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Research Article

Preliminary Phytochemical Screening of Whole Plant Extracts of *Peperomia pellucida* (Linn.) HBK (Piperaceae) and Marsilea quadrifolia Linn. (Marsileaceae)

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ABSTRACT

A number of medicinal plants have been subjected to detailed chemical investigations and this has led to the isolation of pure bioactive molecules which have been pharmacologically evaluated. As a result, new drugs have been discovered, along with new applications. The present study was aimed at investigating the phytochemical constituents of *Peperomia pellucida (P. Pellucida)* and *Marsilea quadrifolia (M. quadrifolia*) to find out their use as medicinal drugs. The screening for phytoconstituents was performed using standard established methods for qualitative analysis. n-hexane, ethyl acetate, ethanol and methanol extracts of both the plants were analyzed for the phytochemical compounds. Phytochemical analysis of crude extracts revealed the presence of tannins, saponins, flavonoids, phenols, phytosterols, steroids, terpenoids, triterpenoids, alkaloids, tropane alkaloids, isoquinoline alkaloids, carbohydrates, glycosides, reducing sugars, lipids, and acids in *P. pellucida* and *M. quadrifolia* in varying concentrations. Carotenoids, anthraquinones, cardiac glycosides, quinones, coumarins, coumarin glycosides, resins, condensed tannins, pseudo tannins, proteins and free Amino acids were found to be absent in *P. pellucida* and *M. quadrifolia.* Phlobatannins and fixed oils and fats were present in *P.pellucida* and absent in *M. quadrifolia*. The powdered drugs were examined for organoleptic and physical parameters. Sequential extraction was carried out in three different ratios for analyzing the experimental yield of the crude extracts. The yield of the crude extract was found to be higher in 1:8 compared to two other ratios. From this study it is suggested that both *P. pellucida* and *M. quadrifolia* have immense medicinal value as a potential drug for the cure of many health related problems as they contain a number of bioactive compounds.

Key words: *Peperomia pellucida; Marsilea quadrifolia;* Phytoconstituents; Organoleptic parameters; Crude extracts; Sequential extraction.

INTRODUCTION

Since prehistoric times, the treatment and cure of diseases has been one of the primary concerns of mankind. Local practitioners have used indigenous plants and herbs for centuries all over the world to treat a variety of ailments and these have exhibited clear pharmacological activities[1]. Among ancient civilizations, India has been known to be rich repository of medicinal plants. Ayurveda, Siddha, Unani and Folk (tribal) medicines are the major systems of indigenous medicines. Among these systems, Ayurveda is most developed and widely practiced in India. Ayurveda dating back to 1500-800 BC has been an integral part of Indian culture. The *Ayurveda* system of medicine uses about 700 species, *Unani* 700, *Siddha* 600, Amchi 600 and modern medicine around 30 species[2, 3,4]. According to data of the Food and Agriculture Organization (FAO), more than 50,000 plant species are being used in the traditional folk medicine throughout the world[5].

Natural bioactive molecules have proved to be lead compounds for the synthesis of various potent drugs, allowing the design and rational planning of new drugs, biomimetic synthesis development and the discovery of new therapeutic properties not yet attributed to known compounds[6]. The plant-derived compounds have a long history of clinical use, better patient tolerance and acceptance. In contrast to synthetic pharmaceuticals based upon single chemicals, many phyto-medicines exert their beneficial effects through the additive or synergistic action of several chemical compounds acting as single or multiple target sites associated with a physiological process. This synergistic or additive pharmacological effect can be beneficial by eliminating the problematic side effects associated with the predominance of a single xenobiotic compound in the body[7]. The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. That the medicinal actions of plants are unique to particular plant species or groups is consistent with this concept as the combinations of secondary products in a particular plant are often taxonomically distinct[8]. Most of these phytochemical constituents are potent bioactive compounds found in medicinal

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Table 1: Organoleptic characters of the powdered drugs of *Peperomia pellucida (Linn.) HBK* and *Marsilea quadrifolia Linn*

Drug	Nature	Color	Odor	Taste	Texture
Peperomia pellucida	Coarse	Olive green	Characteristic	Sour	Rough
Marsilea quadrifolia	Fibrous	Greenish Brown	Characteristic	Slightly sour	Rough

Table 2: The percentage yield of successive extracts of *Peperomia pellucida (Linn.) HBK* and *Marsilea quadrifolia Linn.*

Sequential extraction in three ratios	*Peperomia pellucida* extracts				*Marsilea quadrifolia* extracts			
	H	EA	ETOH	MEOH	H	EA	ETOH	MEOH
1:4 (% w/w yield)	2.40%	3.20%	3.60%	2.80%	1.00%	2.80%	2.40%	2.60%
1:6 (% w/w yield)	1.80%	4.00%	2.20%	4.20%	1.00%	3.60%	2.80%	4.00%
1:8 (% w/w yield	4.20%	6.00%	4.00%	6.00%	2.00%	3.90%	4.00%	5.10%

H= hexane; EA = ethyl acetate; ETOH = ethanol; MEOH = methanol; Yield (%) = (yield weight /sample weight) x 100

Table 3: Physical characteristics of the various extracts of *Peperomia pellucida (Linn.) HBK* and *Marsilea quadrifolia Linn.*

Physical properties	*Peperomia pellucida* extracts				*Marsilea quadrifolia* extracts			
	H	EA	ETOH	MEOH	H	EA	ETOH	MEOH
Time of Extraction	72hrs	72hrs	72hrs	72hrs	72hrs	72hrs	72hrs	72hrs
Color	Yellowish Brown	Yellowish Green	Greenish Brown	Greenish Brown	Golden Yellow	Olive Green	Yellowish Green	Yellowish Green
Consistency	SVS	SVS	SVS	SVS	SVS	SVS	SVS	SVS
Odor	Characteristic	Pungent	Pungent	Pungent	Characteristic	Pungent	Pungent	Pungent

H= hexane; EA = ethyl acetate; ETOH = ethanol; MEOH = methanol; SVS= semi viscous solid

plant parts, which are precursors for the synthesis of useful drugs[9]. It is necessary to identify the Phytochemical constituents of local medicinal plants usually employed for the treatment of diseases by the herbalists[10]. *P. pellucida*, the plant of immense medicinal value is variously known in different Indian and other languages.

In Philippines it is known as Ulasiman-bato,Olasiman-ihalas and Tangontagon. It is known as càng cua (Vietnam); pak krasang (Thailand); suna kosho (Japan); rangu-rangu, ketumpangan ortumpang angin (Bahasa/Malay), rinrin (Nigeria), Pepper elder, silverbush, rat-ear, man-to-man, clearweed (North America), lingua de sapo, herva-de-vidro, herva-de-jaboti or herva-de-jabuti (South America), Its names in Sanskrit are Toyakandha and Varshabhoo. In Malayalam it is known as Mashitandu chedi. In Bengali it is known as Luchi Pata[11].

The plant has a thread like but angular trailing stem. Those growing in rich habitats do have fleshy and stout stems. Its leaves are blunt, heart shaped and in good habitats it grows as a long shrubby looking creeping cover or as an epiphyte. The elongated stems look like a vine with leaves rising 6 to 9 cm above the surface. Both leaves and stems have shiny waxy surfaces. The foliage of the plant looks ornamental. It has been reported to be a tropical perennial. It usually does not exceed 12" in height. The flowers are tiny and unnoticeable and grow in the form of a cord like spike. Inflorescence consists of compact, erect spikes of minute creamy white flowers. It is described in Ayurveda as: Rasa - Katu and Madhur; Guna- Lakhu, rooksha, Teekshna; and Virya – Ushna[12]. The plant is described to pacify vitiated cough, pita, constipation, kidney diseases, urinary retention, dysuria, urinary tract infections, emaciation, edema and general weakness[13]. Infusion and decoction of leaves and stem of fresh plant are eaten as salad for the treatment of gout and arthritis Consumption of *P. pellucida* as green salads in the Philippines and as blanched vegetables in Thailand has been reported[14]. It is also used in teas in Indonesia[15] .

M. quadrifolia is an aquatic fern bearing 4 parted leaf resembling '4-leaf clover' (Trifolium). Leaves floating in deep water or erect in shallow water or on land. Leaflets obdeltoid, to 3/4" long, glaucous, petioles to 8" long; Sporocarp (ferns) ellipsoid, to

Table 4: The analysis of phytoconstituents in the serial extracts of *Peperomia pellucida (Linn.) HBK* and *Marsilea quadrifolia Linn.*

Sl.no	Phytoconstituents	*Peperomia pellucida* extracts				*Marsilea quadrifolia* extracts				Interpretation
		H	EA	ETOH	MEOH	H	EA	ETOH	MEOH	
1	Tannins Ferric Chloride Test	-	-	+	+	-	-	+	+	Blue - black or brownish green precipitate
2	Saponins Sodium Bicarbonate Test for H and EA extracts Foam test for Etoh and Meoh extracts	++	++	++	++	+	+	+	+	Honeycomb like frothing in the first test and layer of foam in the second test
3	Flavonoids	+	++	+	+	-	+	++	++	Yellow coloration
4	Steroids Liebermann Burchard Reaction	+	-	-	-	+	-	-	-	Violet to blue or green
5	Terpenoids Salkowski Test	+	++	+	+	+	-	-	-	Reddish brown colorations of the interface
6	Triterpenoids Liebermann Burchard Test	+	-	-	-	+	-	-	-	Formation of reddish violet color
7	Carotenoids	-	-	-	-	-	-	-	-	Blue color of the interface
8	Anthraquinones Borntrager's Test	-	-	-	-	-	-	-	-	Formation of pink or red color in ammoniacal layer
9	Cardiac glycosides Keller – Killani Test	-	-	-	-	-	-	-	-	Brown ring of the interface .A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form
10	Glycosides Legal test	-	-	+	+	-	-	+	++	Appearance of pink to red color
11	Phlobatannins	+	+	+	+	-	-	-	-	Deposition of a red precipitate
12	Quinones	-	-	-	-	-	-	-	-	Blue green or red color
13	Coumarins	-	-	-	-	-	-	-	-	Formation of yellow color
14	Tropane alkaloids Vitali- Morin's Test	-	-	+	+	+	+	+	+	White precipitate

Table 4: The analysis of phytoconstituents in the serial extracts of *Peperomia pellucida (Linn.) HBK* and *Marsilea quadrifolia Linn.*

Sl. no	Phytoconstituents	*Peperomia pellucida* extracts				*Marsilea quadrifolia* extracts				Interpretation
		H	EA	ETOH	MEOH	H	EA	ETOH	MEOH	
15	Isoquinoline alkaloids	++	+++	+++	+++	++	+++	+++	+++	Orange yellow precipitate
16	Alkaloids									
	Mayer's ReagentTest	-	-	-	-	-	-	-	+++	White or cream precipitate
	Wagner's Reagent Test	-	-	-	+++	-	-	-	++	Reddish brown precipitate
	Dragendorff's Reagent Test	-	-	-	+++	-	-	-	-	Orange yellow precipitate
17	Carbohydrates									
	Molisch's Test	+	-	-	-	+	-	-	-	Reddish violet ring at the junction of two layers
	Fehling's Test	-	-	++	++	++	-	++	++	Appearance of reddish orange precipitate
	Iodine Test	-	-	-	-	-	-	-	-	Formation of blue color, which disappears on heating and reappears on cooling
18	Proteins and free Amino acids									
	Ninhydrin Test	-	-	-	-	-	-	-	-	Appearance of purple color
	Biuret Test	-	-	-	-	-	-	-	-	Appearance of pink or purple color
19	Reducing sugars	+++	+++	+++	+++	+++	+++	+++	+++	Formation of a red color
20	Phenols Ferric Chloride Test	-	-	+	+	-	-	+	+	Appearance of blue or green color
21	Phytosterols	++	+++	+	+	++	+++	-	-	Appearance of a bluish green color
22	Fixed oils and fats Spot Test	+	-	-	-	-	-	-	-	Appearance of oil stains on the paper
23	Coumarin Glycosides Fluorescence Test	-	-	-	-	-	-	-	-	Development of blue-green fluorescence
24	Resins									
	Turbidity Test	-	-	-	-	-	-	-	-	Formation of turbidity
	HCl Test	-	-	-	-	-	-	-	-	Pink color after heating the solution in water bath for 30 minutes
	Ferric chloride Test	-	-	-	-	-	-	-	-	Greenish blue color
25	Lipids Grease Spot Test	+	++	+	+	+	++	+	+	Translucent spot on the paper

Table 4: The analysis of phytoconstituents in the serial extracts of *Peperomia pellucida (Linn.) HBK* and *Marsilea quadrifolia Linn.*

	Phytoconstituents	*Peperomia pellucida* extracts				*Marsilea quadrifolia* extracts				Interpretation
		H	EA	ETOH	MEOH	H	EA	ETOH	MEOH	
26	Condensed tannins (Catechins) Matchstick Test	-	-	-	-	-	-	-	-	Woody pink to magenta color on heating near the flame
27	Pseudotannins (Chlorogenic acid)	-	-	-	-	-	-	-	-	Green color after exposing with air
28	Test for Acids	+	+	-	-	+	+	-	-	Formation of effervescence

(+++) = high concentration; (++) = medium concentration; (+) = low concentration; (-) = absent
H= hexane; EA = ethyl acetate; ETOH = ethanol; MEOH = methanol

3/16" long, dark brown, on stalks to 3/4" long, attached to base of petioles. A juice made from the leaves is diuretic and febrifuge and also used to treat snakebite and applied to abscesses. The plant is anti-inflammatory, depurative, and refrigerant[16]. The plant contains an enzyme named Thiaminase[17]. *M. quadrifolia* is also eaten by various tribal communities such as Kadars, Pulaiyars, Malasars, Malaimalasars, Mudhuvars of Anamailais hills, Western Ghats, Coimbatore district Tamil Nadu, India as per seasonal availability[18]. An investigation has been made on the relevance of *M. quadrifolia Linn.* (Vernacular name - sushni saag) commonly used by 'Ho' tribes in the mining belt of Jaduguda, Jharkhand (India) for its culinary and medicinal properties[19].

M. quadrifolia Linn. has got profound antibacterial, cytotoxic and antioxidant effect and may have potential use in medicine[20]. A medicinal plant can be viewed as a synthetic laboratory as it produces and contains a number of chemical compounds. These compounds, responsible for medical activity of the plant, are secondary metabolites. For example, alkaloids which are nitrogenous principles of organic compounds combine with acids to form crystalline salts[21]. *M. quadrifolia* is enlisted in the Red Data Book of the International Union for Conservation of Nature (IUCN) [22]. In Europe *M. quadrifolia* represents a rare species, included in Red list floras[23]. The current study aims to explore the phytochemical profile of *P. pellucida and M. quadrifolia* and the possibility of commercialization of medicinal and palatable *P. pellucida and M. quadrifolia* as a food medicine in promoting health because of the presence of various phytochemicals which can be the lead compounds for isolating various drugs from these plants which are of high medicinal and culinary value.

MATERIALS AND METHODS

Collection and Identification of plant material: The whole plant of *P. pellucida and M. quadrifolia* were collected from Kalliyad (Thrissur, Kerala, India) in the month of April and May. The plants were identified by Dr. G. Jeya Jothi, Taxonomist and Assistant Professor, Department of Plant Biology and Biotechnology, Loyola College (Madras University), Chennai, India. Voucher specimen of each plant (*P. pellucida* - LCH 128 and *M. quadrifolia* -LCH 129) have been preserved in the Department of Plant Biology and Biotechnology, Loyola College for further reference.

Processing of plant materials: The plant materials were washed thoroughly under running tap water and shade dried for three weeks at room temperature. The dried plant materials of both species were groundseparately to a fine powder using an electric blender and were stored in airtight containers until use.

Organoleptic characters of powdered drug: A small amount of each powdered drug was spread on a white tile and physically examined for general appearance i.e. nature, color, odor, taste and texture[24]. Approximately 2 gm of powdered drug sample was used for the evaluation.

Preparation of plant extract: Four different solvents namely hexane, ethyl acetate, ethanol and methanol were used for the sequential extraction started from low polar to high polar. The sequential extraction was carried out in three different ratios of plant powder to solvent (wt/vol.) [50g of the plant sample was mixed with 200ml of solvent (1:4), 50g of the plant sample was mixed with 300ml of solvent (1:6) and 50g of the plant sample was mixed with 400ml of solvent (1:8)] to find out the highest experimental yield. In each ratio the plant sample weight was kept constant and only the volume of the solvent was increased. The extractions were performed for 72 h at room temperature using an orbital shaker at 120 rpm. The extraction process was carried out in triplicates with each solvent. The extracts were filtered through Whatmann No.1 filter paper and concentrated at reduced pressure using a rotary vacuum evaporator. The dried crude extracts were kept in glass vials and stored in the refrigerator at 4 °C until use.

Determination of extracting values: Extracting values give an idea about the nature of the chemical phytoconstituents present in the crude drug. The use

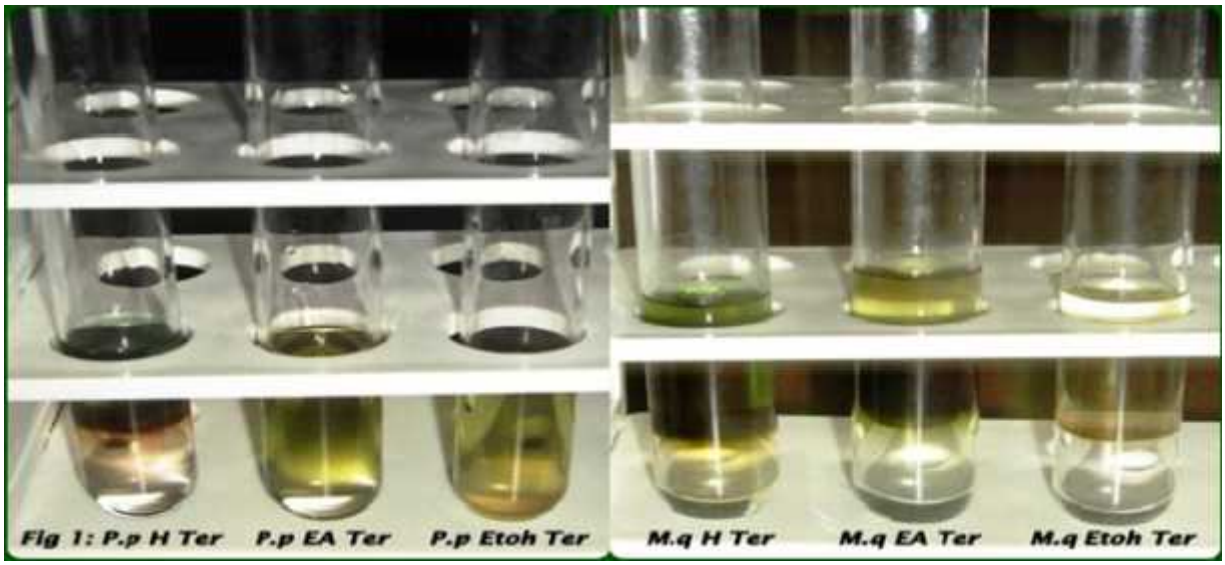

Fig1: Hexane (H), Ethyl acetate (EA) and Ethanol (Etoh) extracts of Peperomia pellucida (P.p) showing Positive test for Terpenoids (Ter); Hexane (H) extract of Marsilea quadrifolia (M.q) showing Positive test for Terpenoids (Ter); Ethyl acetate and Ethanol extracts of Marsilea quadrifolia (M.q) showing Negative test for Terpenoids (Ter).

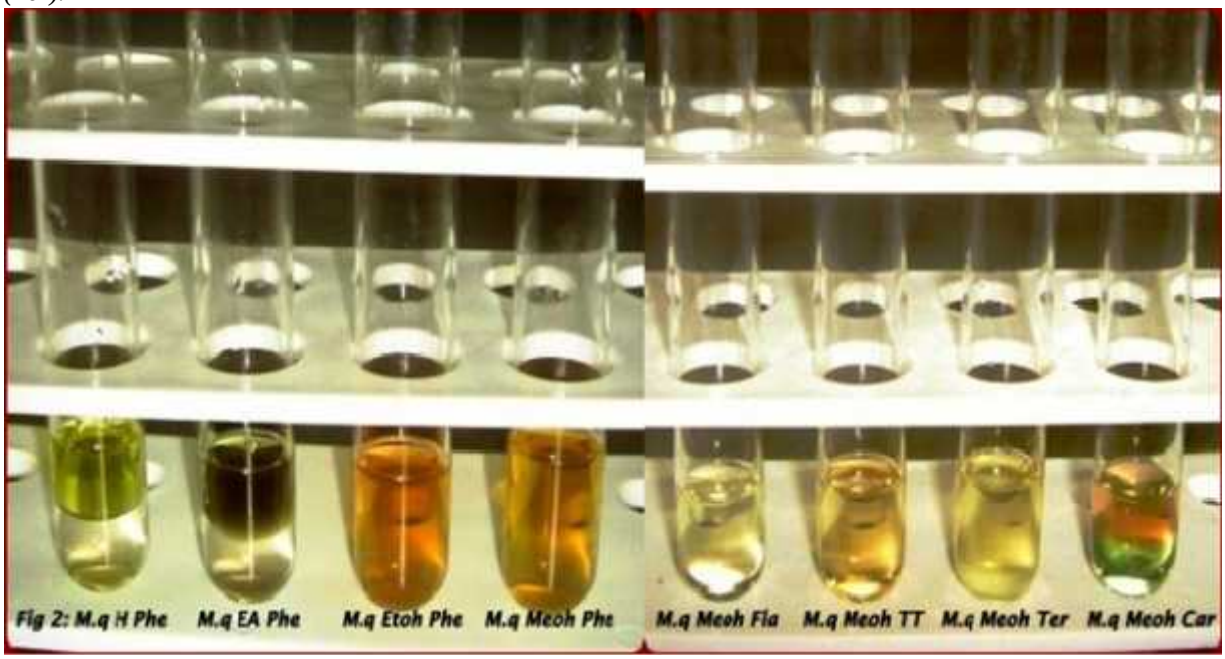

Fig 2: Hexane (H) and Ethyl acetate (EA) extracts of Marsilea quadrifolia (M.q) showing Negative test for Phenols (Phe); Ethanol (Etoh) and Methanol (Meoh) extracts of Marsilea quadrifolia(M.q) showing Positive test for Phenols (Phe); Methanol (Meoh) extract of Marsilea quadrifolia(M.q) showing Positive test for Flavonoids (Fla), Negative test for Triterpenoids (TT),Terpenoids (Ter) and Carbohydrates (Car).

of a specific solvent can be the means of providing information on the quality of a particular drug sample. The extractive values were calculated using the following formula:

Yield (%) = $W_1 / W_2 \times 100$

Where:

W_1 = The weight of the extract after evaporation of hexane/ethyl acetate/ethanol/methanol

W_2 = The dry weight of plant sample

Physical examination of the extracts: The dried extracts of *Peperomia pellucida and Marsilea quadrifolia* were evaluated for physical parameters such as consistency, color, and odor. The time of extraction was 72 hrs.

Preliminary phytochemical screening: The crude extracts were qualitatively tested for the presence of various secondary metabolites using standard established methods. They are as described for alkaloids[25], steroids and phlobatannins[26], phenolics and flavonoids[27], saponins and cardiac glycosides[28], tannins[29], glycosides and carbohydrates[30], phytosterols[31], protein and free amino acids[32, 33], fixed oils and fats[34]. The results of the qualitative chemical tests obtained are shown in Table 4.

Qualitative Phytochemical Tests

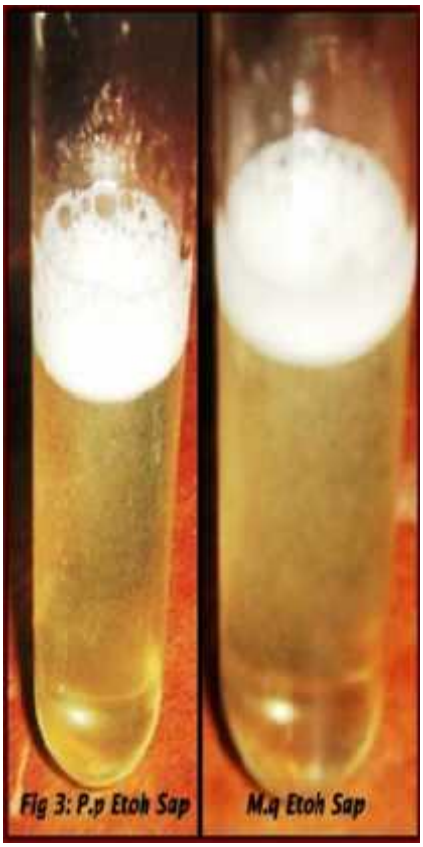

Fig 3: Ethanol (Etoh) extracts of *Peperomia pellucida (P.p)* and *Marsilea quadrifolia (M.q)* showing Positive test for Saponins (Sap).

Fig 4: Methanol (Meoh) extracts of *Peperomia pellucida (P.p)* and *Marsilea quadrifolia (M.q)* showing Negative test for Anthraquinones (A), Steroids (Ste) and Cardiac glycosides (CG) and Positive test for Glycosides (G).

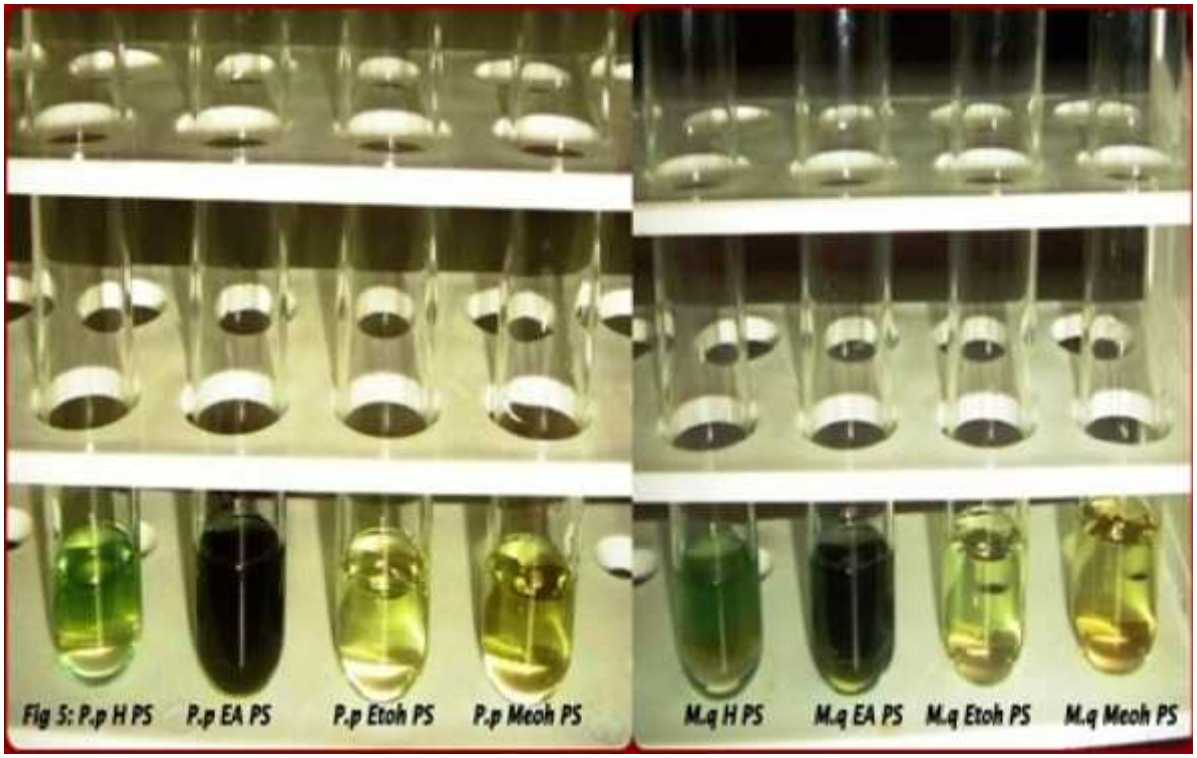

Fig 5: Hexane (H), Ethyl acetate (EA), Ethanol (Etoh) and Methanol (Meoh) extracts of Peperomia pellucida (P.p) showing positive test for Phytosterols (PS); Hexane (H), Ethyl acetate extracts of Marsilea quadrifolia (M.q) showing Positive test and Ethanol (Etoh), Methanol (Meoh) extract showing Negative test for Phytosterols (PS).

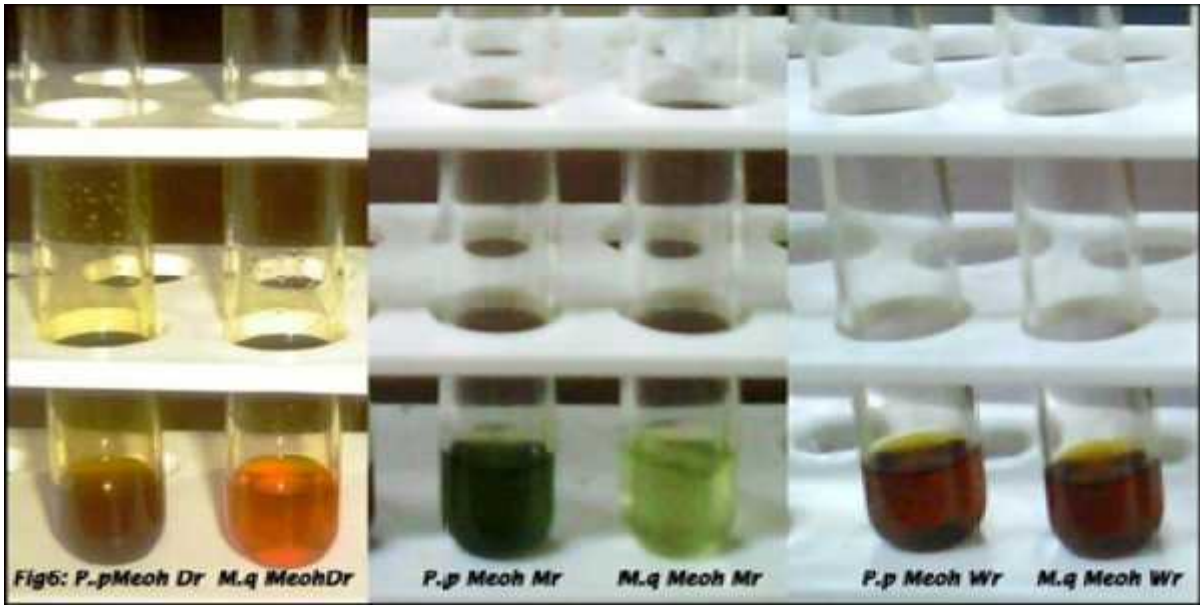

Fig 6: Methanol (Meoh) extracts of Peperomia pellucida (P.p) and Marsilea quadrifolia (M.q) showing Positive test and Negative test for Alkaloids in Dragendorff's reagent (Dr), Negative test and Positive test for Alkaloids in Mayer's reagent (Mr), Positive tests for Alkaloids in Wagner's reagent respectively.

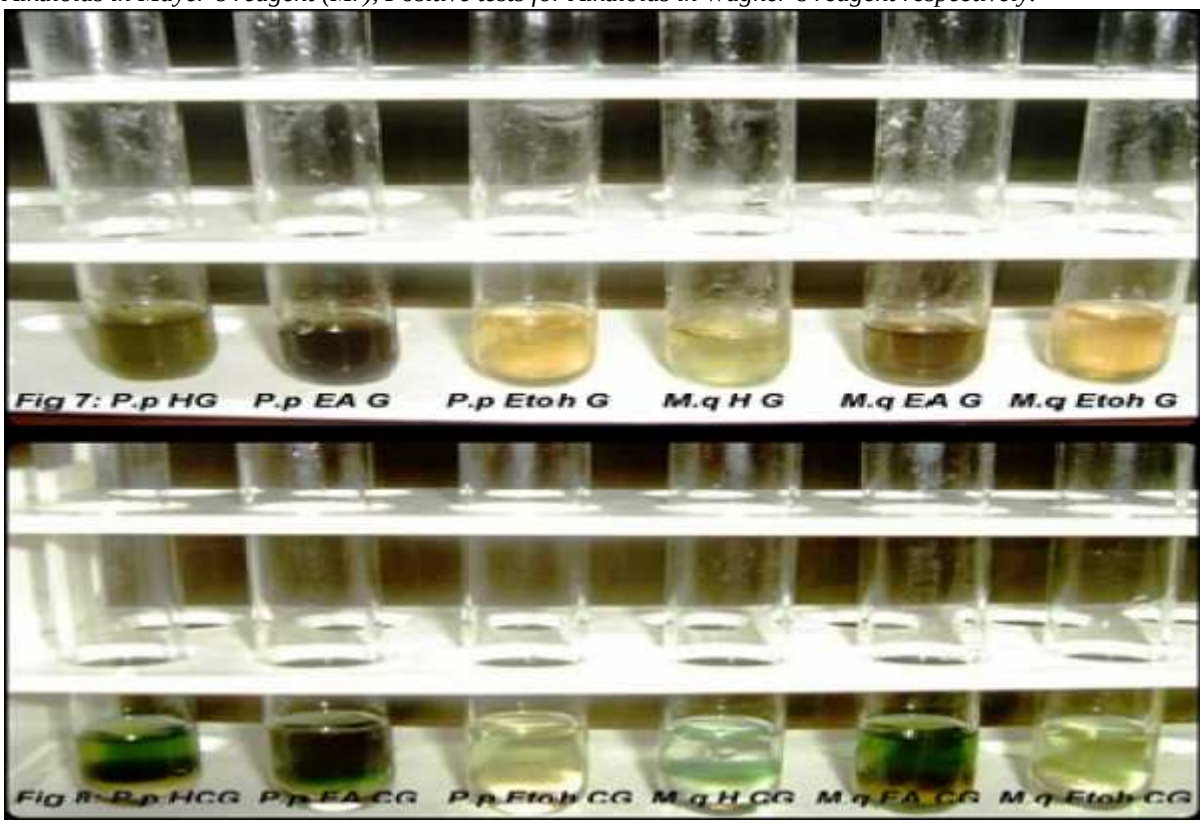

Fig 7: Hexane (H), Ethyl acetate (EA) extracts of Peperomia pellucida (P.p) showing Negative Test and Ethanol (Etoh) extract showing Positive test for Glycosides (G); Hexane (H), Ethyl acetate extracts of Marseille quadrifoila (M.q) showing Negative Test and Ethanol (Etoh) extract showing Positive test for Glycosides (G). Fig 8: Hexane (H), Ethyl acetate (EA) and Ethanol (Etoh) extracts of Peperomia pellucida (P.p) and Marsilea quadrifolia (M.q) showing Negative test for Cardiac glycosides (CG).

Test for Tannins: *Ferric Chloride Test:* To 2ml of the plant extract few drops of ferric chloride were added. Blue-black or brownish green precipitate indicated the presence of tannins.

Test for Saponins: *Sodium bicarbonate test:* To few mg of extract few drops of Sodium bicarbonate were added and shaken well. Formation of honey comb like frothing indicated a positive test for saponins.

Foam test: 1 ml of the alcoholic extract was diluted separately with distilled water to 20 ml and shaken in a graduated cylinder for 15 minutes. A one centimeter layer of foam indicated the presence of saponins.

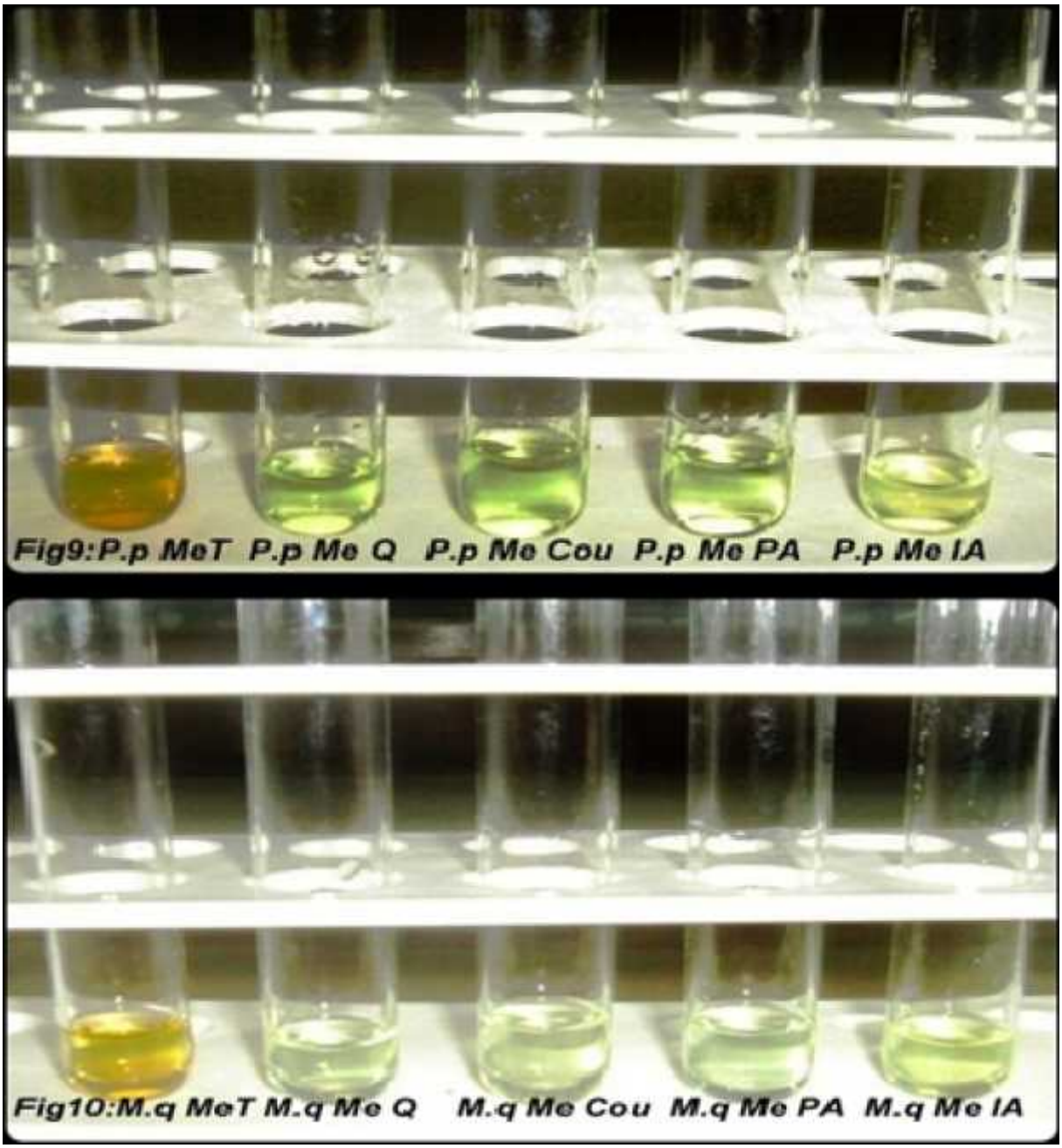

Fig 9:Methanol (Me) extract of Peperomia pellucida (P.p) showing Positive test for Tannins (T), Negative test for Quinones (Q), Coumarins (Cou) and Proteins and Amino acids (PA) and Positive test for Isoquinoline Alkaloids (IA). Fig 10: Methanol (Me) extract of Marsilea quadrifolia (M.q) showing Positive test for Tannins (T), Negative test for Quinones (Q), Coumarins (Cou) and Proteins and Amino acids (PA) and Positive test for Isoquinoline Alkaloids (IA).

Test for Flavonoids: About 5 ml of dilute ammonia solution was added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated H_2SO_4 .A yellow coloration observed in each extract indicated the presence of flavonoids. The yellow coloration disappeared on standing.

Test for Steroids

Liebermann – Burchard Reaction: 2 ml of acetic anhydride was added to 2ml of the extract with 2 ml H_2SO_4. The color changed from violet to blue or green in some samples indicated the presence of steroids.

Test for Terpenoids: *Salkowski* Test: Five ml of each extract was mixed in 2 ml of chloroform, and concentrated H_2SO_4 (3 ml) was carefully added to form a layer. A reddish brown colorations of the interface were formed to show positive results for the presence of terpenoids.

Test for Triterpenoids: *Liebermann Burchard Test:* 2ml of the extract was dissolved in 1ml of chloroform; 1ml of acetic anhydride was added followed by the addition of 2ml of concentrated H_2SO_4; formation of reddish violet color indicated the presence of triterpenoids.

Fig 11: Hexane (H), Ethyl acetate (EA) and Ethanol (Etoh) extracts of Peperomia pellucida (P.p)and Marsilea quadrifolia (M.q)showing Positive test for Isoquinoline Alkaloids (IA). Fig 12: Hexane (H), Ethyl acetate (EA) extracts of Peperomia pellucida (P.p) showing Negative test and Ethanol (Etoh) extract showing Positive test for Tannins (T); Hexane (H), Ethyl acetate (EA) extracts of Marsilea quadrifolia (M.q)showing Negative test and Ethanol (Etoh) extract showing Positive test for Tannins (T).

Test for Carotenoids: 1 g of each specimen sample was extracted with 10 ml of chloroform in a test tube with vigorous shaking. The resulting mixture was filtered and 85 % sulfuric acid was added. A blue color at the interface showed the presence of carotenoids.

Test for Anthraquinones: *Borntrager's Test:* To 1 mg of drug add 5-10 ml of dilute HCl boil on water bath for 10 minutes and filter. The filtrate was extracted with CCl4/benzene and added equal amount of ammonia solution to filtrate and shaken . Formation of pink or red color in ammoniacal layer indicated the presence of anthraquinones.

Test for Cardiac Glycosides: *Keller – Killani Test:* 5ml of each extract was treated with 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was under layered with 1ml of concentrated H_2SO_4. A brown ring of the interface indicated a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer indicated the presence of cardiac glycosides.

Test for Glycosides: *Legal test:* The extract was hydrolyzed with HCl for a few hours in a water bath. To hydrolyzate, 1ml of pyridine was added and a few

Fig 13: Hexane (H), Ethyl acetate (EA) and Ethanol (Etoh) extracts of *Peperomia pellucida (P.p)* showing Positive test for Flavonoids (F); Hexane (H) extract of *Marsilea quadrifolia (M.q)*showing Negative test and Ethyl acetate (EA) and Ethanol (Etoh) extracts showing Positive test for Flavonoids (F). Fig 14: Hexane (H) extract of *Peperomia pellucida (P.p)* showing Positive test and Ethyl acetate (EA) and Ethanol (Etoh) extracts showing Negative test for Steroids (Ste); Hexane (H) extract of *Marsilea quadrifolia (M.q)* showing Positive test and Ethyl acetate (EA) and Ethanol (Etoh) extracts showing Negative test for Steroids (Ste).

drops of sodium nitroprusside solution was added and then it was made alkaline with sodium hydroxide solution. Appearance of pink to red color showed the presence of glycosides.

Test for Phlobatannins: Deposition of a red precipitate when an aqueous extract of plant sample was boiled with 1% aqueous hydrochloric acid was taken as evidence for the presence of phlobatannins.

Test for Quinones: To the test substance, sodium hydroxide was added. Blue green or red color indicated the presence of quinones.

Test for Coumarins: To the sample 10% sodium hydroxide and chloroform were added. Formation of yellow color indicated the presence of coumarins.

Test for Tropane alkaloids: *Vitali – Morin's Test:* Extract was mixed with chloroform, boiled and allowed to extract. The mixture was filtered using a filter paper into a beaker. 2ml of the filtrate was measured into a small crucible and evaporated to dryness. The residue was moistened with a few drops of concentrated Nitric acid and evaporated to dryness on a water bath. A few drops of 10% potassium hydroxide solution in alcohol were added and mixed.

White precipitate indicated the presence of tropane alkaloids.

Test for Isoquinoline alkaloids: The extract was added to 2.5ml of water and 10ml of HCl. The mixture was allowed to stand for 5 minutes, and then filtered. 2ml of the filtrate was taken and a few crystals of potassium chlorate were added. Orange yellow precipitate indicated the presence of isoquinoline alkaloids.

Test for alkaloid: (200mg plant material in 10ml methanol, filtered), 2 ml filtrate + 1% HCl + steam, 1ml filtrate + 6 drops of Mayor's reagent /Wagner's reagent / Dragendroff reagent, creamish precipitate/ brownish- red precipitate/ orange precipitate indicated the presence of respective alkaloids.

Mayer's reagent: Few drops of Mayer's reagent were added in each extract and observed the formation of white or cream colored precipitate.

Wagner reagent: Few drops of Wagner reagent were added in each extract and observed the formation of the reddish brown precipitate.

Dragendorff's reagent: Few drops of Dragendorff's reagent were added in each extract and observed the formation of the orange yellow or a brown colored precipitate.

Test for Carbohydrates: The minimum amount of the extract was dissolved in 5ml of distilled water and filtered. The filtrate was subjected to test for carbohydrates.

Molisch's Test: To about 2 ml of the plant extract few drops of α-naphthol (20% in ethyl alcohol) were added. Then about 1 ml of concentrated sulfuric acid was added along the side of the test tube without mixing. The reddish violet ring appeared at the junction of two layers indicated the presence of carbohydrates.

Fehling's Test: The filtrate was treated with 1 ml of Fehling's A and B solution and heated in a boiling water bath for 5-10min. Appearance of reddish orange precipitate showed the presence of carbohydrates.

Iodine test: It is specific for polysaccharides. Few drops of iodine solution were added to an aqueous solution of the drug. Formation of blue color, which disappears on heating and reappears on cooling, indicated the presence of starch.

Test for Proteins and free amino acids: Various extracts were dissolved in few ml of water and treated with

Ninhydrin Test: 1 ml of the plant extract was treated with a few drops of Ninhydrin reagent. Appearance of purple color showed the presence of proteins and free amino acids.

Biuret test: Equal volume of 5% solution of sodium hydroxide and 1% copper sulphate was added. Appearance of pink or purple color indicated the presence of proteins and free amino acids.

Test for Reducing sugars: 0.5g of the extract in 5ml water was mixed with equal volume of Fehling's A and B solutions, heated in a water bath. Formation of a red color indicated the presence of reducing sugars.

Test for phenols: *Ferric chloride Test:* To test the phenol phytochemical presence, in a test tube 1ml of extract and 2 ml of distilled water were added followed by a few drops of 10% ferric chloride (FeCl3). Appearance of blue or green color indicated the presence of phenols.

Test for Phytosterols: 1gm of the extract was dissolved in a few drops of dry acetic acid; 3ml of acetic anhydride was added followed by a few drops of concentrated sulphuric acid. The appearance of a bluish green color showed the presence of phytosterols.

Test for Fixed oils and fats: *Spot Test:* Small quantity of the various extracts was separately pressed between two filter papers. Appearance of oil stains on the paper indicated the presence of fixed oil.

Test for Coumarin Glycosides: *Fluorescence test:* The alcoholic extract of the drug was mixed with 1N NaOH solution. Development of blue-green fluorescence indicated the presence of coumarin glycosides.

Test for Resins: *Turbidity test:* Resinous drug was extracted with alcohol and water was added in excess to form turbidity, because these are insoluble in aqueous solutions.

HCl test: One gram of the drug was extracted with a few ml of acetone and 3ml of dilute HCl was added. Formation of pink color after heating the solution in water bath for 30 minutes indicated the presence of resins.

FeCl3 test: Few drops of FeCl3 solution were added to the alcoholic extract of the drug. Formation of greenish blue color indicated the presence of resins.

Test for Lipids: *Grease Spot Test:* A simple test for lipid is based on the ability of lipids to produce a translucent spot on the paper.

Test for Condensed Tannins: *Catechin (Matchstick test):* Catechins forms phlorogluecinol when heated in the presence of acids and can be detected by reacting with lignin forming woody red to magenta color. The paste of test drug (tannin) was applied on the rear end of a matchstick and moistened with concentrated HCl. Formation of woody pink to magenta color on heating near the flame indicated the presence of condensed tannins.

Test for Pseudo Tannins: *Chlorogenic acid:* Extracts of drug containing chlorogenic acid on treatment with aqueous ammonia converted to green color after exposing with air.

Test for Acids: Plant extract 0.5 ml was treated with sodium bicarbonate solution. Formation of effervescence indicated the presence of acids.

RESULTS

The analysis of the organoleptic characters of *P. pellucida* showed the powdered drug was olive green in color with characteristic odor, rough texture and sour in taste and the powder of *M. quadrifolia* was greenish brown in color with characteristic odor, rough texture and taste was slightly sour. The results are shown in Table 1.

The whole plant extract of *P. pellucida* and *M. quadrifolia* were prepared in n- hexane, ethyl acetate, ethanol and methanol. The successive extraction was carried out in three different ratios [1:4,1:6 and1: 8]

and the highest yield was reported in 1:8 in both the plants. *P. pellucida* showed the lowest yield in 1:6 and *M. quadrifolia* in 1:4 and 1:6 respectively. Ethyl acetate (6%) and methanol (6%) extracts of *P. pellucida* showed the highest yield in 1:8 ratio. The lowest yield (1:6 ratio) was reported in hexane (1.80%) and ethanol (2.20%) extracts of *P. pellucida* respectively. Ethanol (4.0%) and methanol (5.10%) extracts of *M. quadrifolia* showed the highest yield in 1:8 ratio. Hexane and ethanol extracts of *M. quadrifolia* showed lowest yield 1% and 2.40% in 1:4 and 1% and 2.80% in 1:6 ratio respectively. The results are shown in Table 2.

The dried crude extracts of the drugs were evaluated for physical parameters such as consistency, color and odor. The results are presented in Table 3.

The preliminary phytochemical screening of the whole plant extract of *P. pellucida* and *M. quadrifolia* using different solvents are presented in Table 4. Various Phytochemical tests were performed on the crude extracts, n- hexane, ethyl acetate, ethanol and methanol respectively. The presence of saponins, flavonoids,terpenoids, phlobatannins, isoquinoline alkaloids, reducing sugars, phytosterols and lipids were reported in all extracts of *P. pellucida*. Tannins, glycosides, tropane alkaloids and phenols were reported in ethanol and methanol extracts of *P. pellucida* only. Steroids and triterpenoids were present in hexane extract of *P. pellucida*. Hexane and ethyl acetate extracts of *P. pellucida* reported the presence of acids. Mayer's test for alkaloids answered negative in all the extracts of *P. Pellucida;* Wagner's test and Dragendorff's test for alkaloids answered positive only for methanolic extract and were absent in all other extracts. Molisch's test for carbohydrates answered positive in the hexane extract and negative in other three extracts; Fehling's test showed the presence of carbohydrates in ethanol and methanol extracts and absence in hexane and ethyl acetate extracts and iodine test for carbohydrates answered negative in all the extracts of *P.pellucida*.

Fixed oils and fats were present in the hexane extract of *P. pellucida* and were totally absent in *M. quadrifolia*. Carotenoids, anthraquinones, cardiac glycosides, Quinones, coumarins, coumarin glycosides, resins, condensed tannins, pseudo tannins and proteins and free amino acids were found to be absent in all the extracts of *P. pellucida* and *M. quadrifolia*.

Saponins, isoquinoline alkaloids, tropane alkaloids, reducing sugars, and lipids were present in all four extracts of *M. quadrifolia*. Test for phlobatannins answered negative for all the extracts of *M. quadrifolia*.Tannins, glycosides and phenols were reported only in ethanolic and methanolic extracts of *M. quadrifolia*. Flavonoids were present in three extracts and found to be absent in hexane extract of *M. quadrifolia*. Steroids, terpenoids and triterpenoids were reported only in the hexane extract of *M. quadrifolia*. Hexane and ethyl acetate extracts of *M. quadrifolia* reported the presence of phytosterols and acids. Mayer's test for alkaloids answered positive in the methanolic extract and answered negative in other three extracts of *M. quadrifolia;* Wagner's test for alkaloids answered positive in three extracts and negative in ethyl acetate extract of *M. quadrifolia* and Dragendorff's test for alkaloids answered negative in all four extracts of *M. quadrifolia*. Molisch's test for carbohydrates answered positive in the hexane extract, Fehling's test for carbohydrates showed the presence of carbohydrates in hexane, ethanol and methanol extracts and were absent in ethyl acetate extract of *M. quadrifolia* and iodine test for carbohydrates answered negative in all the extracts of *M. quadrifola*.

DISCUSSION

The active plant constituents are usually the secondary metabolites, derived from biosynthetic pathways present within the plant tissue. Active constituents are responsible for the pharmacological or medicinal activity of the plant extract. Phytochemicals derived from the plants remain the basis for a large proportion of the commercial medications used today for the treatment of a wide range of diseases. The present study undertaken revealed the presence of number of bioactive compounds which can be used as a lead compound for synthesizing drugs for various ailments.

Saponins, flavonoids, terpenoids, phlobatannins, isoquinoline alkaloids, reducing sugars, phytosterols and lipids were present in all extracts of *P. pellucida.* Tannins, glycosides, tropane alkaloids, phenols, steroids, triterpenoids, alkaloids, acids, carbohydrates and fixed oils and fats were present in one or the other extracts of *P. pellucida*. Carotenoids, anthraquinones, cardiac glycosides, quinones, coumarins, coumarin glycosides, resins, condensed tannins, pseudo tannins and proteins and free amino acids were found to be absent in all the extracts of *P. pellucida*. Saponins, isoquinoline alkaloids, tropane alkaloids, reducing sugars, and lipids were present in all four extracts of *M. quadrifolia*. Carotenoids, anthraquinones, cardiac glycosides, quinones, coumarins, coumarin glycosides, resins, condensed tannins, pseudo tannins, fixed oils and fats, phlobatannins and proteins and free amino acids were found to be absent in all the extracts of *M. quadrifolia*. Tannins, glycosides, phenols, flavonoids, steroids, terpenoids and triterpenoids, phytosterols, alkaloids, carbohydrates and acids were reported in one or the other extracts of *M. quadrifolia.*

CONCLUSION

The chemical defense is almost the only effective instrument in the struggle of plants against pathogenic organisms and multiple herbivorous animals. For the most effective defense against pathogens, plants have developed a complicated system comprising structurally different chemicals with different mechanisms of action. These biologically relevant properties of natural products are likely to continue to be sources of new commercially viable drug leads. Since secondary

metabolites from natural sources have been elaborated within living systems, they are often perceived as showing more drug-likeness and biological friendliness than totally synthetic molecules[35]; making them good candidates for further drug development[36, 37]. There are a few accounts of studies carried out using the leaf, stem and root extracts of *P. pellucida* [38, 39, 40] and leaf extract of *M. quadrifolia* [41,42,43]. This is the first report of the analysis with the whole plant extract. The present study provides the Preliminary information on the plants under study in respect of their identification, characterization and standardization as herbal drugs. Both the plants will be further investigated for qualitative and quantitative extraction of reported phytochemicals to explore the possibilities of using it as an herbal medicine on scientific ground.

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REFERNCES

1. Buchman DD, Germerey PB. Herbal Medicine, Publishing Company, New York, 1980.
2. Lozoya M, Lozoya X. Pharmacological properties in-vitro of various extracts of *Mimosa pudica* L. Tepescohuite Arch Invest Mex. 1990; 6:87-93.
3. Jain SK. Dictionary of Indian Folk Medicine and Ethnobotany. Deep Publications, New Delhi. 1991; p232.
4. Kumar VK, Ravi Sankar N, Ramya S, Sahaja RV, Saritha K, Reddy KG, Naidu NV. Phytochemical screening and antimicrobial activity of the leaf extract of *Mirabilis jalapa* against pathogenic microorganisms. Intl. J. Phytomedicine, 2010; 2402-407.
5. Schippmann U, Leaman DJ, Cunningham AB (2002) Impact of Cultivation and Gathering of Medicinal Plants on Biodiversity: Global Trends and Issues, Biodiversity and the Ecosystem Approach in Agriculture, Forestry and Fisheries. Satellite Event on the Occasion of the 9th Regular Session of the Commission on Genetic Resources for Food and Agriculture, Inter-Departmental Working Group on Biological Diversity for Food and Agriculture (October 12–13, 2002), Rome, 2002, pp. 1–21.
6. Elisabetsky E. Pesquisas em Plantas medicinais. Ciencia e Cultura, Journal of the Brazilian Association for the Advancement of Science, 1987, 39(8): 697-702.
7. Tyler VE (1999) Phytomedicines: back to the future. J Nat Prod62:1589–1592.
8. Kaufman PB, Cseke LJ, Warber S, Duke JA, Bri elmann HL (1999) *Natural Products from Plants.* (CRC Press, Boca Raton, FL).
9. Sofowora A. Medicinal plants and Traditional Medicine in Africa. Spectrum Books, Ibadan. 1993; pp 150.
10. Showkat Ahmad Wani, Mir Ashfaq, K. W. Shah and Dharmendra Singh. Phytochemical screening of methanolic extracts of *Podophyllum hexandrum Royle* and *Rheum emodi Wall.* J. Curr. Chem. Pharm. Sc.: 2(2), 2012, 125-128; ISSN 2277-2871.
11. "Pansit-pansitan," *Philippine Medicinal Plants*, August 2012, http://www.stuartxchange.org/Pansit.html, accessed on Jan 3, 2013.
12. *Peperomia pellucida*, an Amazing Wild Medicinal Herb. Ecosensorium, 28 Nov 2010 Studies on ether soluble neutral compounds of *Peperomia pellucida*. Arch Pharm Res. 1983; 6: 133-136. www.ecosensorium.org /2010/11/*Peperomia pellucida.*
13. Complete *Peperomia pellucida* information from Drugs.com. Complete and up to date information about *Peperomia pellucida* - part of the Drugs.com trusted medication database. www.drugs.com/npp/*Peperomia -pellucida.*
14. Staples, G.W., Kristiansen, W.S., 1999. Ethnic Culinary Herbs. University of Hawaii Press, pp. 79.
15. Zakaria, M., Mohd, M.A., 1992. Tumbuhan dan Perubatan Tradisional. Fajar Bakti, Indonesia, pp. 119.
16. Duke. J. A. and E. S. Ayensu, 1985. "Medicinal Plants of China", Reference Publications Inc. ISBN 0-917256-20-4.
17. Schofield, J. J., 1989. "Discovering Wild plants, Alaska, Western Canada, the Northwest". Alaska Northwest Books, G.TE Discovery Publications, Inc. 22023 20th Ave. S.E. Bothell, WA. 98021.
18. Ramachandran, V.S., Wild edible plants of the Anamailais, Coimbatore district Western Ghats, Tamil Nadu. Indian Journal of Traditional Knowledge, 2007, 6 (1), 173-176.
19. Prafulla Soni and Lal Singh; *Marsilea quadrifolia linn.* - A valuable culinary and remedial fern in Jaduguda, Jharkhand, India. Intl. J. Life sciences and Pharma Research, Vol 2/Issue 3/Jul-Sept 2012, ISSN 2250-0480.
20. Ripa, F.A., Nahar, L., Haque, M., Islam, M.M., Antibacterial, Cytotoxic and Antioxidant Activity of Crude Extract of *Marsilea quadrifolia*. European Journal of Scientific Research, 2009, 33 (1): 123-129.
21. Dubey, N.K., Kumar, R. and Tripathi, P., "Globalpromotion of herbal medicines: India's opportunity". Current Science, 2004, 86 (1): 37-41.

22. Tuba, Z., Overview of the flora and vegetation of the Hungarian Bodrogköz. Tiscia, 1995, 29: 11-17.
23. Wraber, T. and Scoberne, P., The Red Data List of Threatened Vascular Plants in Socialistic Republic of Slovenia (Slov.). Nature Conservation, 1989, 14-15, 1-429, Ljubljana.
24. Bhardwaj N.K., Khatri P., Ramawat D., Damor R. and lal M., 2003. Pharmacognostic and phytochemical investigations on the bark of *Tecomella undulate* Seem., international journal of pharma research and development-online, page no. 1-10.
25. Harborne, J.B. Phytochemical methods, In: A guide to modern techniques of plant analysis. J.B. Harborne (ed.), Chapman and Hall, London, 1973; p.279.
26. Trease G.E., and Evans, W.C. Pharmacognosy. 13th edn. Bailliere Tindall, London, 1989, pp 176-180.
27. Awe IS, Sodipo OA (2001). Purification of saponins of root of *Bhighia sapida Koenig-Holl.* Nig. J. Biochem. Mol. Biol. (Proceedings Supplement). 16: 201-204.
28. Sofowara, A., (1993). Medicinal plants and traditional medicine in Africa, Spectrum Books, Nigeria. 2nd Ed. Pp: 10-158.
29. Odebiyi A, Sofowora AE (1978). Phytochemical Screening of Nigerian Medicinal Plants. Part III. Lloydia. 41: 234-246.
30. Ramkrishnan, S., Rajan R.(1994):Text book of medical Biochemistry. Orient Longman, New Delhi. India.
31. Finar, I. L. (1968): Stereo Chemistry and the Chemistry of Natural Products. Vol.2. Longman. Singapur.
32. Fisher, D.D (1968): Protein staining of ribboned epon section for light microscopy. Histochem. 16: 81-96.
33. Ruthmann, A. C. (1970): Methods in cell Research, Cornell University Press, New York. U.S.A.
34. Kokate CK. Practical Pharmacognosy. New Delhi: Vallabh Prakashan; 1999. p. 107-21.
35. Koehn FE, Carter GT (2005) The evolving role of natural products in drug discovery. Nature Reviews Drug Discovery 4, 206-220.
36. Balunas MJ, Kinghorn AD (2005) Drug discovery from medicinal plants. Life Sciences 78, 431-441.
37. Drahl C, Cravatt BF, Sorensen EJ (2005) Protein-reactive natural products. Angewandte Chemie International Edition 44, 5788-5809.
38. Oloyede, G. K.; Onocha, P. A.; Olaniran, B. B. - Phytochemical, toxicity, antimicrobial and antioxidant screening of leaf extracts of *Peperomia pellucida* from Nigeria. Advances in Environmental Biology Volume:5 Issue:12 Pages: 3700-3709 Pubished: 2011.
39. Egwuche, R. U.; Odetola, A. A.; Erukainure, O. L. Preliminary investigation into the chemical properties of *Peperomia pellucida L.* - Research Journal of Phytochemistry Volume:5 Issue:1 Pages: 48-53 Published: 2011.
40. Pulak Majumder; Priya Abraham; Satya, V. Ethno-medicinal, phytochemical and pharmacological review of an amazing medicinal herb *Peperomia pellucida (L.) HBK*. - Research Journal of Pharmaceutical, Biological and Chemical Sciences Volume: 2 Issue: 4 Pages: 358-364 Published: 2011
41. Manjula R and T. Mythili. Improved phytochemical production using biotic and abiotic elicitors in *Marsilea quadrifolia*. INT J CURR SCI 98-101. 2012.
42. Pepsi A., Ben C.P. and Jeeva S. Phytochemical Analysis of Four Traditionally Important Aquatic Species. International Research Journal of Biological Sciences Vol. 1(5), 66-69, Sept. (2012).
43. Ashwini .G, Pranay.P, Thrinath., Karnaker Reddy.T, Giri Prasad.V.S. Pharmacological evalution of *Marsilea qudrifolia* plant extracts against Alzheimer's disease. Int. J. Drug Dev. & Res., April-June 2012, 4 (2): 153-158