant weight. The residue was allowed to cool in suitable desiccator for 30 min and weighed without delay. The percentage of acid insoluble ash was calculated with reference to the air dried drug.

#### **Water Soluble Ash**

To the crucible containing the Total ash, 25 ml of water was added and boiled for 5 minutes. The insoluble matter was collected on an ashless filter paper, washed with hot water & ignited for 15 minutes at a temperature not exceeding 450<sup>0</sup>C. Subtract the weight of this residue obtained from the weight of total ash. The percentage of water soluble ash was calculated with reference to the air dried drug.

### **Determination of Extractive Matter<sup>12</sup>**

**Method: Cold Maceration****Alcohol Soluble Extractive**

Weighed accurately about 4.0g of coarsely-powdered air dried plant material in a glass-stoppered conical flask. It was macerated with 100ml of 90% alcohol for 6 hours shaking frequently and then allowed to stand for 18 hours, filtered rapidly and 25ml of the filtrate was transferred to tared flat bottomed dish and evaporated to dryness on water-bath. Further it was dried at 105<sup>0</sup>C for 6 hours, cooled in desiccator for 30 min and then weighed without delay. Percentage of alcohol soluble extractive was calculated with the reference to the air dried sample.

**Water Soluble Extractive**

Weighed accurately about 4.0g of coarsely-powdered air dried plant material to a glass-stoppered conical flask. Macerated with 100ml of water for 6 hours shaking frequently, and then allowed to stand for 18 hours, filtered rapidly and 25ml of the filtrate was transferred to tared flat bottomed dish and evaporated to dryness on water-bath. Further it was at 105<sup>0</sup>C for 6 hours and cooled in desiccator for 30 min and then weighed without delay. Percentage of water soluble extractive was calculated with the reference to the air dried sample.

**Ether Soluble Extractive**

Weighed accurately about 4.0g of coarsely-powdered air dried plant material to a glass-stoppered conical flask. Macerated with 100ml ether for 6 hours shaking frequently and then allowed to stand for 18 hours, filtered rapidly and 25ml of the filtrate was transferred to tarred flat bottomed dish and evaporated to dryness on water-bath. Further it was at 105<sup>0</sup>C for 6 hours and cooled in desiccator for 30 min and then weighed without delay. Percentage of ether soluble extractive was calculated with the reference to the air dried sample.

**Determination of Foaming Index<sup>13</sup>**

About 1g of the plant material was reduced to a coarse powder (sieve size no. 1250), weighed accurately and transferred to a 500ml conical flask containing 100ml of boiling water, maintained at moderate boiling for 30 min. Cooled and filtered into a 100ml volumetric flask. Sufficient water was added to the filtrate to dilute the volume to 100ml.

The above decoction was placed into 10 stoppered test tubes (height 16cm, diameter 16mm) in a series of successive portions of 1, 2, 3, up to 10ml and the volume of the liquid was adjusted in each tube with water to 10ml. The tubes were stoppered and shaken in lengthwise motion for 15 seconds (2 frequencies per second) it was further allowed to stand for 15 min and the height of foam was measured.

If the height of foam in every test tube was less than 1cm the foaming index was less than 100.

If in any tube the height of the foam of 1 cm is measured, the dilution of the plant material in this tube [a] is the index sought. If this tube is the 1<sup>st</sup> or 2<sup>nd</sup> test tube in series, it is necessary to have an intermediate dilution prepared in a similar manner to obtain a more precise result.

If the height of the foam is more than 1 cm in every test tube the foaming index is over 1000. In this case the determination needs to be made on a new series of dilutions of the decoction in order to obtain results.

$$\text{Foaming index} = 1000/a$$

a = the volume in ml of the decoction used for preparing the dilution in the tube where foaming is observed.

### **Determination of Swelling Index<sup>13</sup>**

Weighed accurately 1g of root powder into a 25ml glass stoppered measuring cylinder. The length of the graduated portion of the cylinder should be about 125mm, the internal diameter about 16mm subdivided in 0.2ml and marked from 0- 25ml in upward direction. 25ml of water was added to the cylinder and shaken thoroughly at intervals of 10 min. for 1 hour. It was then allowed to stand for 3 hours at room temperature. The volume in ml occupied by the plant material, including any sticky mucilage was measured. The mean value of individual determinations, related to 1g of plant material was calculated.

### **Moisture Content<sup>14</sup>**

2g of root powder was taken into glass dish which is previously weighed. This glass dish was kept into the hot air oven at 100-105<sup>0</sup>C. The weight was noted every hour till two successive reading are constant. At last the weight of root was determined and percentage yield was calculated.

### **Fluorescence Analysis<sup>15-17</sup>**

Many herbs show fluorescence when the cut surface or powder is exposed to UV light and this can be useful in their identification. The fluorescence character of the plant powders was studied both in day light and UV light (254 nm and 366 nm) and after treatment with different reagents like sodium hydroxide, hydrochloric acid, nitric acid and ferric chloride etc.

### **Preliminary Phytochemical analysis**

A preliminary phytochemical screening was carried out using the ethanolic extract of the roots by employing the standard procedures<sup>18, 19</sup>.

### **ALKALOIDS**

#### **Dragendroff 's Test:**

To 2mg of the methanolic extract, 5ml of distilled water was added, 2M Hydrochloric acid was

added until an acid reaction occurs. To this 1ml of Dragendroff's reagent was added. Formation of orange or orange-red precipitate indicated the presence of alkaloids.

**Mayer's Test:**

To 2mg of the methanolic extract, a few drops of Mayer's reagent were added. Formation of white or pale yellow precipitate indicated the presence of alkaloids.

**Wagner's Test:**

To 2mg of the methanolic extract, 1ml of Hydrochloric acid was added along with few drops of Wagner's reagent. A yellow or brown precipitate indicated the presence of alkaloids.

**Hager's Test:**

To 2mg of the methanolic extract, was taken in a test tube, a few drops of Hager's reagent were added. Formation of yellow precipitate confirmed the presence of alkaloids.

**CARBOHYDRATES****Molish's Test**

In a test tube containing 2ml of the extract, 2 drops of freshly prepared 20% alcoholic solution of  $\alpha$ -naphthol was added. 2ml of conc. Sulphuric acid was added so as to form a layer below the mixture. Red-violet ring appeared, indicating the presence of carbohydrates, which disappeared on the addition of excess of alkali.

**Benedict's Test**

To 0.5ml of the extract, 5ml of Benedict's solution was added and boiled for 5 minutes. Formation of the brick red colored precipitate indicated the presence of carbohydrates.

**Fehling's Test**

To 2ml of the extract, 1ml mixture of equal parts of Fehling's solution A and B were added and boiled for few minutes. Formation of the brick red colored precipitate indicated the presence of carbohydrates.

**FLAVANOIDS****Shinoda Test**

In a test tube containing 0.5ml of the extract, 10 drops of Hydrochloric acid followed by a small piece of magnesium were added. Formation of pink, reddish, or brown color indicated the presence of flavonoids.

**Lead acetate Test**

To 2ml of plant extract add 1ml of Lead acetate solution. Formation of yellow precipitate indicates the presence of flavonoids.

**Vanillin HCl Test**

Vanillin HCl was added to the alcoholic solution of drug, formation of pink color shows the presence of flavonoids.

### **TRITERPENOIDS & STEROIDS**

#### **Liebermann –Burchard’s**

2mg of dry extract was dissolved in acetic anhydride, heated to boiling, cooled and then 1ml of concentrated sulphuric acid was added along the sides of the test tube. A brown colored ring is formed at the junction of two layers and the upper layer turns green which shows the presence indicated the presence of steroids and formation of deep red color indicates presence of triterpenoids.

#### **Salkowshi Test**

Treat the extract with few drops of conc. Sulphuric acid, red color in the lower layer indicates presence of steroids and formation of yellow colored lower layer the indicates the presence of triterpenoids.

### **TANNINS AND PHENOLIC COMPOUNDS**

To 1-2ml of the extract, few drops of 5%w/v FeCl<sub>3</sub> solution was added. A green color indicated the presence of gallotannins, white brown color indicated the presence of pseudotannins.

To 1-2ml of the extract add lead acetate was added. White precipitate indicated the presence of tannins and phenolic compounds.

### **RESINS**

1ml of the extract was dissolved in acetone and the solution was poured in distilled water. Turbidity indicated the presence of resins.

### **PROTEINS**

#### **Biuret’s Test**

To 1ml of hot extract, 5-8 drops of 10%w/v of sodium hydroxide solution, followed by 1 to 2 drops of 3%w/v of copper sulphate solution were added. Formation of violet red color indicated the presence of proteins.

#### **Millon’s Test**

1ml of the extract was dissolved in 1ml of distilled water and 5-6 drops of millon’s reagent were added. Formation of white precipitate, which turns red on heating, indicated the presence of proteins.

### **GLYCOSIDES**

Determine free sugar content of the extract. Hydrolyze the extract with mineral acids (dil. HCl/dil. H<sub>2</sub>SO<sub>4</sub>). Again determine the total sugar content of hydrolyzed extract. Increase in

sugar content indicates the presence of glycosides in the extract.

### **Test for cardiac glycosides**

#### **Baljet's test**

A thick section shows yellow to orange color with sodium picrate.

#### **Legal's Test**

To aqueous or alcoholic extract, add 1ml pyridine and 1ml sodium nitropruside. Pink to red color appears.

#### **Test for deoxysugars(killer –killani test)**

To 2ml extract add glacial acetic acid, one drop 5% FeCl<sub>3</sub> and conc. H<sub>2</sub>SO<sub>4</sub>. Reddish brown color appears at the junction of the two liquid layers and upper layer appears bluish green.

#### **Libermann's test (test for bufadienoloids)**

Mix 3ml extract with 3ml acetic anhydride. Heat and cool. Add few drops conc. H<sub>2</sub>SO<sub>4</sub>. Blue color appears.

### **Test For Anthraquinone Glycosides**

#### **Borntrager's test for Anthraquinone glycosides**

To 3ml extract add 5ml 5% dil. H<sub>2</sub>SO<sub>4</sub>. Boil and filter. To cold filtrate add equal volume of benzene or chloroform. Shake well. Separate the organic solvent. Add ammonia. Ammonical layer turns pink or red.

#### **Modified Borntrager's test for C-glycosides**

To 3ml extract add 5ml of 5% dil. HCl. Heat for 5minutes in boiling water and cool. To cold filtrate add equal volume of benzene or organic solvent. Shake well. Separate the organic solvent. Add ammonia. Ammonical layer turns pink or red.

### **Test For Saponins**

#### **Foam test**

Shake the drug extract or dry powder vigorously with water. Persistent foam observed.

### **Test For Coumarin Glycosides**

#### **Fluorescence test**

Take moistened powder in a test tube. Cover test tube with filter paper soaked in dilute NaOH. Keep in water bath. After some time expose filter paper to UV light. It shows yellowish-green fluorescence.

#### **FeCl<sub>3</sub> Test**

To the concentrated alcoholic extract of drug few drops of alcoholic FeCl<sub>3</sub> solution was added. Formation of deep green color, which turned yellow on addition of conc. HNO<sub>3</sub> indicates the

presence of coumarins.

### Starch

0.01gms of Iodine and 0.075gms of KI were dissolved in 5ml of distilled water and 2-3ml of extract was added. Formation of blue color indicated the presence of starch.

## RESULTS AND DISCUSSION

The roots of *G. microcos* were subjected to systematic physicochemical, fluorescence and phytochemical screening techniques. Physicochemical characterization of powder of *G. microcos* is shown in Table 1. The physical constant evaluation of the drugs is an important parameter in detecting adulteration or improper handling of drugs<sup>20</sup>. The roots showed less moisture content, it was 7.5% w/w; hence it would discourage the bacterial and fungal growth. The ash value was determined by three different form viz., total ash, water soluble and acid insoluble ash. The objective of ashing the drugs are to remove traces of matter which otherwise interfere in an analytical determination<sup>21</sup>. On incineration crude drugs normally leave ash consisting of carbonates, phosphates, and silicates of sodium, magnesium, potassium and calcium<sup>21</sup>. Fluorescence study is an essential parameter for first line standardization of crude drug<sup>15</sup>. In fluorescence the fluorescent light is always of greater wavelength than the exciting light. Light rich in short wavelengths is very active in producing fluorescence and for this reason ultraviolet light produces fluorescence in many substances which do not visibly fluoresce in daylight<sup>15</sup>. The results of fluorescence analysis were expressed in Table 2. The preliminary phytochemical investigations of powdered roots were performed which shows the presence of Alkaloids, Carbohydrates, Tannins, Steroids, flavanoids and Saponins type of major secondary metabolites which revealed their potent therapeutic activity. The results of the preliminary phytochemical screening were expressed in Table 3.

**Table 1: Physicochemical characterization of powdered roots of *G. microcos***

Sr. No.	Method	Results
1.	Swelling Index	1.3 cm
2.	Extractive Value	
	Alcohol soluble	0.244% w/w
	Water Soluble	0.232% w/w
	Ether Soluble	0.04% w/w
3.	Foaming Index	454.44
4.	Ash Value	
	Total Ash	5% w/w
	Acid Insoluble Ash	2% w/w
	Water Soluble Ash	2.15% w/w
5.	Moisture Content	7.5% w/w

**Table 2: Fluorescence analysis of powdered roots of *G. microcos*.**

Sr. No.	Solvent Treatment	Visible Light	Short UV (254 nm)	Long UV (366nm)
1	Drug	Brown	Brown	Brown
2	Drug+ Dist. H <sub>2</sub> O	Light Yellow	Colorless	Light Blue
3	Drug+ Methanol	Brownish Yellow	Brownish Yellow	Light Blue
4	Drug+ Pet. Ether	Colorless	Colorless	Blue
5	Drug+CHCl <sub>3</sub>	Colorless	Colorless	Colorless
6	Drug+ 10% HCl	Yellow	Light Yellow	Light Yellow
7	Drug+ C. HCl	Brown	Brown	Brown
8	Drug+ 10% H <sub>2</sub> SO <sub>4</sub>	Yellow	Light Yellow	Light Yellow
9	Drug+ c. H <sub>2</sub> SO <sub>4</sub>	Black	Black	Black
10	Drug+ Picric acid	Yellow	Yellow	Yellow
11	Drug+ c. HNO <sub>3</sub>	Orangish Yellow	Yellow	Yellow
12	Drug+ Ammonia	Brown	Brown	Light Green
13	Drug+ 10% NaOH	Reddish Brown	Brown	Brown
14	Drug+50% aq. NaOH	Dark Brown	Brown	Brown
15	Drug+ 50% alc. NaOH	Yellowish Green	Yellowish Green	Yellowish Green

**Table 3: Preliminary Phytochemical Screening of Powdered roots of *G. microcos***

Sr. No.	Tests	Inference
1.	Alkaloids	+ve
2.	Glycosides	-ve
3.	Carbohydrates	+ve
4.	Flavonoids	+ve
5.	Proteins	-ve
6.	Tannins	+ve
7.	Resins	-ve
8.	Saponins	+ve
9.	Triterpenoids	+ve
10.	Steroids	+ve
11.	Starch	-ve

+ = Present , - = Absent

## CONCLUSION

*Grewia microcos* is widely used in traditional medicines having tremendous medicinal potential owing to its multifaced biological functions. Many researchers have been done on this plant but much of the claims are inspired and based on traditional evidence instead of scientific studies. The investigation carried out by us led to certain findings about the physiochemical and phytochemical features which no doubt can be proved beneficial and serve as scientific background for further isolation steps to obtain the lead compounds.

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