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RESEARCH ARTICLE

PHYTOCHEMICAL SCREENING OF METHANOLIC EXTRACT OF AERIAL PARTS OF *TREMA ORIENTALIS*

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Abstract: *Trema orientalis* is a small to medium sized tree belonging to Cannabaceae family. This species was a typical pioneer plant used in traditional medicine for the treatment of ailments in tropical regions, mainly in Asia. This species of Tremawas often used to cure infections and fevers. It is commonly called as gun powder tree, Indian charcoal tree, Indian nettle, oriental Nettle and Pigeon wood. Although there were numerous traditional claims of the medicinal properties of the aerial parts of the plant, the reports of incidences of suspected toxicity in goats necessitated the studies on phytochemical constituents of *T. orientalis*. As per the information received regarding the incidences of the plant toxicity, the ailing goats had exhibited abnormal clinical signs such as in coordination, apathy, tenesmus and paddling movements. An experimental study was undertaken to assess the phytochemical constituents of *T. orientalis*. by using methanol as solvent for the extraction of aerial parts of the plant. The sieved plant powder was soaked in methanol at 1:5 ratio in glass containers for one week and then filtered and subjected to extraction using rotary evaporator. Finally obtained powdery extract was used for phytochemical screening tests. The distilled water was used as solvent for extract for some of the tests. All the tests were performed as per standard protocol using the chemicals and reagents procured from authenticated chemical manufacturers and results were recorded. The Physical characteristics, per cent yield were also estimated in which per cent yield was 12.38, greenish black colour and powdery consistency of extract were also found. The phytochemical screening of *T. Orientalis* revealed the presence of carbohydrates, starch, balsam, flavonoids, glycosides, phenolic compounds, phytosterols, triterpenoids, philobatannins, tannins, saponins and volatile oils, while the extract was negative for the presence of alkaloids, amino acids and protein. The phytoconstituents of the plant might play an important role in therapeutic and toxic properties.

Keywords: *Trema orientalis*, Phytochemical, Therapeutic, Toxic, Methanol

INTRODUCTION

Trema orientalis (L.) Blume is a small to medium sized tree belonging to Cannabaceae family. It is a shade tree with soft leaves that grows quickly, making it best suited for gardens and avenues. It has been used to make paper and poles. It has therapeutic qualities, which have been used medicinally to treat helminthic, inflammatory and respiratory conditions (Saleh *et al*., 2020).

Suspected toxicity conditions in goats after ingestion of *T. orientalis* leaves have been noticed by the veterinarians across Karnataka state particularly in and around Shivamogga and Dharwad district. As per the information received regarding the incidences of the plant toxicity, the ailing goats had exhibited abnormal clinical signs such as in coordination, apathy, tenesmus, paddling movements and coma before death on fifth day of ingestion of the plant.

The separation and identification of biologically active substances and molecules from the medicinal plants have contributed in discovery of new medicinal products, which in turn resulted in improving the pharmaceutical and health care sectors (Pye*et al*., 2017). Due to the safer pharmacological potentiality and significant therapeutic benefits, herbal medicines are in greater demand than ever before. However, in order to put the herbs-based medications on the market as main line therapies, efforts have to be made to investigate, standardize and validate them for their potency, safety and efficacy (Dubey *et al*., 2013).

Thus, in addition to the numerous traditional claims of the medicinal properties of the aerial parts of the plant and the incidences of suspected toxicity in goats, it necessitated the studies on the toxicological aspects and pharmacological properties of *T. orientalis* in laboratory animals. So, an experimental study was undertaken to assess the phytochemical constituents of *T. orientalis*.

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MATERIALS AND METHODS

The fresh aerial parts of *T. Orientalis* plant were gathered from regions of Sorabataluk, Shivamogga district, Karnataka in the months of April and May 2022 and the plant's taxonomic identification was verified by authorized botanist. The plant material was collected, cleansed under running tap water and then dried in the indoors for 20 days. The plant material was first chopped in to small pieces by multipurpose shredder, then mechanically ground in to a coarse powder in heavy duty jumbo flour and spice pulverizer (5HP AC motor) and sieved in to a fine powder, later both of them were stored in airtight containers for further use.

Chemicals

The chemicals employed in the current investigation were commercially purchased from authenticated dealers. Absolute ethanol, acetone, 10% anhydrous ferric chloride (An. $FeCl_3$), concentrated nitric acid (Conc. HNO_3), copper turnings, methanol, picric acid and 10% 1N Sodium hydroxide (NaOH) were procured from Sd Fine Chem. Ltd., Mumbai.

Chloroform, ether and 10% sodium chloride (NaCl) were procured from RFCL Ltd., New Dehli. 10% ammonia solution (NH_4OH), bromine water and concentrated sulphuric acid (Conc.H_2SO_4) by Nice Chemicals Pvt. Ltd, Cochin. α-naphthol, 0.1% ascorbic acid, concentrated 1M Hydrochloric acid (Conc. HCl), copper sulphate ($CuSO_4$), disodium hydrogen phosphate, DPPH (1,1-diphenyl-2-picrylhydrazyl), 1% gelatine, 1% glacial acetic acid, iodine, magnesium ribbon, mercuric chloride, 10% lead acetate, 1% potassium ferricyanide, sodium nitroprusside, sodium bi-carbonate ($NaHCO_3$), sulphanilic acid and Fehling's reagents 1 & 2 from (Hi-media Laboratories Pvt. Ltd.). Ninhydrin, potassium Iodide (KI), potassium chloride (KCl), potassium dihydrogen phosphate, 10% trichloro acetic acid from Merck Pvt. Ltd.

Reagents

Commercially available reagents purchased from Nice Chemicals (P) Ltd. included Barfoed's reagent, Benedict’s reagent, Biuret reagent, Dragendorff's reagent, Hager's reagent, Mayer's reagent, Millon's reagent, Molisch's reagent, Ninhydrin reagent, Robert’s reagent, Seliwanoff's reagent and Wagner's reagent.

Preparation of the extract

By using the particular solvents, the extraction procedures could separate the medicinally useful components of plant species from the inert components. The type of solvent employed in the extraction method is a key factor in the successful evaluation of biologically active chemicals from plant parts. The characteristics of a good solvent is featured by low toxicity, ease of evaporation at low temperatures, rapid physiological absorption of the extract, preservation effect and chemical stability are the characteristics of an ideal solvent in plant extractions. Methanolis used as the solvent to obtain biologically active compounds like anthocyanins, flavones, lactones, phenones, polyphenols, saponins, terpenoids, tannins and xanthoxyllines (Cowan, 1999).

The individual qualities of the bioactive components have been evaluated to determine the solvent system for the extraction process. It is possible to extract the bioactive ingredient from natural compounds using a variety of solvent systems. Polar solvents like methanol, ethanol, or ethyl acetate are used to extract hydrophilic substances. Dichloromethane or a 1:1 combination of dichloromethane and methanol is used to extract highly lipophilic substances. Hexane extraction is occasionally employed to separate chlorophyll (Cos *et al*., 2006).

Soaking

An electronic scale was used to weigh 1kg of fine powder, which was then soaked in 5 litres of methanol (99% SDFCL) at 1:5 ratio in glass containers which were kept closed at room temperature. For the first six hours, the mixture was stirred with an electric orbital shaker (REMI RS-12 plus) to ensure that the solvent and powder were properly mixed. Containers were shaken and stirred three times a day up to one week (Hossain *et al*., 2013).

Filtration and concentration

After one week, the contents were filtered through muslin cloth initially and then with Whatman No. 1 filter paper (Himedia, 24 cm) with the help of Buchner's funnel. As the solvent evaporated and the extract settled, the filtrates were then individually concentrated in vacuum using a Rotary Evaporator (DLAB RE100-Pro, China) at 40 °C with 92 rpm for 1 h. These were concentrated and dried completely in the SLM-INC-OS-250 incubator at 40 °C for one day. The extracts were kept in a refrigerator in airtight containers and aliquots were taken for the production of the pharmacological preparations and to perform phytochemical screening. The pH of the extract was measured using EUTECH pH tutor (Hossain*et al*., 2013).

Calculation of yield

The below formula can be used to estimate the per cent yield (dry weight of the extract) following the solvent extraction.

$$\% \text{ yield} = \frac{\text{Final weight of the extract}}{\text{Initial weight of the powder}} \times 100$$

Phytochemical screening

The Physical characteristics, per cent yield and phytochemical tests were performed on methanol solvent extracts of *T. orientalis*aerial parts for the presence of various phytochemical compounds: Alkaloids, amino acids, anthraquinone, balsam, carbohydrates, fat, flavonoids, glycosides, phenolic

compounds, philobatannins, protein, saponins, sterols, tannins, terpenoids and volatile oils.

Qualitative phytochemical analysis

Organic analysis of primary metabolites

The resultant extracts were subjected to qualitative chemical testing, by utilizing the established techniques for the observation and recognition of various phytochemical compounds (Ahmad *et al*., 2013; Ajayi*et al*., 2018; Ayoade*et al*., 2014; Harborne, 1998; Osanaiye*et al*., 2014; Panchal*et al*., 2015; Parekh and Chanda, 2007; Sasidharan*et al*., 2011; Saxena*et al*., 2013; Shah and Yadav, 2015).

Test for carbohydrates

The METO (100 mg) was mixed in 5 ml of distilled water, then the mixture was filtered. The tests were performed on the filtrate for the presence or absence of phytochemical constituents.

Benedict's test

Approximately 0.5 ml of Benedict's reagent was added to the test tube containing 0.5 ml of the extract filtrate. In a hot water bath, this combination was warmed for about two minutes.

Molisch's test

To 2 ml of extract filtrate two drops of molisch's reagent was mixed. The mixture was thoroughly shaken, to this mixture 1 ml of concentrated H_2SO_4 was slowly added along the walls of test tube and allowed to stand for few minutes.

Fehling's reduction test

Fehling's solutions 1 and 2 were added to 1 ml of the extract filtrate and heated on a water bath for 2 minutes and observed.

Barfoed's test (Monosaccharides)

Barfoed's reagent (1 ml) was added to one ml of the extract filtrate, boiled on water bath for two minutes and then examined.

Seliwanoff's test

To 3 ml of Seliwanoff's reagent, 1 ml of the extract filtrate was added, then boiled on water bath for 1 minute and then observed for colour change.

Test for starch

A test tube containing 5 ml of distilled water was added with the 10 mg of iodine, 75 mg of potassium iodide, and then 3 ml of the METO was also mixed and shaken well.

Test for proteins

Biuret test

The extract (50 mg) was dissolved in 2 ml of distilled water and filtered. This was followed by addition of 2 ml of biuret reagent.

Millon's test

To 2 ml of the extract filtrate in a test tube, few drops of Millon's reagent was added and observed for changes.

Xanthoproteic test

To 1 ml of concentrated HNO3, 2 ml of the extract filtrate was added and then heated for 3 minutes, before cooling at room temperature subsequently 0.5 ml of NaOH was added.

Test for amino acids

A test for amino acids was performed on the extract filtrate prepared by dissolving 100 mg of the extract in 10 ml of distilled water and filtering it through Whatman filter paper No. 1.

Ninhydrin test

To 2 ml of the extract filtrate in a test tube, few drops of the Ninhydrin reagent was added and the solution was inspected for colour changes.

Nitric acid test

To 2 ml of the extract filtrate in a test tube few drops of concentrated HNO3 was poured slowly along the walls of test tube.

Tests for gums and mucilage

An amount of the extract (100 mg) was diluted in 10 ml of distilled water to this 25 ml of absolute alcohol was added and continuously stirred, then monitored for the formation of precipitate.

Qualitative analysis of secondary metabolites

Test for alkaloids

To 100 mg of the extract, 5 ml of 1.5% HClwas added and filtered. Using several reagents, the filtrate was examined for the presence of alkaloids.

Mayer's test

To 3 ml of filtrate in a test tube Mayer's reagent was added. Then it is examined for change in colour.

Wagner's test

The test tube with 2 ml of the extract filtrate was added with a few drops of Wagner's reagent and the mixture was inspected for colour change.

Hager's test

To 2 ml of the extract filtrate in the test tube, 2 ml of Hager's reagent was mixed and observed for colour change.

Dragendorff's test

The extract filtrate (2 ml) was exposed to 2% H2S04 for two minutes. Then 2 ml of Dragendorff's reagent was added and solution was examined.

Test for flavonoids

Aqueous sodium hydroxide test

After treating the extract with 10% NaOH solution, yellow colour appeared, then few drops of conc. HCl was added and observed for colour change.

Sulphuric acid test

Several drops of concentrated H2SO4 were added to 2 ml of the extract filtrate and then observed.

Magnesium and Hydrochloric acid reduction

The extract (100 mg) was immersed in 5 ml of ethanol (95%), then few pieces of magnesium turnings and 5-6 drops of conc. HCl were added. The appearance of scarlet pink or crimson red colour indicates the presence of flavanolglucosides.

Lead acetate test

The lead acetate solution (10%) was added to 1 ml of the extract and the reaction was examined.

Alkaline reagent test

To the aqueous extract solution, 10% ammonium hydroxide solution was added and boiled for few minutes. It was observed for the presence of yellow fluorescence.

Test for glycosides

The plant extract (500 mg) was digested with 20 ml of 0.1 N HCl on a water bath for two hours and then the mixture was filtered using Whatman No. 1 filter paper. The filtrate was used to detect the presence of glycosides.

Borntrager's test

To 2 ml of extract filtrate 3 ml of chloroform was added and mixed well. The 10% ammonia solution was added after isolating the chloroform layer formed earlier.

Keller-Killiani test

To 2 ml of extract filtrate, 2 to 3 drops of glacial acetic acid and few drops of 5% FeCl3 were added. After few seconds, 2 ml of concentrated $H_2SO_4$was added to the filtrate.

Legal's test

With few drops of 10% NaOH solution, the concentrated extract was made alkaline. Immediately freshly made sodium nitroprusside solution was added to the solution.

Test for phenolic compounds

Lead acetate test

To 50 mg of the extract, 5ml of distilled water was added, subsequently 3 ml of 10% lead acetate solution was added.

Gelatine test

Gelatine solution (1% w/v) containing NaCl (10% v/v) was added to 5 ml of crude plant extract and observed for the presence of white precipitate.

Test for tannins

Ferric chloride test

To 50 mg of the extract 2 ml of distilled water was added, followed by the addition of 2 ml of neutral 5% ferric chloride solution and observed.

Bromine water test

The plant extract (500 mg) was sprinkled on 5 ml of bromine water in a test tube and examined for changes.

Test for saponins

Froth test

The extract filtrate was diluted with distilled water up to 20 ml. The solution was gently stirred in a graduated cylinder for 15 minutes. The presence of saponins is indicated by a 2 cm layer of foam.

Test for phytosterols and triterpenoids

The extract (500 mg) was dissolved in 10 ml of chloroform and then filtered. The filtrate was screened for the detection of Triterpenoids and phytosterols.

Leibermann's - Burchard's test

The extract filtrate (2 ml) was mixed with 2 ml of acetic acid, then the solution is chilled using ice. Then two drops of concentrated $H_2SO_4$was added along the sides of test tube.

Salkowaski test

To 2 ml of extract filtrate, 3 ml of conc. H_2SO_4 was cautiously added and shaken well. Afterwards left to stand for few minutes.

Test for fixed oils and fats

Oily spot test

A little amount of the extract is placed between two filter papers and then pressed gently. Oily stain indicates the presence of fixed oils.

Test for Philobatannins

The extract (500 mg) was dissolved in distilled water and filtered. The filtrate was boiled with 2% HCl solution.

Test for Balsam

To 4 ml of the extract filtrate, 3 drops of alcoholic FeCl3 solution was added and heated for few seconds.

Test for Volatile oils

To 2 ml of the extract filtrate few drops of diluted NaOH and 1M HCl were added and shaken well. Then observed for the formation of white precipitate.

Test for Anthroquinones

The extract (500 mg) was heated with 10 ml of H_2SO_4 and filtered when it is hot. The filtrate was shaken with 5 ml of chloroform. The chloroform layer formed was drawn into another test tube and 1 ml of 10% NH_4OH was added. The resultant solution was tested for colour changes (Ayoola*et al*., 2008).

RESULTS

Phytochemical screening

Physical nature and pH of the extract

Physical characteristics and percentage yield of methanolic extract of *T. orientalis*aerial parts is presented in table (I). The percentage yield of methanolic extract was 12.38% w/w. The pH of methanolic extract of *T. orientalis*was acidic and it was 5.88.

Organic analysis

A preliminary phytochemical screening of the methanolic extract of *T. orientalis*was performed and the findings are demonstrated in table (II) and plates (1 to 4).

Table 1. Physical characteristics and per cent yield of methanolic extract of aerial parts of *T. orientalis*

Sl. No.	Details	Results
1	Solvent used	Methanol
2	Extract colour	Greenish black
3	Consistency	Powder
4	Appearance	Cake like
5	% Yield (w/w)	12.38
6	pH	5.88

Qualitative analysis of primary metabolites

Test for carbohydrates

Benedict's test

A distinctive red precipitate was formed indicating the presence of sugars in METO.

Molisch's test

At the intersection of two fluids, a violet colour ring was formed, which indicated the presence of carbohydrates in METO.

Fehling's reduction test

Brick red precipitate was formed at the bottom of test tube, suggested the presence of carbohydrates in METO.

Barfoed's test

The presence of carbohydrates (monosaccharides) in METO was revealed by the formation of red precipitate.

Seliwanoff's test

Appearance of rose red colour indicated the presence of ketones in METO.

Test for starch

Formation of navy-blue colour indicated the presence of starch in METO.

Test for proteins

Biuret test

Absence of purple or pink colour in the ethanolic layer inferred that the test was negative for the presence of proteins in METO.

Millon's test

Absence of development of white precipitate suggested the absence of protein in METO.

Xanthoproteic test

Non development of brownish yellow or reddish orange colour revealed that METO tested negative for proteins.

Test for amino acids

Ninhydrin test

The non-appearance of blue or violet colour revealed the absence of amino acids in METO.

Nitric acid test

The yellow colour was not formed, which indicated that METO tested negative for protein and free amino acids.

Gums and mucilage

No white or cloudy precipitation was developed indicating that the test was negative for the presence of gums and mucilages.

Qualitative analysis of secondary metabolites

Test for alkaloids

Mayer's test:

The absence of white or cream-coloured turbid precipitate indicated that alkaloids were absent in METO.

Wagner's test

The lack of development of reddish-brown coloured precipitate inferred the absence of alkaloids in METO.

Hager's test

The absence of yellow precipitate indicated that METO tested negative for alkaloids.

Dragendorff's test

The lack of formation of reddish-brown precipitate indicated the absence of alkaloids in METO.

Test for flavonoids

Aqueous sodium hydroxide test

The appearance of strong fluorescent yellow, which later turned colourless, confirmed the presence of flavonoids in METO.

Sulphuric acid test

The development of orange coloured precipitate indicated the presence of flavonoids in METO.

Magnesium and Hydrochloric acid reduction

The emergence of pink to crimson red colour indicated the presence of flavanolglucosides in METO.

Lead acetate test

A favourable indicator for the presence of flavonoids in METO is the formation of yellow precipitate

Alkaline reagent test

The intense yellow colour formation suggested the presence of flavonoid content in METO.

Test for glycosides

Borntrager's test

The emergence of deep red to pink colour revealed the presence of glycosides in METO.

Keller-Killiani test

At the interphase of the two aqueous layers, a reddish-brown colour developed, which indicated the presence of glycosides in METO.

Legal's test

The development of blue colour demonstrated the presence of glycosides in METO.

Test for phenolic compounds

Lead acetate test

A curdy white precipitate appeared, indicating the presence of phenolic compounds in METO.

Gelatine test

The formation of bulky white precipitate suggested the presence of phenol in METO.

Test for tannins

Ferric chloride test

The development of a dark green colour was detected, which indicated the presence of tannins in METO.

Bromine water test

The presence of tannins in METO was confirmed by the decolourization of bromine water.

Test for saponins

Froth test

The development of a persistent froth that remained relatively stable for five minutes made it evident that saponins were present in METO.

Test for phytosterols and triterpenoids

Leiberman-Bucharat test

At the intersection of two layers, a deep red ring was noticed which indicated the presence of triterpenes in METO.

Salkowaski test

The lower layer turned reddish brown which revealed the presence of sterols in METO.

Test for fixed oils and fats

Oily spot test

There was no oily stain on filter paper which indicated the absence of fixed oil and fats in METO.

Test for Philobatannins

The formation of dark red precipitate indicated the presence of philobatannins in METO.

Test for Balsam

The appearance of dark green colour revealed the presence of balsam in METO.

Test for Volatile oils

The formation of cloudy white precipitate indicated the presence of volatile oils in METO.

Test for Anthraquinones

The lack of pink or violet colour at the upper layer of liquid revealed the absence of anthraquinones in METO.

Inference

The methanolic extract of aerial parts of *T. orientalis*subjected to preliminary phytochemical screening, revealed the presence of phytoconstituents such as anthraquinones, balsam, carbohydrates, flavonoids, glycosides, phenolic compounds, phytosterols and triterpenoids, saponins, starch, tannins, and volatile oils. The interpretations were shown in table II and plates (1, 2, 3, and 4).

Table 2. Preliminary phytochemical screening of methanolic extract of aerial parts of *T. orientalis*

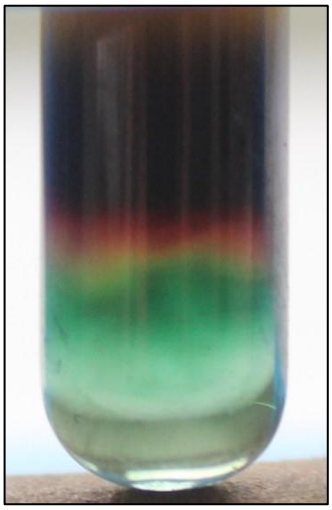
Sl. No.	Tests	Result
	Primary metabolites	
1	**Test for carbohydrates**	
	A. Benedict's test	**Positive**
	B. Molisch's test	**Positive**
	C. Fehling's test	**Positive**
	D. Barfoed's test	**Positive**
	E. Seliwanoff's test	**Positive**
2	**Starch test**	**Positive**
3	**Test for proteins**	
	A. Biuret test	**Negative**
	B. Millon's test	**Negative**
	C. Xanthoproteic test	**Negative**
4	**Tests for amino acids**	
	A. Ninhydrin test	**Negative**
	B. Nitric acid test	**Negative**
5	**Tests for Gums and mucilage**	**Negative**
	Secondary metabolites	
6	**Test for alkaloids**	

	A. Mayer's test	**Negative**
	B. Wagner's test	**Negative**
	C. Hager's test	**Negative**
	D. Dragendorff's test	**Negative**
7	**Test for flavonoids**	
	A. Aqueous NaOH test	**Positive**
	B. Sulphuric acid test	**Positive**
	C. Mg-HCl reduction test	**Positive**
	D. Lead acetate test	**Positive**
	E. Alkaline reagent test	**Positive**
8	**Test for glycosides**	
	A. Borntrager's test	**Positive**
	B. Keller-Killiani test	**Positive**
	C. Legal's test	**Positive**
9	**Test for phenolic compounds**	
	A. Lead acetate test	**Positive**
	B. Gelatine test	**Positive**
10	**Test for tannins**	
	A. Ferric chloride test	**Positive**
	B. Bromine water test	**Positive**
11	**Test for saponins**	
	Froth test	**Positive**
12	**Test for phytosterols and triterpenoids**	
	A. Leiberman-Bucharat test	**Positive**

	B. Salkowaski test	**Positive**
13	**Test for fixed oils and fats**	
	Oily spot test	**Negative**
14	**Test for Philobatannins**	**Positive**
15	**Test for Balsam**	**Positive**
16	**Test for Volatile oils**	**Positive**
17	**Test for Anthraquinones**	**Negative**

Plate 1. Interpretation of phytochemical tests of METO

1A. Benedict's test: +ve



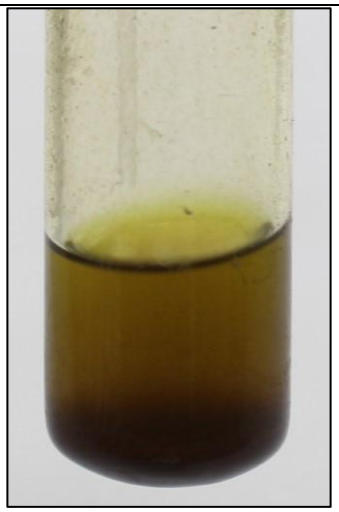
1B. Molisch's test: +ve

1C. Fehling's test: +ve

1D. Barfoed's test: +ve

1E. Seliwanoff's test: +ve

2. Test for starch: +ve



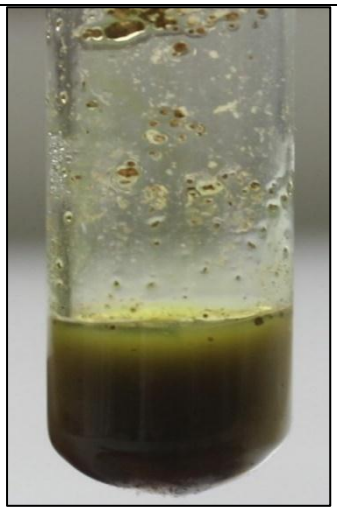
3A. Biuret test: -ve

3B. Millon's test: -ve

3C. Xanthoproteic test: -ve

Plate 2. Interpretation of phytochemical tests of METO

4A. Ninhydrin test: -ve



4B. Nitric acid test: -ve

5. Gums &Mucilages: -ve

6A. Mayer's test: -ve

6B. Wagner's test: -ve

6C. Hager's test: -ve

6D. Dragendorff's test: -ve

7A. Aq NaOH test: +ve

7B. H_2SO_4 test: +ve

Plate 3. Interpretation of phytochemical tests of METO

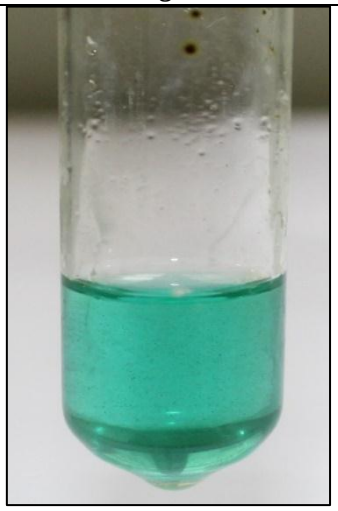
7C. Mg-HCl test: +ve

7D. Lead acetate test: +ve

7E. Alk reagent test: +ve

8A. Borntrager's test: +ve

8B. Keller-Killiani test:+ve



8C. Legal's test: +ve

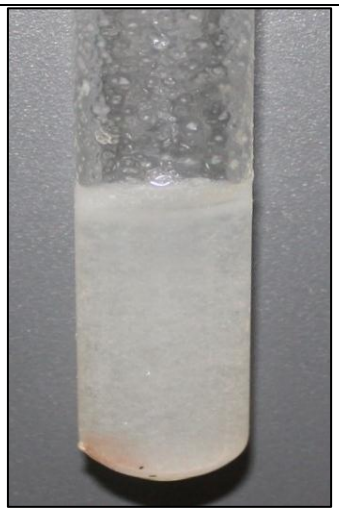
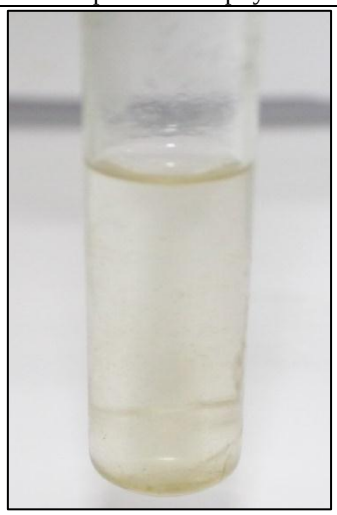
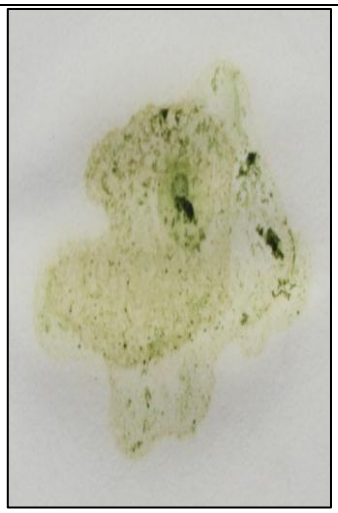
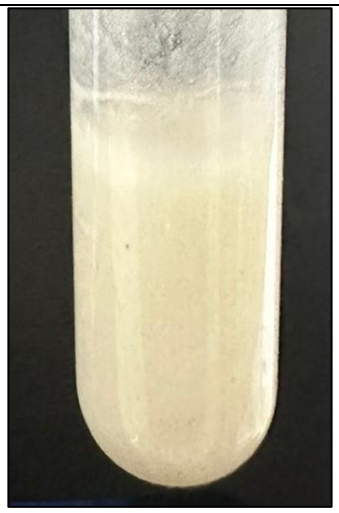
		
9A. Lead acetate test: +ve	**9B. Gelatine test: +ve**	**10A. $FeCl_3$ test: +ve**

Plate4. Interpretation of phytochemical tests of METO

		
10B. Bromine water test: +ve	**11. Froth test: +ve**	**12A. Leiberman-Bucharat test: +ve**
		
12B. Salkowaski test : +ve	**13. Oily spot test: -ve**	**14.Philobatannins test: +ve**

		
15. Balsam test: +ve	**16. Volatile oils test: +ve**	**17. Anthraquinones test:-ve**

DISCUSSION

Trema orientalis plant was often claimed to be used in conventional medicine to treat a variety of diseases as it was revealed to contain several phytochemical elements having therapeutic properties.

The screening of the methanolic extract of aerial parts of *T. orientalis* for the phytochemical constituents had revealed the presence of carbohydrates, starch, balsam, flavonoids, glycosides, phenolic compounds, phytosterols and triterpenoids, philobatannins, tannins, saponins and volatile oils. Alkaloids, amino acids and protein were not prevalent in the extract. The findings of the present study were in agreement with the previous reports of Ajayi *et al*. (2018) and Panchal *et al*. (2015), who had reported the presence of the similar phytoconstituents, the phytochemical screening of *T. orientalis*.

CONCLUSION

The phytochemical analysis of the methanolic extract of *T. orientalis* aerial parts (METO) demonstrated the acidic pH and powdery consistency. The preliminary phytochemical screening of METO revealed the presence of carbohydrates, starch, balsam, flavonoids, glycosides, phenolic compounds, phytosterols, triterpenoids, philobatannins, tannins, saponins and volatile oils, while the extract was negative for the presence of alkaloids, amino acids and protein. The phytoconstituents of the plant which might play an important role in therapeutic and toxic properties need to be identified for further exploration of the active toxic principle.

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