

PHYSICOCHEMICAL AND PHYTOCHEMICAL ANALYSIS OF TRIPHALA IN AQUEOUS AND METHANOLIC EXTRACTS

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ABSTRACT

Triphala is an ayurvedic provision made out of three equivalent ratios of herbal dried fruits indigenous to the Indian subcontinent namely Terminalia chebula, Emblica officinalis, and Terminalia bellirica. Triphala is the most widely recognized element of more than 400 conceptualizations demonstrated for various illnesses. The present examination plans to give a review of the compound constituents present in the primitive distills of dried powdered Triphala with exceptional accentuation on their pharmacological activities. The powdered Triphala distills have been riddled for phytochemical

constituents in two unique solvents water and methanol. Preliminary phytochemical investigation divulged the comportment of compounds like alkaloids, flavonoids, tannins, phenols, saponins, diterpenes, sugars, and terpenoids and so on.

KEYWORDS: Ayurveda, Triphala, organoleptic, phytochemical analysis.

INTRODUCTION

Nature gives all the answers for humanity issues such as the ayurvedic medication inferred from the animals, plants, and marine. Ayurveda is the well known conventional Indian medicinal system being used for a great many years. Numerous raw products such as plants, animals, and minerals have been utilized for the intervention of human illness. The advanced pharmaceutical industry has recognized the significance of Ayurveda and subsequently now number of medications is being prefaced utilizing these natural products for preparing effectual medications against numerous illnesses.^[1]

Today, pharmaceutical researchers are encountering trouble in distinguishing new lead structures, layouts, and frameworks in the limited universe of chemical diverseness. Various synthetic medications have contrary and insufferable side effects. We are acquiring increasingly more reliant upon and have turned out to be basically inebriated with medications. Numerous medications are giving out because of indecorous or harmful effects, and the pursuance for beneficial and more secure medications proceeds considerably more forcefully. In India, corroboration and utilization of therapeutic plants began amid the Vedic time of which in excess of 100 medicinal plants are found. Charaka Samhita and Sushruta Samhita compromise definite depictions of more than 800 medicative herbs and more than 8000 conceptualizations. The utilization of natural medicine is picking up force in this period on account of the side effects of synthetic drugs, and the wellbeing, productivity and promising capability of plant inferred medication. Triphala is a tridoshic rasayana having an adjusting and rejuvenating outcome on the three built-in components that oversee the human life. Triphala is wealthy in antioxidants, antibacterial, antiviral and against malignancy properties. Triphala is customarily been utilized as purgative in chronic constipation, colon clean up, digestion troubles and poor food acculturation. The current work endeavours to assess the organoleptic and fundamental phytochemical characters of the dried mesocarp of *Terminalia bellirica*, *Terminalia chebula*, and *Embllica officinalis*.^[2]

METHODS AND MATERIALS

Preparation of powder

All the raw materials were cleaned to remove any foreign materials and dust. The samples were subjected to organoleptic and phytochemical study so as to generate inputs that can be considered for laying down standards in respect of this plant. The fruits were dried in shade. The dried pericarp and mesocarp of fruits were pulverised into fine powder using a stainless steel electrical blender, passed through #100 mesh sieves and stored in an airtight container for physicochemical and phytochemical screening.

Preparation of aqueous and alcoholic extracts of powder^[3,4,5,6]

An accurately weighed 50 gm of powder formulation was subjected to hot percolation in a Soxhlet extractor (Sisco) with 500 ml of distilled water and 75% of methanol at 80°C as solvents to get aqueous and alcoholic extract respectively. The resultant extract was concentrated using rotary vacuum evaporator (Buchi type – Sisco) below 40°C. This concentrated extract is evaporated to dryness over a water bath and stored in desiccator using

indicator silica gel. The amount of extract obtained with solvent was weighed and calculated the percentage extractive yield.

Table 1: Organoleptic characters of extracted powder.

S. No	Parameters	<i>Terminalia bellirica</i>	<i>Terminalia chebula</i>	<i>Emblica officinalis</i>
1	Odour	Aromatic	Pungent	Aromatic
2	Colour	Yellowish brown	Yellow	Yellowish green
3	Taste	Astringent	Astringent	Sour
4	Consistency	Solid - powder	Solid - powder	Solid - powder

Table 2: Results of extract preparations.

S. No	Extract	Color	Type of extract	Consistency	% yield
1	Aqueous	Brownish	Crude	Solid	47.83%
2	Methanol	Dark brown	Crude	Solid	51.72%

Physicochemical parameters^[7,8]

1. Total ash

2 g of the powdered drug was accurately weighed in a silica crucible which was previously ignited and weighed. The powdered drug was spread as a fine layer on the bottom of the crucible. Crucible was incinerated at a temperature not exceeding 450°C until free from carbon. The crucible was cooled and weighed. The procedure was repeated to get a constant weight. The percentage of total ash was calculated with reference to their air dried drug.

$$\text{Total ash \%} = \frac{z - x}{y} * 100$$

x = weight of empty dish.

y = weight of Triphala

z = weight of empty dish + ash (after incineration)

2. Acid insoluble ash

The ash obtained in the determination of total ash was boiled for 5 min with 25 ml of dilute HCl. The insoluble matter was collected on an ashless filter paper (Whatmann) and washed with hot water. The insoluble ash was transferred to a preweighed silica crucible and then ignited, cooled and weighed. The procedure was repeated to get a constant weight.

$$\text{Acid insoluble ash} = \left(\frac{a}{y}\right) * 100$$

a = weight of acid insoluble residue.

y = Weight of Triphala used

3. Water soluble ash

The water insoluble matter was collected on an ashless filter paper and ignited in an electric furnace at 450°C in silica crucible until it reaches a constant value. The weight of insoluble matter was subtracted from the weight of total ash to indicate the weight of water soluble ash.

$$\text{Water soluble ash} = \frac{a - b}{y} * 100$$

a = weight of residue after incineration.

b = weight of water insoluble residue.

y = weight of Triphala used.

4. Water soluble extractive value

10 gm of dried coarsely powdered drug was macerated with 100 ml of distilled water in a flask for twenty four hours shaking intermittently. The solution was filtered and 25 ml of the filtrate was evaporated in a shallow dish. It was dried and weighed. The percentage of water soluble extractive was calculated with reference to the dried drugs.

$$\% \text{ of water soluble extractive} = \frac{\text{weight of the residue}}{\text{weight of the drug}} * 100$$

5. Alcohol soluble extractive value

About 10 gm of coarsely powdered drug was accurately weighed in a conical flask. 100 ml of alcohol as added and weighed. It was shaken well and allowed to stand for 1 hour. It was refluxed for 6 hours, cooled and weighed. The extracted powder was dried in an oven. The content of extractable matter was calculated.

$$\% \text{ of alcohol soluble extractive} = \frac{\text{weight of the residue}}{\text{weight of the drug}} * 100$$

6. Loss on drying

The powdered drug sample of 10 gm was placed on a tarred evaporating dish at 105°C for 6 h and weighed. The drying was continued until two successive readings matched each other or the difference between two successive weighing was not more than 0.25% of constant weight.

$$\% \text{ Loss on drying} = \frac{W_{\text{initial}} - W_{\text{final}}}{W_{\text{initial}} - W_{\text{tare}}} * 100$$

Table 3: Results of physiochemical evaluation of Triphala extract.

S. No	Parameters	Results
1	Total ash	19.21% ± 0.65
2	Acid insoluble ash	3.62% ± 1.1
3	Water soluble ash	5.23% ± 0.93
4	Water soluble extractives	46.2% w/w
5	Alcohol soluble extractives	64.6% w/w
6	Loss on drying	5.2% ± 0.34 w/w
7	Foreign matter	Nil
8	pH (solution)	6.2

Preliminary phytochemical screening^[1,8,9]**1. Tests for alkaloids**

a. Dragendorff's Test: 2 ml of the extract was treated with a few drops of Dragendorff's reagent (solution of potassium bismuth iodide). Formation of orange brown precipitate indicates the presence of alkaloids.

b. Mayer's Test: 2 ml of the extract was treated with a few drops of the Mayer's reagent (Potassium mercuric iodide solution). Formation of a cream coloured precipitate indicates the presence of alkaloids.

2. Test for Glycosides

a. Keller-Kiliani Test: The extract was mixed with 2 ml of glacial acetic acid containing 1 or 2 drops of freshly prepared ferric chloride solution. The mixture was shaken well and was carefully poured into a test tube containing concentrated sulphuric acid along the sides. Formation of a brown ring at the junction indicates the presence of cardiac glycosides.

3. Test for flavonoids

a. Shinoda Test: Crude extract was mixed with a few small pieces of Magnesium ribbon for a minute and a few drops of concentrated HCl was added drop wise into this mixture. Development of pink scarlet colour or light red colour after a few minutes indicates the presence of flavonoids.

b. Lead acetate Test: Small quantity of the extract was treated with a few drops of lead acetate solution. Formation of yellow colour or yellow creamy precipitate indicates the presence of flavonoids.

c. Alkaline reagent Test: The extract was mixed with 2% NaOH solution. Intense yellow colouration which loses the intensity on the addition of dilute acid indicates the presence of flavonoids.

4. Test for tannins

a. Ferric chloride Test: 2 ml of freshly prepared ferric chloride solution was added to 2 ml of the concentrated extract. Formation of dark blue or green or black colour indicates the presence of tannins.

5. Test for phenols

a. Ferric chloride Test: To 2 ml of the extract, 2 ml of freshly prepared ferric chloride solution was added. The development of blue-green or black colour indicates the presence of phenols.

6. Test for saponins

a. Froth test: 2 ml of the extract was mixed with 20 ml of distilled water in a graduated test tube and shaken well for 10 minutes. Formation of 1 cm thick froth indicates that the sample contains saponins.

7. Test for sterols

a. Liebermann-Burchard Test: 2 ml of the extract was mixed with a few drops of acetic anhydride. It was boiled and cooled and concentrated sulphuric acid was added along the sides of the test tube carefully. A brown ring at the junction of two layers and the upper layer turning green indicates the presence of sterols.

8. Test for diterpenes

a. Copper acetate Test: 2 ml of the extract was mixed with 3-4 drops of copper acetate solution and shaken well. The formation of green colour indicates the presence of diterpenes.

9. Test for carbohydrates

a. Molisch's test: 2 ml of the extract was taken in a test tube and few ml of Molisch's reagent was added along the sides. Formation of violet ring at the junction indicates the presence of carbohydrates.

b. Fehling's test: 1 ml each of Fehling's solution A and B were mixed and boiled for one minute. Equal volume of the extract was added and then boiled in a water bath for 5 minutes. Formation of reddish brown colour indicates the presence of reducing sugar.

c. Iodine test: 1 or 2 drops iodine solution was added to 1ml of the extract. Formation of dark blue colour indicates the presence of carbohydrates.

10. Test for proteins and amino acids

a. Ninhydrin test: 3 ml of the extract was boiled with 3 drops of 5% Ninhydrin solution. Formation of blue or violet colouration indicates the presence of amino acids.

b. Xanthoproteic test: The extract was treated with a few drops of concentrated nitric acid. Formation of yellow colour indicates the presence of proteins.

11. Test for terpenoids

a. Salkowski test: 5 ml of the extracts were mixed with 2ml of chloroform and 3ml of conc. H₂SO₄ solution. A reddish brown colour at the interphase indicates the presence of terpenoids.

Table 4: Results of phytochemical screening of Triphala extracts.

S. No	Name of tests	Results	
		Aqueous Extract	Methanolic extract
Test for alkaloids			
1	Dragendroff's	-ve	+ve
2	Mayer's	-ve	+ve
Test for glycosides			
3	Keller-Kiliani	+ve	-ve
Test for flavonoids			
4	Shinoda	-ve	-ve
5	Alkaline Reagent	+ve	+ve
6	Lead Acetate	+ve	-ve
Test for tannins			
7	FeCl ₃	+ve	+ve
Test for phenols			
8	FeCl ₃	+ve	+ve
Test for saponins			
9	Froth test	+ve	+ve
Test for sterols			
10	Libermann - Burchard Test	-ve	-ve
Test for diterpenes			
11	Copper acetate test	+ve	+ve
Test for carbohydrates			
12	Molisch's test	+ve	-ve
13	Fehling's test	+ve	+ve
14	Iodine test	+ve	+ve
Test for proteins & aminoacids			
15	Ninhydrin test	+ve	-ve
16	Xanthoproteic test	+ve	+ve
Test for Terpenoids			
17	Salkowiski Test	+ ve	+ve

PHYTOCHEMICAL CONSTITUENTS OF TRIPHALA**1. Tannin^[10]**

It has a property of astringency. It exposes anti-microbial action by demobilizing microbial enzymes, adhesion etc. It possess an extensive array of anti-infective, stimulation of phagocytic cells, host-arbitrated neoplasm activity etc.

2. Alkaloid – Quinone^[10,11]

Alkaloids are produced by a large variety of organisms including bacteria, fungi, plants, and animals. They can be purified from crude extracts. The possible ranges of quinone anti-microbial effects are declamatory. Vitamin K is a composite naphthoquinone with anti-hemorrhagic process.

3. Flavonoids^[10,11]

Flavones are phenolic constructions curbing one carbonyl group. These are very effective antimicrobial substances may since they are synthesized by plants in response to microbial infection.

4. Phenol - Gallic acid^[10,11]

This is a vernacular phytochemical constituent present in all three herbaceous plants used in Triphala. It oppresses the ontogenesis of cancer cells. It has antioxidant and immunomodulatory properties.

5. Ascorbic acid^[10]

Juice of amla has mellowest vitamin C of 478.56 mg/ 100 ml. The nutritional lineament of ascorbic acid content is enhanced when it is coalesced with other herbaceous fruits. Ascorbic acid defenses for about approximately 45 – 70% of antioxidant activity. Along with bioflavonoid, it facilitates in hastening up the sanative process.

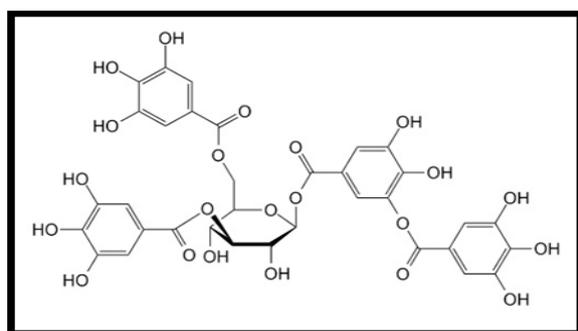


Fig 1: Structure of Tannic acid

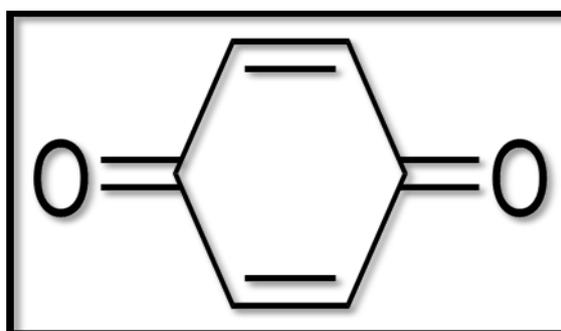


Fig 2: Structure of quinone

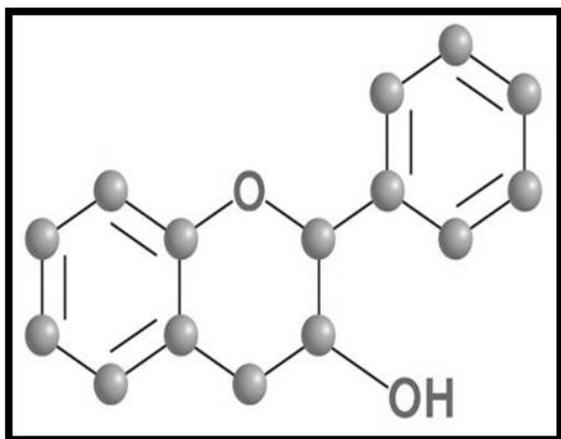


Fig 3: Structure of flavonoids

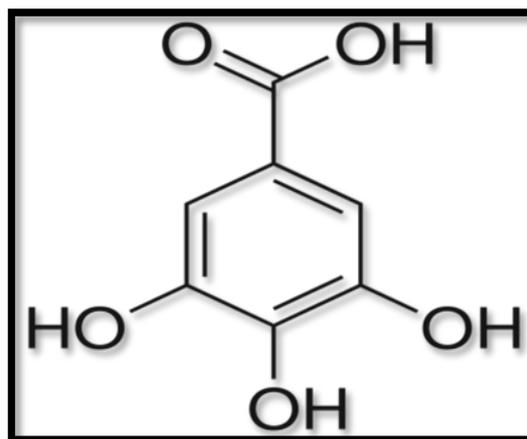


Fig 4: Structure of gallic acid

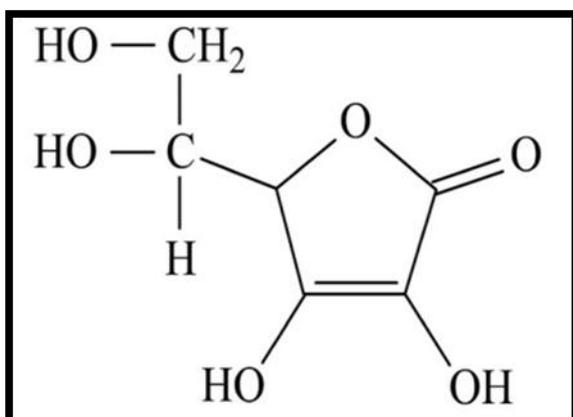


Fig 5: Structure of ascorbic acid

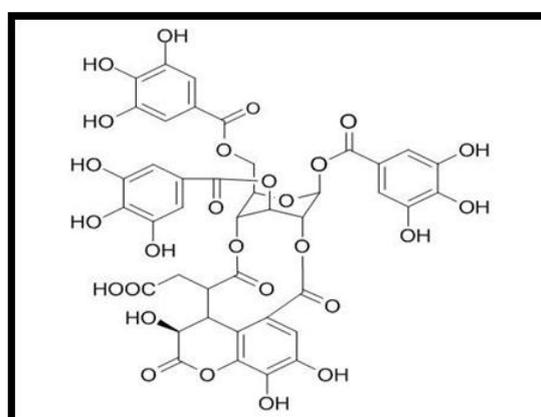


Fig 6: Structure of chebulinic acid

6. Hydrolyzable tannin - Chebulinic acid^[12]

It is an ellagitannin present in the fruits of *Terminalia chebula*. It is an anti-hyperglycaemic, anti-fungal, anti-bacterial etc. It showed many bioactivities including inhibition of cancer cell growth. It helps in removing toxins and fat from body resulting in their reduced absorption.

7. Chebulagic acid^[13]

Chebulagic acid is a benzopyran tannin and an antioxidant that has many potential uses in medicine. It has been found to be immunosuppressive, hepatoprotective, and a potent alpha-glucosidase inhibitor, a human gut enzyme useful in diabetic studies. It has been shown to be active against *Staphylococcus aureus* and *Candida albicans*. It is found in the plants *Terminalia chebula* Retz.

8. Epicatechin^[14]

It exerts powerful antioxidant activity. It is described as slightly astringent but not bitter. It reduces the risk of cardiovascular diseases and cancer.

9. Soluble sugar^[15]

Being a composite mixture of three dried fruits, Triphala powder is extremely copious in sugar concentration. Triphala powder samplings of herbal practitioners and local fabricators were enormous in total soluble sugar concentration when compared to the samples of multinational and territorial fabricators.

10. Ellagic acid^[16]

Ellagic acid is a natural phenol antioxidant found in numerous fruits and vegetables. The antiproliferative and antioxidant properties of ellagic acid have prompted research into its potential health benefits. Ellagic acid is the dilactone of hexahydroxydiphenic acid.

11. Saponins^[17]

These are a class of chemical compounds found in particular abundance in various plant species. More specifically, they are amphipathic glycosides grouped phenomenologically by the soap-like foam they produce when shaken in aqueous solutions, and structurally by having one or more hydrophilic glycoside moieties combined with a lipophilic triterpene or steroid derivative.

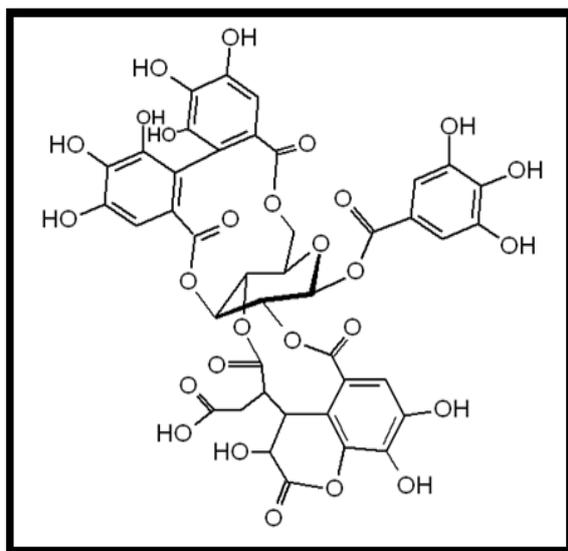


Fig 7: Structure of chebulagic acid

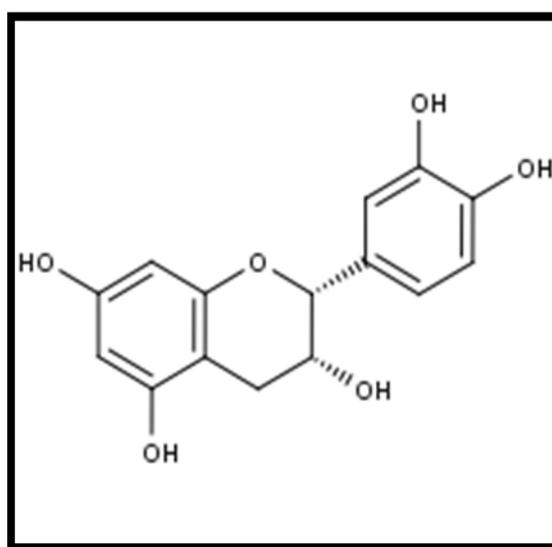


Fig 8: Structure of epicatechin

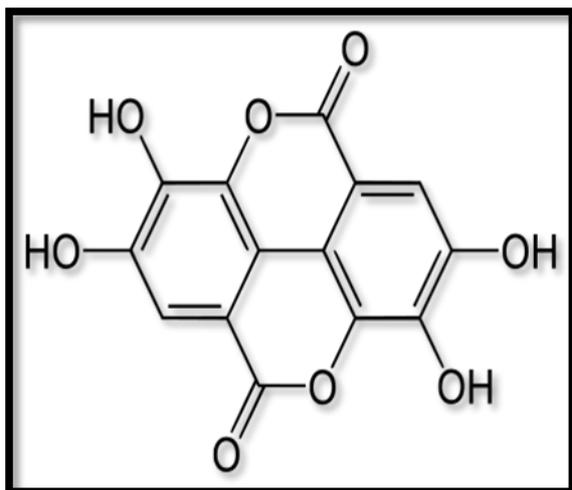


Fig 9: Structure of ellagic acid

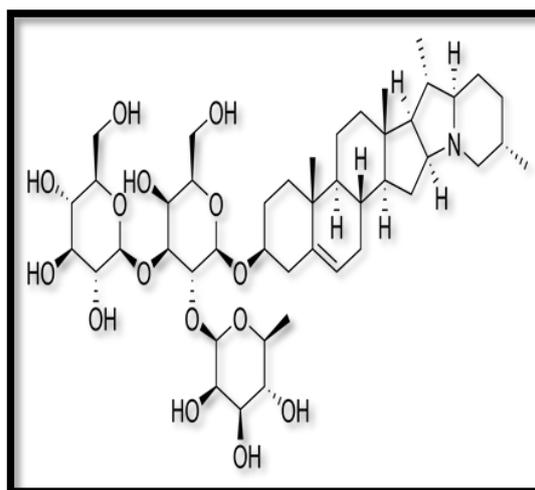


Fig 10: Structure of Saponin

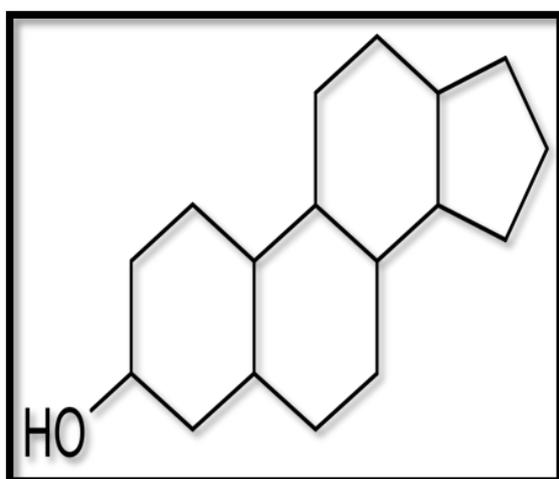


Fig 11: Structure of Sterol

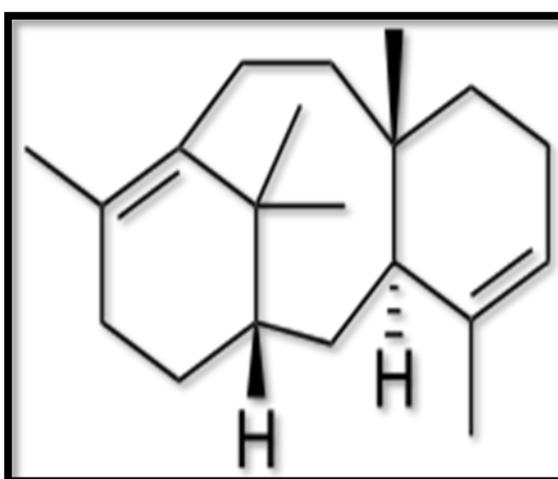


Fig 12: Structure of diterpenes

12. Sterols^[18]

They are also known as steroid alcohols, are a subgroup of the steroids and an important class of organic molecules. They occur naturally in plants, animals, and fungi, with the most familiar type of animal sterol being cholesterol. Cholesterol is vital to animal cell membrane structure and function and a precursor to fat-soluble vitamins and steroid hormones.

13. Diterpenes^[19]

Diterpenes are a class of chemical compounds composed of two terpene units, often with the molecular formula $C_{20}H_{32}$. Diterpenes consist of four isoprene subunits. They are biosynthesized by plants, animals and fungi via the HMG-CoA reductase pathway, with geranyl-geranyl pyrophosphate being a primary intermediate. Diterpenes form the basis

for biologically important compounds such as retinol, retinal, and phytol. They are known to be antimicrobial and anti-inflammatory.

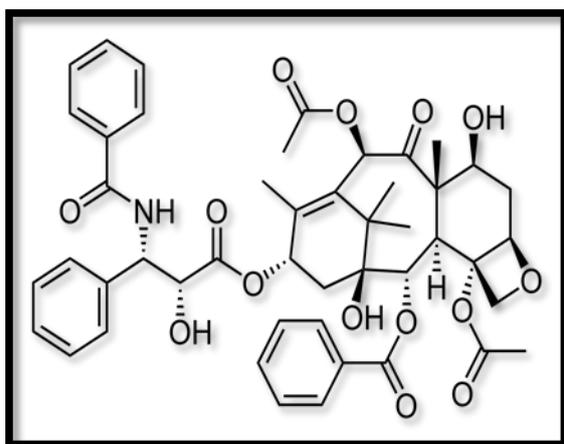


Fig 13: Structure of terpenoids

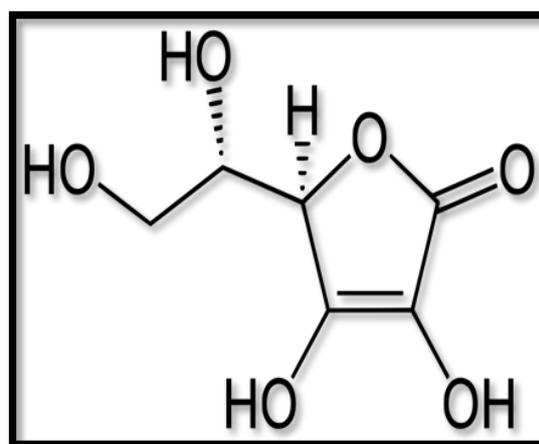


Fig 14: Ascorbic acid

14. Terpenoids^[20]

Plant terpenoids are used for their aromatic qualities and play a role in traditional herbal remedies. Terpenoids contribute to the scent of eucalyptus, the flavours of cinnamon, cloves, and ginger, the yellow color in sunflowers, and the red color in tomatoes.

CONCLUSION

On the basis of the results obtained, the present work conclude that the test extracts of Triphala powder are rich in phytochemical constituents even though the screening of the samples had shown variation in their phytochemical constituents with the presence and or absence of some components. Most components were present in both water and methanol extracts. The test samples were found to be suitable in the elucidation of bioactive components which could be used effectively in the treatment of several ailments but the efficacy of the products can only be judged by doing the pharmacology of which is suggested as future scope of research and development. This obtained information also will be helpful as a primary platform for further phytochemical, pharmacological studies, and future researches of this species.

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