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(RESEARCH ARTICLE)

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Preliminary phytochemical, antibacterial and pharmacological screening of the whole plant of *Merremia umbellata*

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Abstract

The present investigation of crude extracts from the whole plant of *Merremia umbellata* was done by cold maceration with methanol and pet ether solvent and was subjected to preliminary phytochemical, pharmacological screening, and antibacterial against the selected Gram-positive and Gram-negative bacteria. The Phytochemical studies indicate the presence of Flavonoids steroids, alkaloids, carbohydrates, Triterpenoids and glycosides, tannins etc. The antibacterial activity of the methanolic and pet ether extract was studied by the cup and plate method against *streptococci, staphylococci, E. coli* and proteus and the plant *Merremia umbellata* have wider activity also the methanolic extract plant has a wider analgesic activity.

Keywords: *Merremia umbellata*; Phytochemical; Antibacterial; Analgesic; Diuretic; FT-IR

1. Introduction

The whole plant of *Merremia umbellata* possesses several medicinal properties and analgesics. Also, the plant is useful in the treatment of rheumatism; neuralgia; headache; etc. It is also used for auriculae ulcers caused in the ear. The leaves have emollient properties, and the dried powdered leaves are sniffed up the nose as a treatment for epilepsy. The pounded leaves are used to poultice burns; abscesses; ulcers; sores; and scalds. And the poultice leaves are combined with Curcuma powder (Curcuma longa) and applied on the hands and the feet. The seeds when soaked in water yield mucilage which is used for the treatment of cutaneous diseases. Tubers are mildly drunk as a remedy for haematuria. A paste or powder made of the root and mixed with Java flour and water is applied as a poultice to swellings. The latex of the root is taken as a purgative. It is also aimed to carry out the Antibacterial and Pharmacological screening of methanolic and Petroleum ether of *Merremia umbellata*. Our present study is to carry out phytochemical; antibacterial and Pharmacological evaluation of the methanolic and Petroleum ether extracts and to study their characterization. [1,2,3]

2. Material and methods

2.1. Plant collection [1,2,3]

The plant of *Merremia umbellata* was collected during the month of January from Marthandam, Kanyakumari (Dt), and it was authenticated by Dr K. Lingakumar. Head and Associated professor of Botany, ANJA College, Sivakasi. The

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collected material was dried at room temperature under shade for 15 days then it was blended into coarse powder by the mechanical grinder. The powder drug was passed through sieve No.22 to get uniform particle size.

2.2. Physical evaluation

2.2.1. Determination of ash value

The ash value is an important parameter for the evaluation of crude drugs, due to the variation of value within wide limits. The ash of any organic material is composed of inorganic material (metallic salts and silica) most direct contamination. Sand or earth can be immediately detected by ash value. Ashing involves the oxidation of the components of the products. A high ash value involves contamination, substitution, adulteration, or carelessness in the preparation of crude drugs for marketing. The following four different methods are adopted.

Determination of total ash

The total ash measures the total amount of material remaining after ignition. This includes both "Physiological ash" which is derived from the plant tissue itself and “non-physiological ash” which is the residue of the extraneous matters (e.g.) sand and soil adhering to the plant surface. About 2gm of ground air-dried material was placed in a silica crucible and ignited in an electrical burner at 85°C for 15 minutes, until it is white, which indicates the absence of Carbon, cooled in a desiccator, and weighed, the percentage of total ash was calculated concerning air dried drug.

Determination of Acid insoluble ash

This method is designed to measure the amount of silica present, especially in the sand and siliceous earth. The total ash was taken in a silica crucible, 25ml of hydrochloric acid was added, covered with watch glass with 5ml of hot water and added this liquid to the silica crucible. The insoluble matter was collected in a Whatman filter paper, wash with hot water, ignited, cooled in a desiccator, and weighed. The percentage of acid ash was calculated concerning the air-dried drug.

Determination of Water-soluble ash

The total ash was taken in a silica crucible this 25ml of water was added and boiled for 5 minutes, and the insoluble was collected in a Whatman filter paper. wash with hot water, ignited in a silica crucible at 85° c for 15 minutes. The weighed of this ash was subtracted from the total ash. The percentage of water-soluble ash was calculated in mg/gm of air-dried material.

2.2.2. Determination of Moisture content (Loss on drying)

About 2gm of ground air-dried material was taken in a previously dried China dish, this was placed in a hot oven and heated at 100°C for 1 hour. Then it was cool to room temperature and weighed. The percentage moisture content was calculated concerning air-dried material.

2.2.3. Determination of Sulphated ash

About 1 gm of powder was weighed in a silica crucible. The powder was moist with 1 ml of concentrated Sulphuric acid and ignited and cooled until the difference of weight was not more than 0.5gm and calculate the percentage of Sulphated Ash concerning air-dried drug.

2.2.4. Determination of Crude fibre content:

About 2 grams of powder was weighed accurately and transferred to a porcelain dish. 50ml of 10% Nitric acid was added and boiled for 30 seconds with constant stirring and filtered through fine mesh cotton cloth. The residue was washed with 100ml boiling water. The material from the cloth was collected in a porcelain dish and boiled with 50ml of 2.5% Caustic soda. Then using a fine-meshed cotton cloth the liquid is filtered. The residue was washed with 100ml of boiling water. Then the fibre was collected in a watch glass dried at 105°C and weighed. From the weight of the residue, the crude fibre content was calculated.

2.3. Preliminary phytochemical studies [7]

The various extracts of the whole plant of *Merremia umbellata* were subjected to qualitative analysis to identify the presence of various phytoconstituents like Alkaloids, Carbohydrate, Glycosides, Flavonoids, Steroids, Triterpenoids, Tannins etc., in the crude extracts.

2.4. Test for alkaloids

A small portion of the extracts was dissolved in a suitable solvent and each extract was stirred separately with a few drops of dilute Hydrochloric acid and filtered. The filtrate was tested for alkaloids by using the following reagents.

- Mayer's reagent (Potassium mercuric iodide): the appearance of yellow precipitate
- Dragendroff's reagent (Potassium bismuth iodide): the appearance of orange blown precipitate
- Hager's reagent (Saturated picric acid): the appearance of yellow precipitate
- Wagner's reagent (Iodine potassium iodide) appearance of reddish-brown precipitate

2.5. Test for carbohydrates

A small quantity of extracts was dissolved separately in 5 ml of distilled water and filtered. The filtrates were subjected to the following test to detect the presence of Carbohydrates.

2.5.1. Molisch's Test:

The filtrate was treated with 2-3 drops of 1% of Alcoholic alpha naphthol solution and 2ml of Conc. Sulphuric acid was added along the sides of the test tube. The appearance of the Brown ring at the junction of two liquids shows the presence of Carbohydrates:

2.5.2. Fehling's Solution

To a solution of a substance, add a mixture of equal parts of Fehling's solution A and B and the test tube was heated in a water bath. The appearance of Brick red colour shows the presence of Carbohydrates.

2.5.3. Barfoed's Test

To a small portion of the substance, Barfoed's solution was added. The appearance of red precipitate shows the presence of carbohydrate

2.5.4. Benedict's Test

To a small portion of the substance, Benedict's solution was added and mixed well and it was allowed to cool. The appearance of yellow precipitate shows the presence of carbohydrate

2.6. Test for gum and mucilage

To a small number of extracts and add 25ml of absolute alcohol and then it was filtered. The precipitate was examined for its swelling properties. To the small number of extracts and add Ruthenium solution.

2.7. Test for saponins

2.7.1. Foam Test

1 ml of the test solution was taken in a measuring cylinder. To this, 20ml of distilled water was added and shaken well. The formation of the 1 cm layer of foam shows the presence of Saponins.

2.7.2. Haemolysis Test

The extracts of the plant were separated over a glass slide to form a thin film layer on which a drop of human blood was placed and separated over the extract layer. After 30 minutes, the slide was examined under a microscope for changes in the structure and shape of the red blood cells. Control was always maintained to see the change in red blood cell structure for haemolysis.

2.8. Test for fixed oils and fats

2.8.1. Spots Test

A few drops of 0.5N Alcoholic potassium hydroxide were added to a small quantity of various extracts along with a drop of Phenolphthalein. The mixture was heated in a water bath for 1-2 hours. Formation of soap or partial neutralization of alkali indicates the presence of Fixed oil and Fats.

2.8.2. Using NaOH

The extracts were taken, and it was added with 1 ml Copper sulphated and 10% NaOH and shaken well. The appearance of blue colour shows the presence of Fixed oil and Fats.

2.9. Test for triterpenoids

2.9.1. Salkowski Test

The extracts were taken, and it was added with Chloroform and Sulphuric acid fluorescence of the solution was noted.

2.10. Test for flavonoids:

2.10.1. With aqueous NaoH

Colour change from Blue to Violet- Anthocyanin

Colour change from Yellow to Orange-Flavanones

2.10.2. Concentrated sulphuric acids

Colour change from Yellow to Orange- Anthocyanin Colour change from Orange to Crimson- Flavanones.

2.10.3. Shinodas's Test

The extracts are dissolved in Alcohol, to that piece of Magnesium followed by Conc. Hydrochloric acid dropwise is added and heated. The appearance of the Magenta colour shows the presence of Flavonoids.

2.11. Test for glycosides

2.11.1. Legal Test

To the hydrolysate. 1ml Pyridine and a few drops of Sodium nitroprusside solution were added and then it was made alkaline with Sodium hydroxide solution. The appearance of Pink to Red colour shows the presence of Glycosides.

2.11.2. Bontrager's Test

The hydrolysate was treated with Chloroform and the chloroform layer was separated. To this equal quantity of dilute Ammonia solution was added ammoniacal layer acquired Rose pink colour showing the presence of Glycosides.

2.11.3. Modified Borntrager's Test

The powdered drug is boiled with a few ml of dilute Hydrochloric acid and 5 ml of 5% Ferric chloride solution. Contents are cooled and shaken with an organic solvent. The organic layer is separated and an equal volume of ammoniacal solution is added to this ammoniacal layer shows showing to Red colour.

2.11.4. Baljet's Test

To the extracts, Sodium picrate solution was added. The appearance of Yellow to Orange colour shows the presence of Glycosides.

2.12. Test for phenolic compounds and tannins

Small quantities of alcohol and aqueous were taken in water and the presence of Phenolic compound and Tannins were tested by adding dilute solution and (5%) (10%) Lead acetate solution. The appearance of bluish-black colour shows the presence of Phenolic compounds and Tannins.

2.13. Test for proteins and amino acids

Dissolve small quantities of various extracts in a few ml of water and treated with the following reagents,

2.13.1. Million's Reagent

The appearance of red colour shows the presence of Proteins and Free amino acids.

2.13.2. Biuret Test

Equal volumes of 5% sodium hydroxide solution and 1% copper sulphate solution are added. The appearance of pink or purple shows the presence of Proteins and Free amino acids

2.14. Antibacterial activity [5,6]

The Antibacterial activity of the extract was studied by the Cup and Plate method against *Streptococci, Staphylococci, E. coli, and Proteus*.

2.15. Cup-plate method

Petri dish plates were previously sterilized, and Mueller Hinton agar was transferred into the Petri dish plates and allowed for solidification. The plates were swabbed uniformly with 24 hours culture the cup was made by using a sterile borer. The different concentrations of extracts, standards and control were poured into the respective cups accordingly. Then the Petri dish plates were incubated at 37°C for 24 hours. After the zone of inhibition formed by the standard and extracts were measured.

2.16. Diuretic activity [9,10]

2.16.1. Preparation of Samples

Methanol and Pet. ether extracts were taken for Diuretic studies. Both Methanol and Pet. ether extracts were dissolved in 0.1N Sodium hydroxide. Both extracts were prepared in concentrations of 200 mg/kg and 400 mg/kg.

2.16.2. Procedure

The method of Lipschitz was employed for the assessment of Diuretic activity. Six groups (each containing two rats) were fasted and deprived of water for 18h before the experiments. On the day of the experiment, all the animals are weighed and noted. Control animals received saline only and 2nd group with Frusemide (STD), 3rd 4th 5th 6th groups are treated with plant extracts. The group are treated immediately after dosing, the rats were placed in metabolic cages (2 in each cage) specially designed to separate urine and faces. Animals were kept at a room temperature of 25 ± 0.5°C throughout the experiment. Collect the urine in the measuring cylinder up to 5hrs after dosing. Measure the total volume of urine for both control and treated groups of animals. The Methanolic and Pet. Ether extracts (200 mg/kg and 400 mg/kg) were compared with standard diuretic agents like Frusemide (10 mg/kg body weight).

2.17. Analgesic activity

2.17.1. Preparation of Samples

Methanol and Pet. ether extracts were taken for Analgesic activity. Both Methanol and Pet. ether extracts were dissolved in 0.IN Sodium hydroxide. Both extracts were prepared at a concentration of 200 mg/kg.

2.17.2. Procedure

Weigh and mark animals and take basal reaction time to radiant heat placing the dip. Three to four cm area of the tail was marked and immersed in the water bath thermostatically maintained at 55°C and the withdrawal time of the tail from hot water (in seconds) was noted as the reaction time tail-flick latency. The maximum cut-off time for immersion was 15 seconds to avoid injury to the tissues of the tail. The initial reading was taken immediately before the administration the of test and standard drugs and then 1st hour, 2nd hour, 3rd hour and 4th hour after the administration. After the drug administration, the tail on time reaches in seconds. It is considered May analgesia and the tail is removed from the source of heat to avoid tissue damage. Calculate the percentage increase in reaction time of each time interval.

3. Results and discussion

3.1. Physical Evaluation

The result obtained from the physical evaluation of *Merremia umbellata* in total ash, water-soluble ash, acid-soluble ash, sulphated ash, loss on drying and crude fibre content was shown in below Table 1.

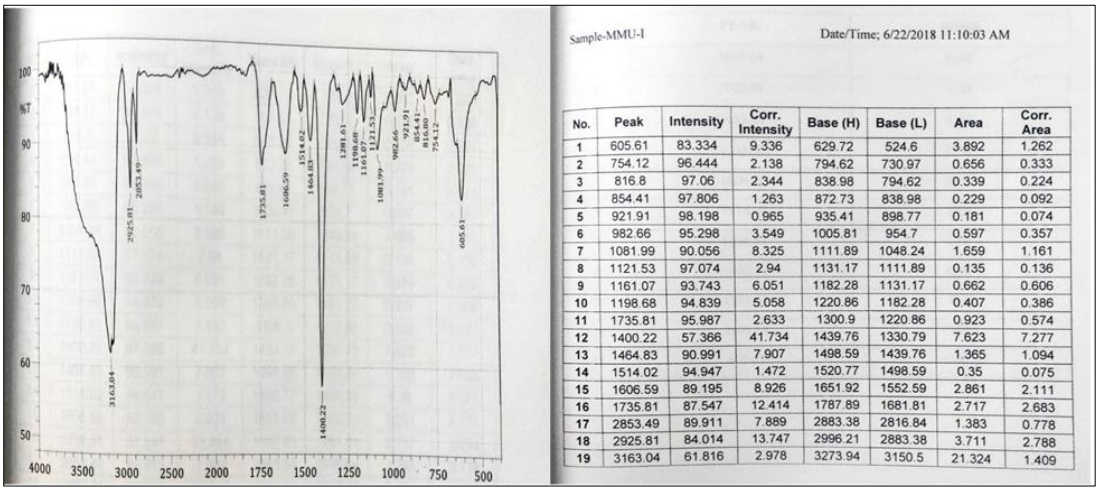
Table 1 Physical evaluation of *Merremia umbellata*

S.no	Physical evaluation	Experiment			
		1	2	3	Average
1	Total ash	7.5	7.7	7.3	7.5
2	Water soluble ash	4.5	4.4	4.6	4.5
3	Acid soluble ash	1	0.9	1.1	1
4	Sulphated ash	16.5	16.4	16.5	16.5
5	Loss on drying	4.5	4.7	4.5	4.5
6	Crude fibre content	45	46	44	45

3.2. FT-IR SPECTROSCOPY

The FT-IR of methanolic extract and pet ether extract of the *M. umbellata* was studied there was no interaction was shown in the methanol and pet. ether of *M. umbellata* the result of FT-IR was given below in Tables 2 and 3.

3.2.1. Methanolic extract



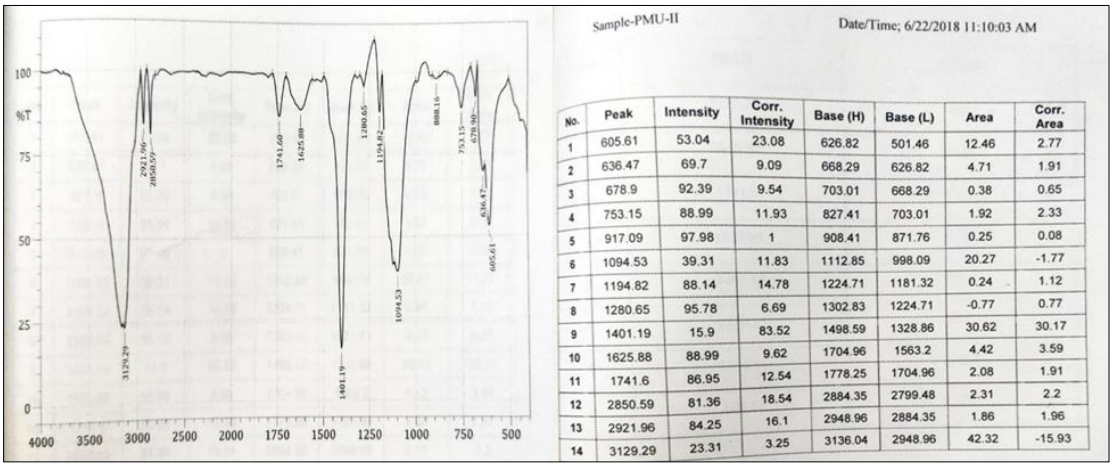
No.	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	605.61	83.334	9.336	629.72	524.6	3.892	1.262
2	754.12	96.444	2.138	794.62	730.97	0.656	0.333
3	816.8	97.06	2.344	838.98	794.62	0.339	0.224
4	854.41	97.806	1.263	872.73	838.98	0.229	0.092
5	921.91	98.198	0.965	935.41	898.77	0.181	0.074
6	982.66	95.298	3.549	1005.81	954.7	0.597	0.357
7	1081.99	90.056	8.325	1111.89	1048.24	1.659	1.161
8	1121.53	97.074	2.94	1131.17	1111.89	0.135	0.136
9	1161.07	93.743	6.051	1182.28	1131.17	0.662	0.606
10	1198.68	94.839	5.058	1220.86	1182.28	0.407	0.386
11	1735.81	95.987	2.633	1300.9	1220.86	0.923	0.574
12	1400.22	57.366	41.734	1439.76	1330.79	7.623	7.277
13	1464.83	90.991	7.907	1498.59	1439.76	1.365	1.094
14	1514.02	94.947	1.472	1520.77	1498.59	0.35	0.075
15	1606.59	89.195	8.926	1651.92	1552.59	2.861	2.111
16	1735.81	87.547	12.414	1787.89	1681.81	2.717	2.683
17	2853.49	89.911	7.889	2883.38	2816.84	1.383	0.778
18	2925.81	84.014	13.747	2996.21	2883.38	3.711	2.788
19	3163.04	61.816	2.978	3273.94	3150.5	21.324	1.409

Figure 1 FT-IR of methanolic extract

Table 2 FT-IR data of methanolic extract

S.no	Peak	Bond
1	3163.04	O-H
2	2925.81	C-H
3	2853.49	C-H
4	1735.81	C=O
5	1006.59	C=C
6	1514.02	NO2
7	1464.83	CH3
8	1400.22	C=C
9	1281.61	C-F
10	1198.68	C-OH
11	605.61	C-CL

3.2.2. Pet ether extract



No.	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	605.61	53.04	23.08	626.82	501.46	12.46	2.77
2	636.47	69.7	9.09	668.29	626.82	4.71	1.91
3	678.9	92.39	9.54	703.01	668.29	0.38	0.65
4	753.15	88.99	11.93	827.41	703.01	1.92	2.33
5	917.09	97.98	1	908.41	871.76	0.25	0.08
6	1094.53	39.31	11.83	1112.85	998.09	20.27	-1.77
7	1194.82	88.14	14.78	1224.71	1181.32	0.24	1.12
8	1280.65	95.78	6.69	1302.83	1224.71	-0.77	0.77
9	1401.19	15.9	83.52	1498.59	1328.86	30.62	30.17
10	1625.88	88.99	9.62	1704.96	1563.2	4.42	3.59
11	1741.6	86.95	12.54	1778.25	1704.96	2.08	1.91
12	2850.59	81.36	18.54	2884.35	2799.48	2.31	2.2
13	2921.96	84.25	16.1	2948.96	2884.35	1.86	1.96
14	3129.29	23.31	3.25	3136.04	2948.96	42.32	-15.93

Figure 2 FT-IR of Pet. ether extract

Table 3 FT-IR of Pet. ether extract

S.no	Peak	Bond
1	3129.29	OH
2	2850.59	C-H
3	1741.60	C=O
4	1625.88	C=C
5	1401.19	C=C
6	1280.65	C-F
7	1094.53	C-OH
8	605.61	C-CL

3.3. Preliminary phytochemical studies

Table 4 Phytochemical studies of *Merremia umbellata*

S.no	Active principle	Extraction	
		Methanol	Pet. ether
1	Alkaloids	-	-
2	Carbohydrates	+	+
3	Gum and mucilage	-	-
4	Saponins	+	-
5	Fixed oil	+	+
6	Triterpenoids	-	+
7	Flavonoids	+	+
8	Glycosides	+	-
9	Phenolic compounds	+	-
10	Protein and amino acids	-	-

+ indicates the presence of constituents; - indicates the absences of constituents

The result obtained from the methanolic and pet ether extracts of the whole plant of *M.umbellata* were subjected to qualitative analysis to identify the presence of various phytoconstituents was obtained below in Table 4.

3.4. Anti-bacterial activity

The Antibacterial activity of Methanolic and P. ether extract of *Merremia umbellata* against various strains of Gram-negative organisms (E. coli) than Gram-positive organisms (*Streptococci* and Staphylococci). By standard well-plate method. When compared with standard (ciprofloxacin) only Methanolic extract showed activity against Gram-negative organisms and Gram-positive organisms. Pet. ether extract does not show any antibacterial activity towards any tested organism. The result confirms that the plant *Merremia umbellata* has wider antibacterial activity.

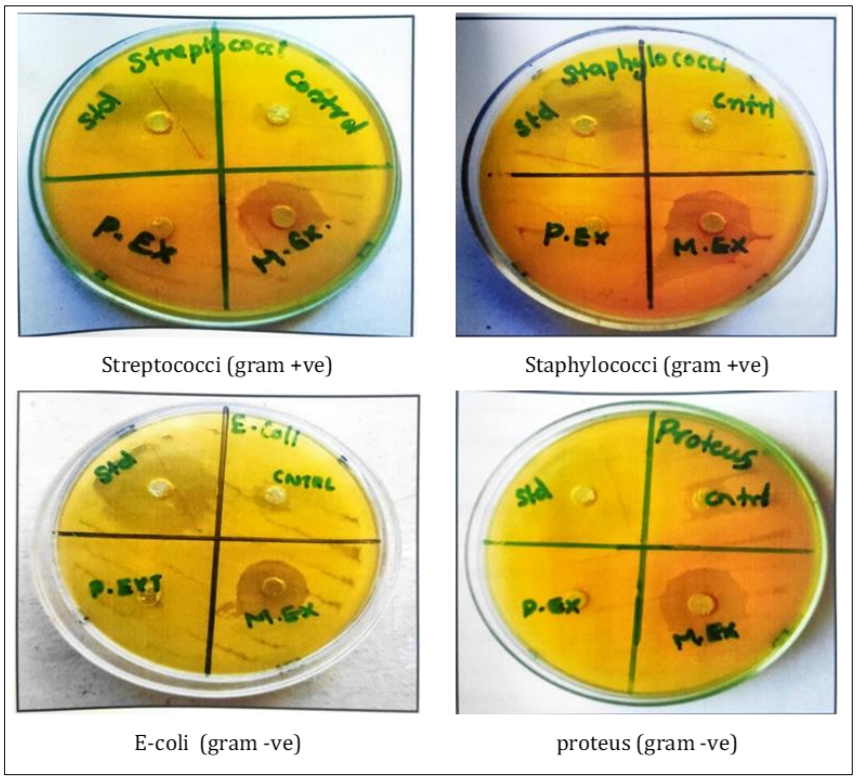


Figure 3 Well plate method for M. Umbellata

Table 5 Zone of inhibition of extracts of M. umbellata

Name of organism	Zone of inhibition (mm)			
	Standard	Control	Methanol	Pet. ether
Streptococci	16	3	8	-
Staphylococci	15	3	10	-
E-coli	17	4	11	-
Proteus	16	3	9	-

3.5. Diuretic activity [8]

The result obtained with an evaluation of the diuretic activity of Methanolic and Pet. Ether extracts of the entire plant of *M. Umbellata* was shown below in Table 6. The results confirm that the plant *M. umbellata* does not have Diuretic activity.

Table 6 Diuretic activity of M. Umbellata

Group	Animal weight(gm)	Drug and dose	Volume of urine			
			1Hr	2Hr	3Hr	4Hr
1	190 gm 140 gm	Control (i.p)	1	1.8	3.1	4.5
2	200 gm 190 gm	Frusemide 10 mg/kg (i.p)	4	6.4	8.9	11
3	180 gm 200 gm	Methanol extract 200 mg/kg (i.p)	0.3	0.8	1.1	1.4
4	200 gm 180 gm	Methanol extract 400 mg/kg (i.p)	0.2	0.5	0.7	1
5	160 gm 200 gm	Pet. ether extract 200 mg/kg (i.p)	0.1	0.2	0.4	0.5
6	210 gm 170 gm	Pet. ether extract 400 mg/kg (i.p)	0.4	0.6	1	1.6

3.6. Analgesic activity [4]

Table 7 Analgesic activity of *M. umbellata*

S.no	Animal weight (mg/kg)	Drug and dose (mg/kg)	Basal reaction time (sec)					After drug administration (hours)			
			1	2	3	4	5	1hrs	2hrs	3hrs	4hrs
1	190 gm	Control (p.o)	3	4	5	5	5	4	5	4	5
	140 gm		4	3	4	5	4	5	4	5	4
	190 gm		4	3	4	4	5	5	4	5	4
			3.6	3.3	4.3	4.6	4.6	4.6	4.3	4.6	4.3
2	160 gm	Indomethacin 100 mg/kg (p.o)	4	3	5	5	5	7	8	8	9
	200 gm		3	4	4	4	5	6	7	9	9
	170 gm		3	3	4	5	4	7	7	8	9
			3.3	3.3	4.3	4.6	4.6	6.6	7.3	8.3	9
3	180 gm	Methanol ext. 200 mg/kg	3	4	4	5	6	7	7	7	8
	200 gm		3	4	5	5	7	7	7	7	7
	230 gm		4	4	5	5	6	6	6	8	7
			3.3	4	5	4.3	5	6.3	6.6	7.3	7.3
4	210 gm	Pet. ether ext. 200 mg/kg (p.o)	4	3	4	5	5	5	6	8	8
	180 gm		3	4	4	4	4	5	5	6	6
	1990 gm		4	3	4	4	4	5	6	6	6
			3.6	3.3	4	4.3	4.3	5	5.6	6.6	6.6

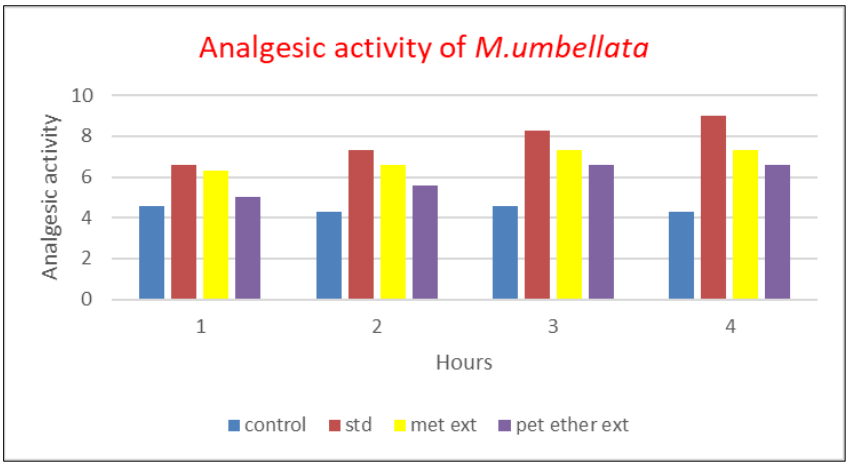


Figure 4 Analgesic activity of M. umbellate

The Methanolic extract of *M. umbellata* has more Analgesic activity than Pet. ether extract when compared with the standard drug of Indomethacin. The result confirms that the plant *M. umbellata* has wider Analgesic activity. Table 7 and a graphical representation of analgesic activity was mentioned below in Table 7.

4. Conclusion

The present study confirmed that *M. umbellata* has wider antibacterial activity against gram-negative and gram-positive organisms and it has analgesic activity, and the Phytochemical study confirmed the presence of various phytoconstituents like alkaloids, carbohydrates, flavonoids etc.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest.

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