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THE PHYTOCHEMICAL SCREENING OF THE PERICARP OF FRUITS OF TERMINALIA *CHEBULA* Retz.

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ABSTRACT

The medicinal properties of a plant are attributed to the presence of non-nutritive phytochemicals, the secondary metabolites. The extracts of the dried powder of the pericarp of fruits of *Terminalia chebula* Retz obtained using petroleum ether, chloroform, ethyl alcohol and water in succession were tested along with positive controls and blanks for the presence of tannins, alkaloids steroids, triterpenoids, flavonoids, hydroxy-anthraquinones, cardiac glycosides, saponins, and for carbohydrates (glucose and fructose), proteins, amino acids, and fixed oils & fatty acid. The results suggest the presence of diverse active phytochemicals having selective solubility in solvents of varying polarities used in succession. However, the tests for proteins, cardiac glycosides and fixed oils and fats were negative in all the extracts. The use of positive control and blank, a first approach in such studies provided unbiased accuracy in grading the intensity of the reaction. The selective solubility in solvents of varying polarities may probably is responsible in conferring a wide spectrum of biological activities attributed to the fruits of this medicinal plant. In addition the data suggest that the fruits of *T.chebula* are highly nutritious and could be a source of dietary supplement being rich in carbohydrates and amino acids. The successive extraction using solvents of varying polarities can maximize the exploitation of 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, medicinal values and therapeutic usages.

KEYWORDS: **Pericarp, Fruits, *Terminalia chebula* Retz, Phytochemical analysis, Successive extraction**

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INTRODUCTION

Fruits of *Terminalia chebula* is yellowish-brown, ovoid, generally 20-35 mm long and 13-25 mm wide, wrinkled and ribbed longitudinally.The pericarp of the dried ripe fruits of *T.chebula* is fibrous 3-4 mm thick, non adherent to the seed with astringent taste used traditionally in preparation of many ayurvedic formulations.[1,2.] Several recent reviews have documented an extensive information on morphological characteristics, phytochemical, ethnobotanical, biochemical, auyrvedic, pharmacological activities and the medicinal uses of *Terminalia chebula.*[1-8.] The literature documents that the fruits of *T.chebula p*ossesses antiviral, antibacterial, antioxidant, antidiabetic, immunomodulatory, anticancer, antimutagenic, anticarcinogenic, antifungal,cytotoxic,radioprotective, anticaries, cardioprotective, anticonvulsant, antihelmintic, hepatoprotective, retinoprotective, antiarthritic, antiaging,antiphyletic,laxative,antisplasmodial, chemopreventive, anxiolytic, hypolipidemic, antinciceptive, antiulcer, hypocholesterolemic, antispermatogenic antidepressant, cutaneous wound healing and molluscicidal activities.[5-10.] A regular intake of *T.chebula* fruits have been reported to prevent *salmonellae* infection and reduce the risk of typhoid.[11] The methanolic extract of *T chebula* has been suggested to be useful in controlling infectious diseases in diabetic patients [12] and the aqueous extract of fully ripe fruits of *T chebula* has been reported to act as a free radical scavenger with potential antioxidant effects on erythrocytes of aged rats.[13] Recently it has been demonstrated that the ethanolic extract of *T.chebula* fruit exhibits antidiabetic activity suggesting that its constituents possesses insulin like action and ability to promote insulin release.[14] The study of phytoconstituents is highly essential for plant materials used for the preparation of compound formulation of drugs to isolate and characterize the chemical constituents[15].Earlier studies have recorded the presence of phytochemicals in the fruits of *T.chebula* extracts obtained using methanol,[12, 16] ethanol,[17] petroleum ether, chloroform, dimethylformamide ethanol or water[18] and petroleum ether, chloroform or ethanol[19] each used separately. However, no report documents the screening of active principles in the successive extracts of the pericarp of the fruits of *T.chebula.* The present study aimed to conduct a preliminary phytochemical analysis of the successive extracts of the pericarp of the fruits of *T. chebula* using petroleum ether, chloroform, ethanol and water solvents in succession.

MATERIALS AND METHODS

Plant material

The matured, dried, fruits of *T. chebula Retz* with five ribs (Fig 1) were collected from a local Ayurvedic shop.The fruits were authenticated by Dr.Krishna kumar, taxonomist, Department of Botany, Mangalore University and a voucher specimen is deposited in the laboratory.

Figure 1
The Fruits of Terminalia chebula exhibiting five ribbed pericarp.

Preparation of successive extracts of the pericarp of fruits of Terminalia chebula

The dried pericarp of the *T. chebula* fruits was homogenized into a fine powder and stored in air-tight bottles. The powdered pericarp (75 gm) was extracted using petroleum ether, chloroform; ethanol and water in succession using soxhlet apparatus till the colour of the solvent became clear. The liquid extract obtained in each case was filtered (Whatman filter No. 1) dried, weighed (Table -1) and stored in refrigerator at 4^0 C.

Table 1

The yield and colour of the successive extracts of pericarp of fruits of Terminalia chebula obtained following extraction with petroleum ether, chloroform, ethanol and water solvents in succession.

Solvent Used	Sample Taken (Gram)	Boiling point	Total Hrs of Extraction	Yield (Gram)	Yield /100 g	Color of the extract
Petroleum Ether	75	60 C	5hrs	0.156	0.208	Dark green
Chloroform	74.5	60 C	4 hrs	0.186	0.249	Yellow brown
Alcohol	74.34	72 C	5 ½ hrs	36.561	49.180	Dark brown
Aqueous	58.03	80 C	6 ½ hrs	14.726	25.376	Dark brown
Residue left	40.304					

Phytochemical analysis

A stock concentration of 1 % (W/V) was prepared using the respective solvent in each case.These extracts along with positive and negative controls were tested for the presence of active phytochemicals viz: tannin, alkaloids, phytosterols, triterpenoids, falvonoids, cardiac glycosides, anthroquinone glycosides, saponins and carbohydrates, proteins, amino acids and fixed oils & fats following standard methods[20-21] as briefed below:

Phytochemical Evaluation

I. TANNINS

1. Ferric chloride Test: To 2 ml of test solution, add few drops of 5% ferric chloride solution. The blue color indicates the presence of hydrolysable tannins while the green color indicates the presence of condensed tannins.

2. Gelatin Test: To 1 ml of test solution, add 5 drops of 1% gelatin containing 10% sodium chloride. Formation of white precipitates confirms as the positive test.

II. ALKALOIDS

Approximately 50 mg of each extract is dissolved in 5 ml of distilled water and 2M hydrochloric acid is added until an acid reaction occurs and filtered.The filtrate was tested for the presence of alkaloids:

1. **Dragendorff's Test**: To 2 ml of the filtrate, add 1 ml of Dragendorff's reagent by the side of the test tube. Formation of orange or orange reddish brown precipitate indicates the test as positive.

2. Mayer's Test: To 1 ml of test solution or filtrate, add a drop or two of the Mayer's reagent along the side of the test tube. A white or a creamy precipitate indicates the test as positive.

3. Hager's Test: To 1 ml of test solution or filtrate, a drop or two of Hager's reagent is added. Formation of yellow precipitate indicates the test as positive.

III. PHYTOSTEROLS

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

IV.TRITERPENOIDS

1.Salkowski Test : Approximately 2 mg of dry extract is shaken with 1 ml of chloroform and add few drops of concentrated sulfuric acid is added along the sides of the test tube**.** Red brown color at the interface indicates the presence of triterpenoids.

V.FLAVONOIDS

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

2. Alkaline reagent test- To 1 ml of test solution added 5 drops of 5% sodium hydroxide. An increase in the intensity of yellow colored solution is seen which becomes colorless on addition of few drops of 2 M hydrochloric acid.

VI. SAPONINS

1. Foam Test: 5 ml of the test solution is taken in a graduated cylinder and shaken well for five minutes .A stable foam is formed.

2. **Olive oil Test**: Few drops of olive oil is added to 2ml of the test solution and shaken well. A soluble emulsion is formed.

VII. CARDIAC GLYCOSIDES

:**Keller -Killiani Test:** To a little of dry extract added 0.4 ml of glacial acetic acid and a few drops of 5% ferric chloride solution. Subsequently add 0.5 ml of concentrated sulfuric acid along the side of the test tube carefully. The blue colour in acetic acid layer confirms the test.

VIII. ANTHRAQUINONE GLYCOSIDES

Hydroxyanthraquinone Test: To 1 ml of extract, add few drops of 10% potassium hydroxide solution**.** The Red color produced confirms the test.

IX.TEST FOR CARBOHYDRATES:

1. Molisch"s test: To 1 ml of test solution add few drops of 1 % alpha-napthol and 2-3 ml concentrated sulfuric acid along the sides of test tube. Reddish violet or purple ring formed at the junction of two liquids.

2. Barfoed's test: To 2ml of reagent add 2 ml of test solution, mix & keep in boiling water bath for 1 min. Cool & add phosphomolybdic acid drop wise until the solution is clear. A deep blue color indicates the presence of monosaccharide and light blue color indicates the presence of disaccharide.

3. Seliwanoffs test: To 3 ml of Seliwanoffs reagent added 1 ml of the test sample and heated on a water bath for one minute. Formation of rose red color confirms carbohydrate

4. Fehlings Test: Dissolved 2 mg dry extract in1 ml of water and added 1ml of Fehling's(A+B) solution , shake and heat on water bath for 10 minutes –Brick red precipitates formed confirms the test

.IX.TEST FOR PROTEINS

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

X .TEST FOR AMINO ACIDS

1. Xanthoproteic test: Added 1ml of concentrated nitric acid to 3 ml of the test solution, shaked and heated for 1 min and cooled. A change of yellow (acid media) to orange color (alkaline media) on adding 1 ml of 40% NaOH solution confirms the presence of proteins with amino acids carrying aromatic groups.

1. **Sakaguchi test:** To 3ml of test solution added 2 drops of 40%NaOH,4 drops of alpha naphthol and 10 drops of bromine water and mixed. Red color confirms the test

2. 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. Red color confirms the test

XI. FATS AND FIXED OILS: To 5 drops of sample add 1 ml of 1% copper sulphate solution and a few drops of 10% sodium hydroxide.A clear blue solution formation indicates the presence of fats

RESULTS AND DISCUSSION

Terminalia chebula (English name: Chebulic myrobalan) a native plant of India is an important medicinal plant . The fruits are astringent, purgative, laxative, gastroprotective and are used to alleviate asthma, piles and coughing.[22] The pericarp of the dried ripe fruit is

used widely for therapeutic purposes[2, 4] A composite mixture of *T.chebula* (Haritaki) , *Terminalia bellerica* (Bibhitaki) and *Emblica officinalis* (Amla) called triphala is used for the treatment of many chronic diseases, heart ailments and hepatic diseases. [2,5,7] Medicinal plants are of prime importance to the health of individuals and communities and the medicinal values of these economically important plant species is due to the presence of certain secondary metabolites which produce a definite physiological action on human body [23.] The yield and phytochemical analysis of successive fractions of various extracts of fruits of *T. chebula* obtained in the present study using petroleum ether, chloroform ethanol and water (aqueous) solvents along with blank and control are as detailed in Table 1 and 2 and Figs 2 to 22. The present study records the highest yield in the case of ethanol followed by water, chloroform and petroleum ether solvents used in succession (Table.1).The phytochemical evaluation for various phytoconstituents in successive fractions of fruits of T. *chebula* were graded as very high (++++) ; high (+++) ; moderate (++) ; low (+) ; negligible (±) 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 2

List of phytochemicals in successive extracts of pericarp of fruit of Terminalia chebula using petroleum ether, chloroform, ethanol and water in succession along with blank (water) and positive control*

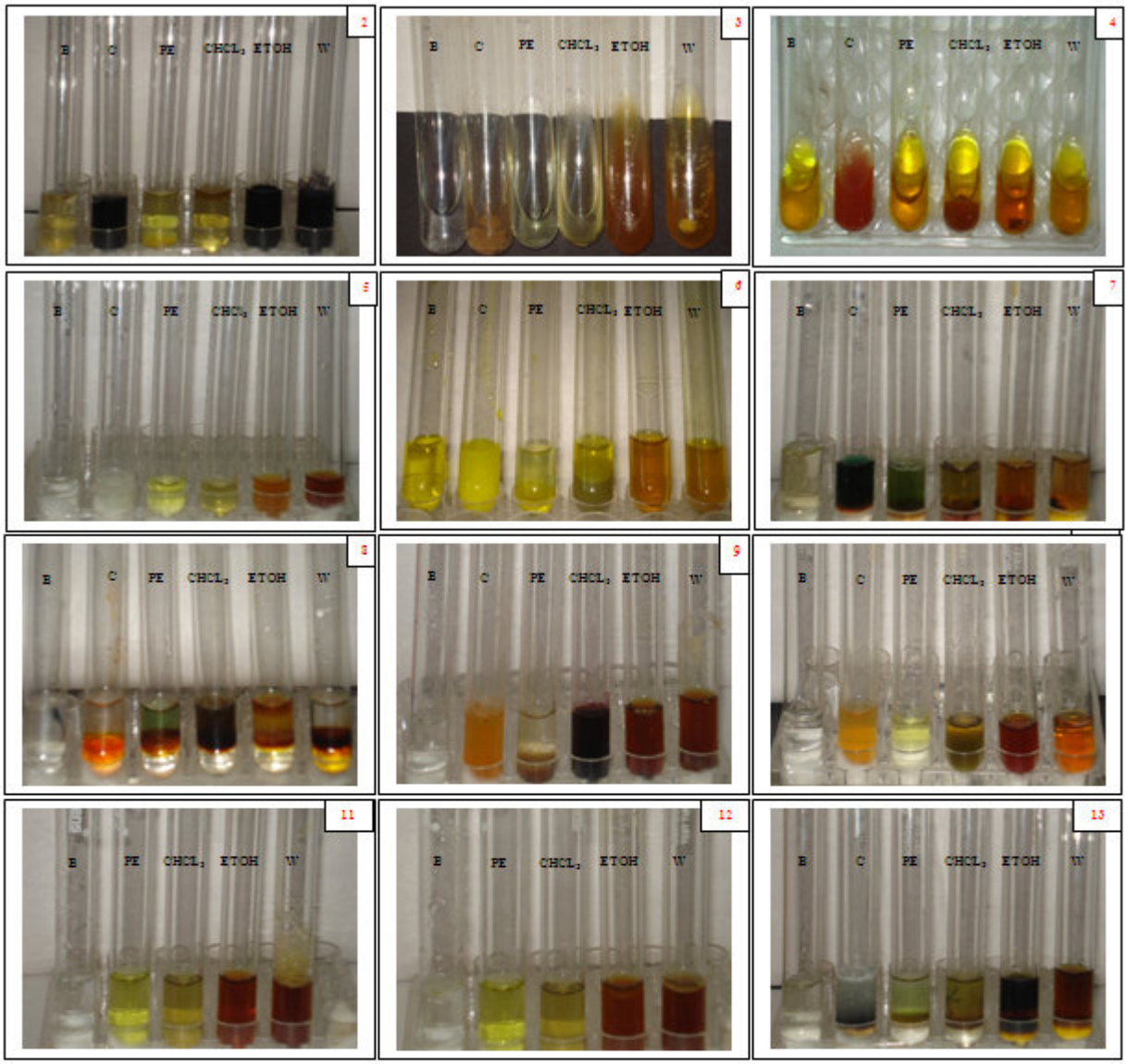
TEST		BLANK	CONTROL	PETROLEUM ETHER	CHLOROFORM	ETHANOL	WATER
TANNINS	Ferric chloride test	_	++++	_	_	+++	+++
	Gelatin Test	_	++	_	_	++	++
ALKALOIDS	Dragendorff's test	_	+++	+	+++	++	+
	Mayer's Test	_	+++	_	_	±	±
	Hager's test	_	++++	_	+++	±	+
PHYTOSTEROLS	Liebermann-Burchard Test	_	+++	++	+	_	_
TRITERPENOIDS	Salkowski Test	_	++	++	±	++	++
FLAVONOIDS	Shinoda Test	_	+++	_	+++	++	++
	Alkaline reagent Test	_	+++	_	+	+++	++
SAPONINS	Foam test	_	++++	_	_	_	++
	Olive oil test:	---	+++	_	_	_	++
CARDIAC GLYCOSIDES	Keller Killani test	_	++++	_	_	_	_
HYDROXYANTHRO-QUINONES GLYCOSIDE		_	++++	_	++	++	+++
CARBOHYDRATES	Molisch's test	_	++++	+	++	++++	++++
	Fehling's test	_	+++	_	+	+++	++
	Barfoed's test	_	++++	_	_	++++	++++
	Seliwanoff's test	_	++++	+	+++	++	++
PROTEINS	Biuret Test	_	+++	_	_	_	_
AMINOACIDS	Xanthoproteic test	_	++++	_	_	++++	+++
	Sakaguchi test	_	++++	_	_	+++	++++
	Millon's test	_	++++	_	_	++	_
FIXED OILS & FATS		--	++++	--	--	--	--

++++ (Very High) +++ (High), ++ (Moderate)+ (low) ± (negligible) and - (Nil)

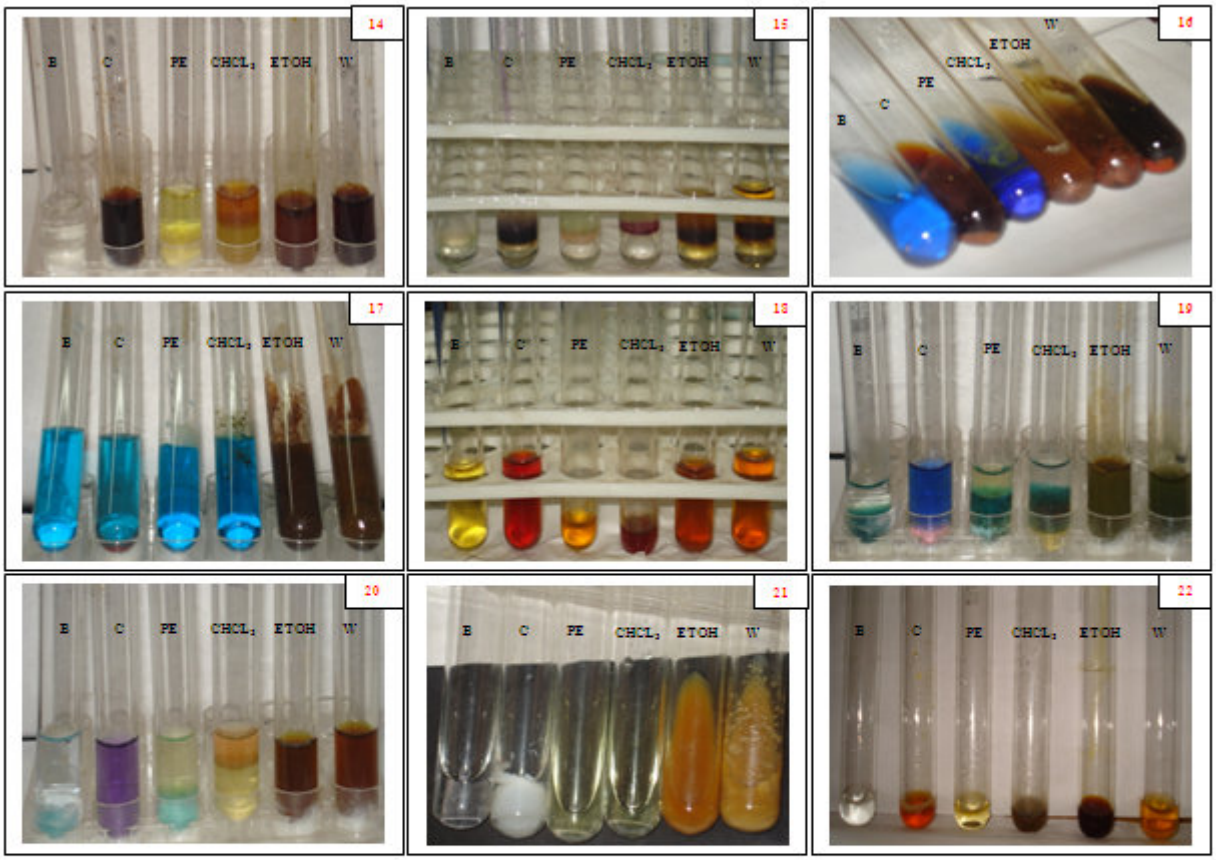
***Positive controls:* 1.Tannins -Tannic acid., 2. Alkaloids -Quinine sulphate., 3.Phytosterols –Cholesterol., 4. Triterpenoids – cholesterol., 5. Flavonoids -Lemon skin juice., 6. Saponins –foam test: pea powder & olive oil test: oat powder., 7.-Cardiac glycosides - *Calotropis latex.*, 8. Hydroxy anthroquinone glycosides- Hydroquinone., 9. Molisch's and Barford 's test –Glucose 10. Fehling and Seliwanoff test- fructose.,11.Biuret test-Bovine.serum albumin.,12.Xanthoproteic test – Tyrosine., 13.Sakaguchi – Arginine.,14. Millon's - Tyrosine and 15. Fixed oils & fatty acids – Glycerine.**

The phytochemical tests employed indicate the presence of hydrolysable tannins in ethanol and aqueous extracts and absence in the petroleum ether and chloroform extracts. The Dragendorff's, test for alkaloids was positive in all the extracts whereas the Hager's was highest in chloroform and negligible in ethanol and low in aqueous extracts whereas Mayer's test was negative in petroleum ether and chloroform extracts and negligible in ethanol and aqueous extracts. The Liebermann-Burchard's test for phytosterols was positive in petroleum ether and chloroform and negative in ethanol and aqueous extracts. The Salkowski test for triterpenoid was positive in all extracts exhibiting negligible amount of triterpenoids in chloroform extract. The shinoda and alkaline reagent test for flavonoids were negative in petroleum ether extract only. The shinoda test indicated the highest amount of flavonoids in chloroform whereas the alkaline reagent test exhibited the highest amount in ethanol extract. The foam and olive oil tests for saponin's were positive only in aqueous.extract. The hydroxyanthroquinone test was positive in chloroform, ethanol and aqueous extracts and negative in petroleum ether extract. The Molisch tests for carbohydrates indicated the presence of a very low amount of carbohydrates in petroleum ether compared to other extracts. However, Fehling's tests was negative only in petroleum ether and Barfoed's test was negative both in the case of petroleum ether and chloroform extracts exhibiting the absence of the reducing sugars. The Seliwanoff's test indicated the presence of keto sugars in all the extracts exhibiting low amount in petroleum ether extract. The Xanthoproteic (specific for nitro derivatives of the aromatic amino acids) and Sakaguchi tests (specific for guanidine group containing amino acid arginine) were positive in ethanol and aqueous extracts whereas Millons test (hydroxyl phenol group of tyrosine) was positive only in ethanol extract. Tests for protein (Biuret), cardiac glycosides (Kellar-Killiani's) and fats and fixed oils were negative in all the successive extracts.

Earlier reports[12, 16, 17-19] on preliminary phytochemical analysis have documented the presence of phytoconstituents in extract of fruits of *T. chebula* using single solvent. However, in present study, the phytochemical analysis of successive re-extracts obtained following extraction using petroleum ether, chloroform, ethanol and water points to the presence of diverse active principles having selective solubility in solvents of varying polarities. Basic phytoinvestigations of the extracts for their major phytocompounds is vital as the active principles of many drugs are these secondary metabolites found in plants.[24] The selective solubility of bioactive constituents in the present study points to the presence of diverse phytochemicals suggesting that the secondary metabolites vary widely which probably is responsible in conferring a wide spectrum of biological activities[25]. In addition the data suggest that the fruits of *T. chebula* is highly nutritious and could be a source of dietary supplement being rich in carbohydrates and amino acids[26]



Figs 2 to 13: Demonstration of phytochemicals in blank (**B**) positive control (**C**) and the successive extracts of *Terminalia chebula* in petroleum ether (**PE**), chloroform (**$CHCL_3$**), ethanol (**ETOH**) and water (**W**) exhibiting (**2**) -**Ferric chloride** and (**3**) - **Gelatin test** for **tannins** (positive in ethanol and aqueous extracts)., (**4**)- **Dragendorff's** test (positive in all exhibiting highest activity in chloroform extract)., (**5**) - **Mayer's** (negative in petroleum ether and chloroform extracts and negligible in ethanol and water extract) and (**6**)- **Hager's** test (Highest in chloroform and negative in petroleum ether and negligible in ethanol and aqueous extract) **for alkaloids** ., (7)- **Liebermann** test for **steroids** (positive in petroleum ether and chloroform extracts)., (**8**) - **Salkowski** test for triterpenoids (positive in all extracts exhibiting negligible amount in the chloroform extract)., (**9**)- **Shinoda** and (**10**) - **Alkaline** reagent test **for flavonoids** (positive in chloroform, ethanol and water extracts and absent in petroleum ether extract)., (**11**) - **Foam and** (**12**)- **Olive oil test for saponins** (positive in water extracts only) and (**13**)- **Keller Kiilani** test for cardiac glycosides (negative in all extracts).



Figs 14 to 22: Demonstration of phytochemicals in blank (**B**) positive control (**C**) and the successive extracts of *Terminalia chebula* in petroleum ether (**PE**), chloroform (**$CHCL_3$**),ethanol (**ETOH**) and water (**W**) exhibiting (**14**) **hydroxyanthroquinone** test **for glycosides** (positive in chloroform, ethanol and aqueous extracts)., (**15**) **Molisch** test (positive in all exhibiting low amount in petroleum ether extract)., (**16**) **Fehling test** (positive in chloroform, ethanol and aqueous extracts).,(**17**) **Barfoed's test** (negative in petroleum ether and chloroform extracts) and (**18**) **Seliwanoff's test** (positive in all extracts) **for carbohydrates** (**19**) **Saponification** test for **fats & fixed oils** (negative in all extracts)., (**20**) **Biuret** test -(negative in all extracts) for proteins (**21**) **Millon's** test (positive in ethanol extract only) and (**22**) **Xanthoproteic** test (positive in ethanol and aqueous extracts) **for amino acids.**

CONCLUSION

The phytochemical analysis of successive re-extracts obtained following extraction using petroleum ether, chloroform, ethanol and water in succession points to the presence of diverse active principles having selective solubility in solvents of varying polarities which probably may be responsible in conferring a wide spectrum of biological activities attributed to the fruits of *T chebula*. In addition, the successive extraction using solvents of varying polarities can maximize the usage of diverse bioactive compounds. The data also suggest that the pericarp of the fruit of *T.chebula* is highly nutritious and could be a source of dietary supplement being rich in carbohydrates and amino acids. The present information would be of help to isolate and characterize diverse pharmacologically active principles from the successive extracts.

CONFLICT OF INTEREST DECLARED NONE

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