

PHYTOCHEMICAL ANALYSIS OF SUCCESSIVE REEXTRACTS OF THE LEAVES OF *MORINGA OLEIFERA* LAM.

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ABSTRACT

Objective: The present study aims to conduct a preliminary phytochemical analysis of the successive reextracts of the leaves of *M. oleifera* using petroleum ether, chloroform, and ethanol and water solvents in succession

Methods: The extracts of the dried powder of the leaves of *M. oleifera* Lam obtained using petroleum ether, chloroform, ethyl alcohol and water in succession were tested along with positive control and blank for the presence of tannins, alkaloids, steroids, triterpenoids, flavonoids, hydroxy-antraquinones, cardiac glycosides, saponins, and for carbohydrates (glucose and fructose), proteins, amino acids, and fixed oils & fatty acid

Result: The results suggest the presence of tannins, alkaloids, steroids, triterpenoids, flavonoids, hydroxy-antraquinones, cardiac glycosides, saponins, and carbohydrates (glucose and fructose) having selective solubility in selected solvents of varying polarities used in succession. However, the tests for proteins and fixed oils and fats were negative in all the extracts.

Conclusion: The selective solubility of the phytochemicals is probably responsible in conferring a wide spectrum of biological activities attributed to the leaves of *M. oleifera* suggesting the importance of the solvent as a decisive factor and also suggest the importance of the employed test also as a decisive factor for confirming the presence of a phytoconstituent. The data in addition indicates that the leaves of *M. oleifera* are highly nutritious and could be a source of dietary supplement being rich in carbohydrates, proteins and amino acids. In addition, the present study suggests that the successive reextractions using solvents of varying polarities would maximize the exploitation of the diverse bioactive compounds. The present information would be of help to isolate and characterize the diverse pharmacologically active principles of importance supporting their varied biological activities and the medicinal values.

Keywords: Leaves, *Moringa oleifera*, Phytochemical analysis, Successive reextraction

INTRODUCTION

Moringa oleifera (Family: Moringaceae) commonly called as Drumstick tree, Horseradish tree or Ben tree is an important medicinal herb referred as a miracle tree [1] as all the parts of the plant possess nutritional and medicinal properties [2,3].

M. oleifera is an important medicinal plant and phytochemical investigations and isolated principles from them would be of use to achieve lead molecules in the search of novel herbal drugs [2]. Earlier studies have reported the presence of various phytoconstituents present in the leaves of *M. oleifera* extracts using various solvents [4 to 8]. However, there is no report documenting the screening of the bioactive principles in the successive extracts of the leaves of *M. oleifera* using solvents of varying polarities in succession. The present study therefore, aimed to conduct a preliminary phytochemical analysis of the successive reextracts of the leaves of *M. oleifera* using petroleum ether, chloroform, ethanol and water solvents in succession.



Fig. 1: Leaves of *Moringa oleifera* (drumstick) tree

MATERIAL AND METHODS

Preparation of successive extracts

The mature leaves of *Moringa oleifera* (Fig 1) collected locally were washed with water, shade dried and grounded into fine powder. 75g of the dried leaf powder was reextracted using petroleum ether, chloroform, ethanol, and water in succession using soxhlet apparatus. Each extract obtained following successive extraction was filtered using Whatman No 1 filter paper, dried to a semisolid mass using water bath and the yield of each extract thus obtained was recorded and stored in a refrigerator at 4°C till further use.

Phytochemical analysis

A stock concentration of 1 % (W/ V) of each successive extract obtained using petroleum ether, chloroform, ethanol and water was prepared using the respective solvent. These extracts along with positive and negative controls were tested for the presence of active phytochemicals viz: tannins, alkaloids, phytosterols, triterpenoids, flavonoids, cardiac glycosides, anthraquinone glycosides, saponins, carbohydrates, proteins, amino acids and fixed oils & fats following standard methods [9 and 10] as briefed below:

I. Tannins

1. Ferric chloride Test: Added a few drops of 5% ferric chloride solution to 2 ml of the test solution. Formation of blue color indicated the presence of hydrolysable tannins.

2. Gelatin Test: Added five drops of 1% gelatin containing 10% sodium chloride to 1 ml of the test solution. Formation of white precipitates confirmed the test.

II. Alkaloids

Approximately 50 mg of extract was dissolved in 5 ml of distilled water. Further 2M hydrochloric acid was added until an acid reaction occurred and filtered. The filtrate was tested for the presence of alkaloids as detailed below

1. Dragendorff's Test: To 2 ml of the filtrate was added 1 ml of Dragendorff's reagent along the side of the test tube. Formation of orange or orange reddish brown precipitate indicated the test as positive.

2. Mayer's Test: To 1 ml of test solution or filtrate was added a drop or two of the Mayer's reagent along the sides of the test tube. A white or a creamy precipitate confirmed the test as positive.

3. Hager's Test: To 1 ml of test solution or filtrate, a drop or two of Hager's reagent was added. The formation of yellow precipitate indicated the test as positive.

4. Wagner Test: Two drops of Wagner's reagent was added to 1ml of the test solution along the the side of the test tube. The formation of yellow or brown precipitate confirmed the test as positive for alkaloids.

III. Phytosterols

1. Liebermann-Burchard's Test: The extract (2 mg) was dissolved in 2 ml of acetic anhydride, heated to boiling, cooled and then 1 ml of concentrated sulfuric acid was added along the side of the test tube. A brown ring formation at the junction and the turning of the upper layer to dark green color confirmed the test for the presence of phytosterols.

IV. Triterpenoids

1. Salkowski Test: Approximately 2 mg of dry extract was shaken with 1 ml of chloroform and a few drops of concentrated sulfuric acid were added along the side of the test tube. A red brown color formed at the interface indicated the test as positive for triterpenoids.

V. Flavonoids

1. Shinoda test: A few magnesium turnings and 5 drops of concentrated hydrochloric acid was added drop wise to 1 ml of test solution. A pink, scarlet, crimson red or occasionally green to blue color appeared after few minutes confirmed the test.

2. Alkaline reagent test:- Addition of 5 drops of 5% sodium hydroxide to 1 ml of the test solution resulted an increase in the intensity of the yellow color which became colorless on addition of a few drops of 2 M hydrochloric acid which indicated the presence of flavonoids.

3. Lead acetate test:- A few drops of 10% lead acetate added to 1ml of the test solution resulted in the formation of yellow precipitate confirmed the presence of flavonoids.

VI. Saponins

1. Foam Test: 5 ml of the test solution taken in a test tube was shaken well for five minutes. Formation of stable foam confirmed the test.

2. Olive oil test: - Added a few drops of olive oil to 2ml of the test solution and shaken well. The formation of a soluble emulsion confirmed the test.

VII. Cardiac glycosides

1. Keller -Killiani test: Added 0.4 ml of glacial acetic acid and a few drops of 5% ferric chloride solution to a little of dry extract. Further 0.5 ml of concentrated sulfuric acid was added along the side of the test tube carefully. The formation of blue colour in acetic acid layer confirmed the test.

VIII. Anthraquinone glycosides

Hydroxyanthraquinone Test

To 1 ml of the extract, added a few drops of 10% potassium hydroxide solution. The Formation of red color confirmed the test.

IX. Test for carbohydrates

1. Molisch's test: To 1 ml of test solution added a few drops of 1 % alpha-naphthol and 2-3 ml concentrated sulfuric acid along the side of test tube. The reddish violet or purple ring formed at the junction of two liquids confirmed the test.

2. Barfoed's test: 2ml of reagent was added to 2 ml of the test solution, mixed & kept a in boiling water bath for 1 min. Red precipitate formed indicates the presence of monosaccharides..

3. Seliwanoffs test: To 3 ml of Seliwanoffs reagent was added to 1 ml of the test sample and heated on a water bath for one minute. The formation of rose red color confirmed carbohydrates

4. Fehlings test: Dissolved 2 mg dry extract in 1 ml of distilled water and added 1ml of Fehling's(A+B) solution, shooked and heated on a water bath for 10 minutes. The brick red precipitate formed confirmed the test

IX. Test for proteins

1. Biuret test: To 2 ml of the test solution added 5 drops of 1% copper sulphate solution and 2 ml of 10% NaOH .Mix thoroughly. Formation of purple or violet color confirmed proteins.

X. Test for amino acids

1. Millon's test: Added 5 drops of millons reagent to 1 ml of test solution and heated on a water bath for 10 min, cooled and added 1% sodium nitrite solution. Appearance of red color confirmed the test.

XI. Fats and fixed oils

To 5 drops of the sample was added 1 ml of 1% copper sulphate solution and a few drops of 10% sodium hydroxide. The formation of a clear blue solution confirmed the test.

RESULTS AND DISCUSSION

Basic phytoinvestigations of the extracts for their major phytochemicals is vital as the active principles of many drugs are these secondary metabolites found in plants.

The yield obtained for each successive extracts of the leaves of *M. oleifera* in present study using petroleum ether, chloroform ethanol and water (aqueous) is recorded to be highest in the case of ethanol followed by water, petroleum ether and chloroform used in succession (Table.1)

The phytochemical evaluation of various phytoconstituents in successive extracts of the leaves of *M. oleifera* along with control and blank were graded as very high (++++); high (+++); moderate (++) ; low (+) and nil (-) based on the intensity of the colored reaction product of the test compared to control in each case (Table-2 and Figures 2 to 22).

Table 1: The yield and colour of the extracts of *Moringa oleifera* leaves obtained following extraction with petroleum ether, chloroform, ethanol and water in succession.

Solvent Used	Sample (Gram)	Boiling point	Total Hrs of Extraction	Yield (Gram)	Yield /100g	Color of extract
Petroleum Ether	75	60° C	3.5hrs	3.58	4.77	Dark green
Chloroform	72	60° C	6 hrs	2.47	3.43	Yellow brown
Alcohol	70	72° C	7 hrs	14.00	20.00	Dark green
Aqueous	55	80° C	3 hrs	6.48	11.78	Dark green
Residue left	42					

Table 2: Levels of phytochemicals in the successive extracts of leaves of *Moringa oleifera* obtained using petroleum ether, chloroform, ethanol and water in succession along with blank (water) and positive control*.

Test		Blank	Control	Petroleum ether	Chloroform	Ethanol	Water
Tannin	Ferric chloride	-	++++	-	-	++	+
	Gelatin	-	++	-	-	+++	+
Alkaloids	Dragendorff's	-	+++	-	-	+++	+
	Hager's	-	++++	-	-	+	-
	Mayer's	-	+++	-	-	+	-
	Wagner's	-	+++	-	-	+	-
Phytosterols	Liebermann-Burchard	-	++++	+++	+++	-	-
Triterpenoids	Salkowski	-	+	-	-	++++	+++
Flavonoids	Shinoda	-	++	-	-	+++	+
	Lead acetate	-	++++	-	-	+++	++++
	Alkaline reagent	-	++++	-	-	+++	+++
Saponins	Foam	-	++	-	-	-	-
	Olive oil	-	+++	-	-	-	++
Cardiac Glycoside	Keller Killani	-	++++	+++	++++	++	-
Hydroxyanthraquinone		-	++++	-	+++	++	+++
Carbohydrates	Molisch's	-	++++	-	-	++++	+++
	Barfoed's	-	+++	-	-	+	-
	Fehling's	-	+++	-	-	+++	+++
	Seliwanoff's	-	++++	+++	+	+++	++
Proteins	Biuret	-	+++	-	-	-	-
Amino acid	Millon's	-	+++	-	-	++++	+
Fixed Oils And Fats		-	++++	-	-	-	-

++++ (Very High) +++ (High), ++ (Moderate) + (low) ± (negligible) and - (Nil). Positive controls: **1.**Tannins-Tannic acid, **2.**Alkaloids-Quinine sulphate., **3.**Phytosterols- Cholesterol., **4.**Triterpenoids- Cholesterol. I, **5.**Flavonoids-Lemon skin juice., **6.** Saponins -Foam test-Pea powder and olive oil test - Oat powder., **7.** Cardiac glycosides-*Calotropis* latex, **8.** Anthraquinone glycosides-Hydroquinone; **9.** Carbohydrates: Molisch's, Barfoed's, and Fehling's test-Glucose., Seliwanoff's test - Fructose; **10.**Proteins: Biuret test- Bovine serum albumin **11.** Amino acids: Millon's test- Tyrosine and **12.**Fats & fixed oils -Glycerin.

The phytochemical tests employed indicated the presence of hydrolysable tannins in ethanol and aqueous extracts and their absence in petroleum ether and chloroform extracts. The Dragendorff's test for alkaloids was positive in ethanol and aqueous extracts whereas the Hager's, Mayer's and the Wagner tests exhibited the presence of low amount of alkaloids only in the ethanol extract. The Liebermann-Burchard's test for phytosterols was positive in petroleum ether and chloroform extracts and negative in ethanol and aqueous extracts. The Salkowski test for triterpenoid was positive only in ethanol and aqueous extracts. The lead acetate and alkaline reagent tests indicated the presence of high amount of flavonoids in ethanol and water extracts whereas the Shinoda test exhibited the presence of low amount of flavonoids in aqueous extracts. The Foam test was negative whereas the olive oil tests for saponin's was positive in aqueous.extract. The kellar killani test for cardiac glycoside was positive in petroleum ether, chloroform and ethanol extracts and negative in aqueous extracts Hydroxyanthroquinone test was positive in chloroform, ethanol and water extracts only. The Molisch and Fehling's test indicated the presence of high amount of carbohydrates in the ethanol and aqueous extracts whereas the Barfoed's test exhibited low amount of carbohydrates in ethanol extract and negative in aqueous extract. The Seliwanoff's test indicated the presence of keto sugars in all extracts exhibiting lowest amount in chloroform extract. Millon's test for amino acid (hydroxyl phenol group of tyrosine) was positive in ethanol and aqueous extracts. The tests for protein (Biuret) and fats and fixed oils were negative in all the successive extracts.

The ethanolic extract of *M. oleifera* leaves has been demonstrated to exhibit anthelmintic activity against Indian earthworm[11], antifungal activity against dermatophytes [12], antifertility [13 and 14] and hypoglycemic potential [15] A study on evaluation of *M.*

oleifera leaves extract on ovariectomy induced bone loss in rats records that the ethanolic extract of *M. oleifera* leaves possess osteoprotective effect comparable with estradiol [16] and has been reported to reduce cyclophosphamide induced immunodepression by stimulating cellular and humoral immunity in mice [17]

The aqueous extract of *M oleifera* leaves have been demonstrated to exhibit protective effect on ulcerated gastric tissue induced by aspirin, cerebral nodular lesion and cold stress in rats [18] , wound healing property in rats[19] significant hypoglycemic and antidiabetic potential [15],antifertility activity [13,14] and the regulatory control on thyroid hormone status in adult Swiss rats [20]

Earlier reports on preliminary phytochemical analysis have documented the presence of phytoconstituents in the leaves of *M oleifera* using a single solvent. However, in present study, the phytochemical analysis of successive re-extracts obtained following extraction using petroleum ether, chloroform, and ethanol and water points to the presence of diverse active principles having selective solubility in successive solvents of varying polarities used in succession suggesting the importance of the solvent as a decisive factor [6]. Further the data also suggest the importance of a particular test employed also as a decisive factor for confirming the presence of a phytoconstituent. Further it suggested that the leaves of *M. oleifera* are highly nutritious and could be a source of nutritional supplement and as a growth promoter being rich in carbohydrates and amino acids [21].Further the data suggest that the successive reextractions using solvents of varying polarities would maximize the exploitation of the diverse bioactive compounds. The present information thus would be of help to isolate and characterize the diverse pharmacologically active principles of importance supporting their varied biological activities and the medicinal values.

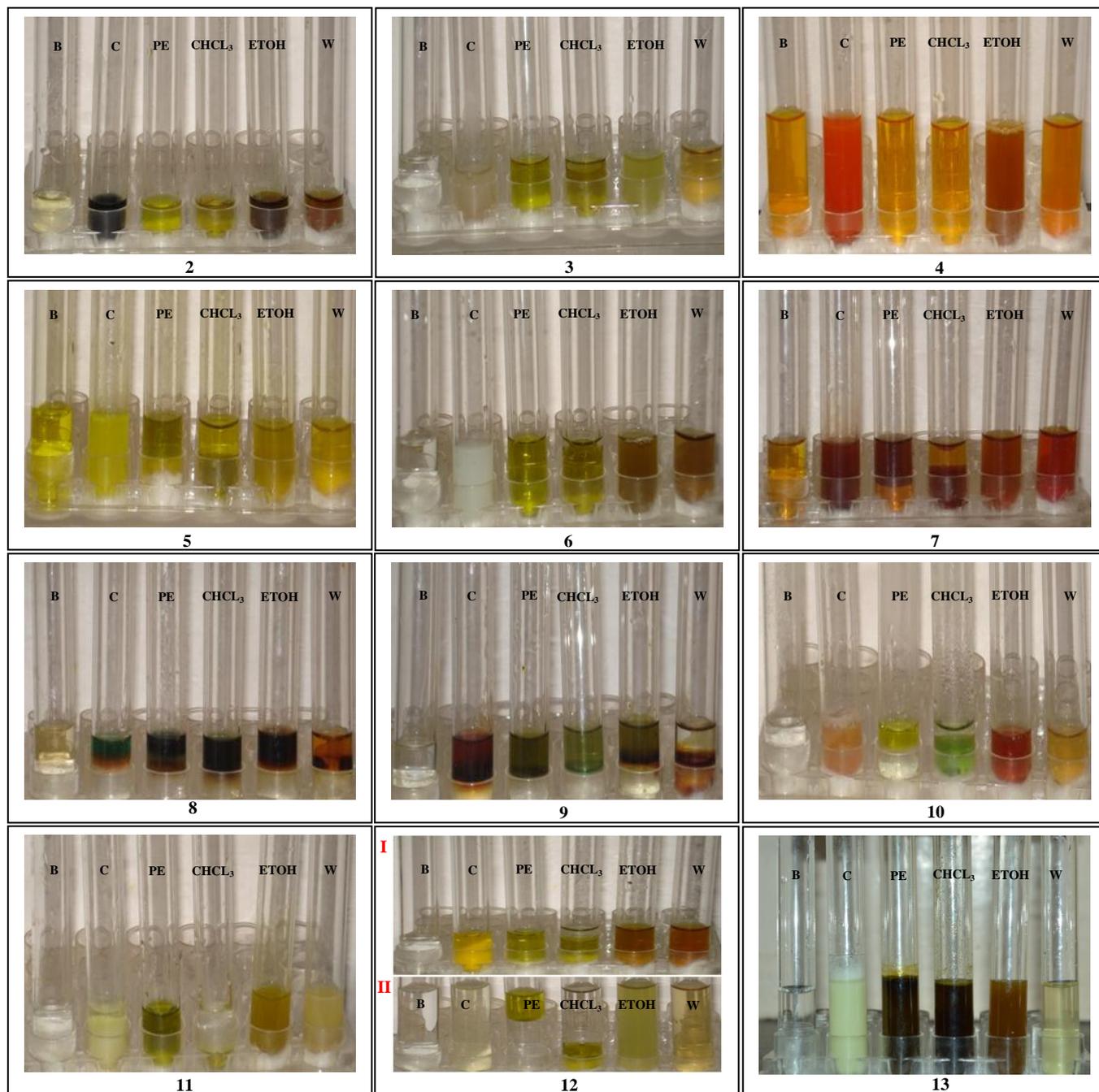
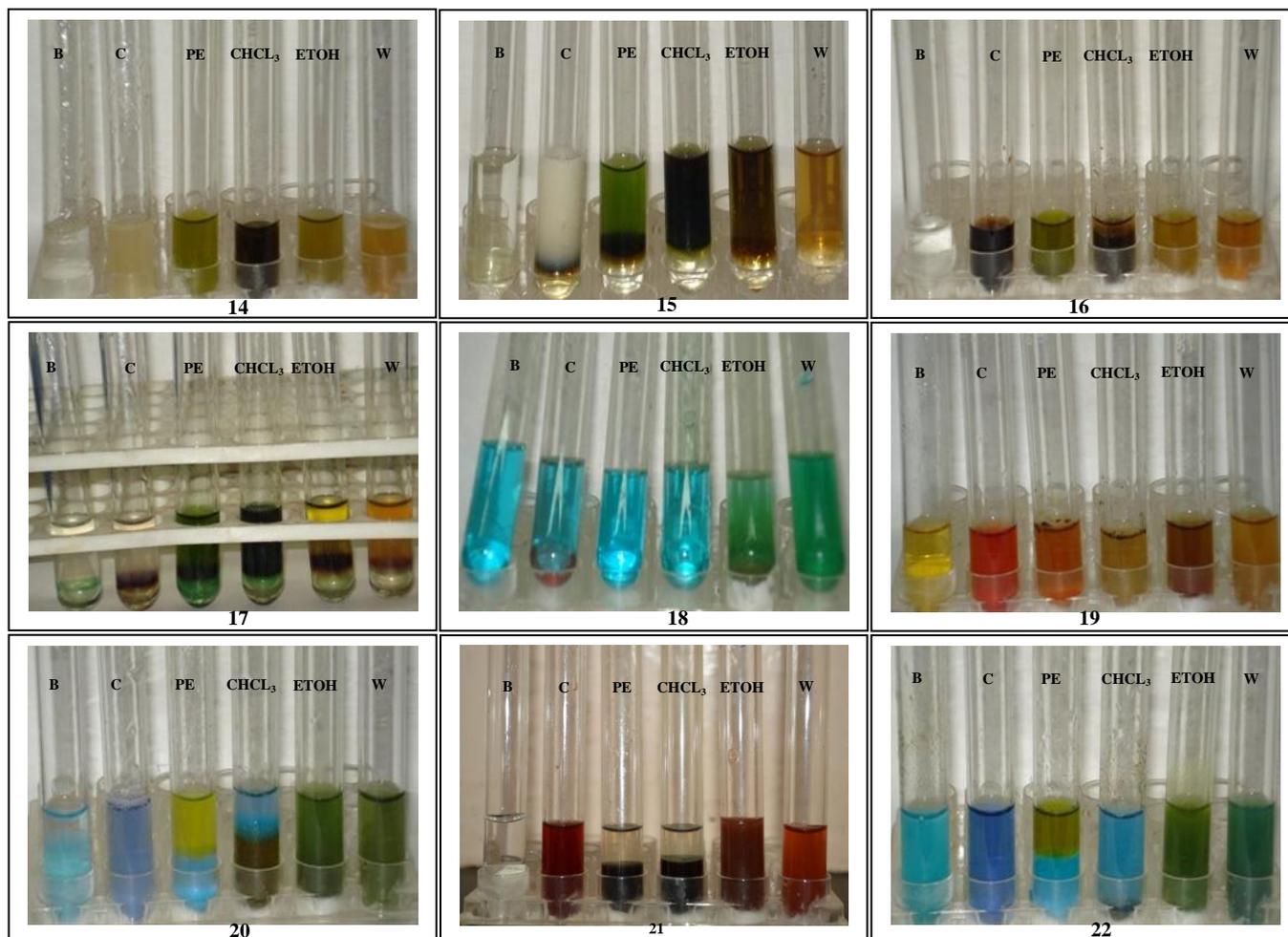


Fig. 2 to 13: Demonstration of phytochemicals in blank (B) positive control (C) and the successive extracts of *Moringa oleifera* in petroleum ether (PE), chloroform (CHCl₃), ethanol (EtOH) and water (W) exhibiting (2) -Ferric chloride and (3) - Gelatin test for tannins (high in ethanol and low in aqueous extracts) ; (4)- Dragendorff's test (high in ethanol and low in water extracts) ; (5) - Hager's (6) Mayer's and (7)- Wagner's test (positive in ethanol only) for alkaloids ; (8) Liebermann- Burchard test (positive in petroleum ether and chloroform extracts) for phytosterols ; (9) Salkowski test for triterpenoids (high in ethanol and aqueous extract); (10) Shinoda test (high in ethanol and low in aqueous extract) ; (11) lead acetate test and (12) - Alkaline reagent test (I) - before the addition and (II) - after the addition of acid (high in ethanol and aqueous extracts) for flavonoids and (13)- Foam test (negative in all extracts) for saponins .



Figs 14 to 22: Demonstration of phytochemicals in blank (B) positive control (C) and the successive extracts of *Moringa oleifera* using petroleum ether (PE), chloroform (CHCl₃), ethanol (ETOH) and water (W) exhibiting (14) Olive test (positive in aqueous extract) for saponins; (15) Keller Killani test (positive in petroleum ether, chloroform and ethanol extract) for cardiac glycosides; (16) Hydroxyanthraquinone test (positive in chloroform, ethanol and aqueous extracts); (17) Molisch test (positive in ethanol and aqueous extracts); (18) Barfoed's test (positive in ethanol extract only); (19) Seliwanoff's test (positive in all extracts) for carbohydrates; (20) Biuret test (negative in all extracts) for proteins; (21) Millon's test (very high in ethanol and moderate in aqueous extract) for amino acid and (22) Saponification test (negative in all extracts) for fats & fixed oils

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