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QUALITATIVE PHYTOCHEMICAL SCREENING OF METHANOL, ETHANOL AND WATER EXTRACT OF LEAVES OF *PEPEROMIA PELLUCIDA* BY COLD MACERATION PROCESS

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ABSTRACT
Phytochemicals are the substances that have biological activity, produced mainly by plants that contribute to their colour, smell andtaste. Alkaloids, flavonoids, tannin, carotenoids, antioxidants and phenolic compounds are some of the bioactive substances that are derived from the plants that may have therapeutic significance.The active ingredients are pharmacological suitable for treating various bacterial and fungal infections including the chronic-degenerative diseases like diabetes and cancer. The present study deals with the phytochemical screening and therapeutic importance from *Peperomiapellucida.* The plants were collected, dried, powdered and extracted with methanol, ethanol and distilled water by cold maceration method for 7 days. The extract was filtrated and evaporated, and screened to identify the phytochemicals present in the plant by performing various tests which include test for alkaloids, flavonoids, carbohydrates, phenolic compounds, tannins, mucilage, terpenoids, coumarins, saponins, proteins and amino acids, glycosides and volatile oils.The phytochemical investigation of the *P. pellucida* extracts of methanol, ethanol and aqueous showed the presence of tannins, diterpenes, caumarins, saponins, proteins and glycosides. The methanol extract of *P.pellucida*shows the carbohydrate whereas the ethanolic and aqueous extracts didn't show any carbohydrate moiety. Moreover, all three types of extracts are shown the absence of alkaloids and flavonoids. Due to the presence of most pharmacological active molecules like tannins, diterpenes, caumarins, saponins, proteins and glycosides in *P. pellucida*may help to treats the various chronic disorders.Based on the presence of bioactive lead molecules in *P. pellucida*; the extended animal studies will be useful for the exploration of molecular mechanism and specific disease management. Further works to be need in the future to correlate the lead compounds with its biological activity.

Keywords: *Peperomiapellucida,*Cold Maceration, Phytochemical Screening

INTRODUCTION
Phytochemicals are the chemical compounds thatnaturally found in plants. Chemical compounds are responsible forthe colour, odour, flavor and organoleptic properties [1].The phytochemicals are biological activitybut not documented as abasics nutrient. The phytochemicals are available in the form of dietary supplement; however the possible health benefits are consumption whole plant[2].Phytochemicals have a wide range of activities, which support the immunity against various diseases. The phytochemicals like flavonoids, alkaloids, saponins, tannins, glycosides, carbohydrates, phenols, phytosterols, amino acid and protein etc. are well known that it have the medicinal property as well asit exhibit physiological activity[3,4]. Medicinal plantsare importance to health for the individual and societies[5,6].For the human body the medicinal value of plants lies with some active chemical compoundswhich produce the physiological action. For the medicinal purposes many of these indigenous plants are used. Themost significant chemically activeconstituents of plants are tannin, alkaloids, flavonoids and phenolic compounds [3,7].

Peperomiapellucida is also called as Silverbush;it belongs to the family Piperaceae. *Peperomiapellucida* is an herbaceous plant found in many parts of Asiancountries and SouthAmerican. The plant is eminent by its heart-shaped, small flowers, shallow roots, and fleshy leaves with lush and juicy stems[8, 9].*Peperomiapellucida* has been

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widely used for various medicinal practices and is used conventionally for the treatment of different conditions such as conjunctivitis, convulsions, fever, headache, gout;rheumatic pains and skin diseases[10,11].Preliminary phytochemical screening is an important step in the detection of bioactive compounds present in the medicinal plants and consequentlyit may lead to drug development and discovery [12,13].With this, the present study was carried out to determine the phytochemistry of the crude extracts of methanol, ethanol and aqueous of *Peperomiapellucida* by using cold maceration process. During the course of study three extracts of the *Peperomiapellucida* were selected for their qualitative analysis.

MATERIAL AND METHODS

Plant material

Leaves of *Peperomiapellucida* were collected from the residential area located at JalanPenghulu Him, Sungai Petani, Kedah. The plant material was authenticated by Dr. Mari Jothi,(M.D Research Scholar), Government Siddha Medical College and Hospital, Tirunelveli, Tamil Nadu, India.

Washing and drying of plant

The *Peperomiapellucida* plant samples were collected and washed under running water to remove the unwanted materials and dust. The washed plant samples were placed on the tray to dry in the shade. Drying is an important step to prevent fungal growth and should be done in the shade besides; dried plant material enables the solvent to freely bond to the phytochemicals based on its polarity.

Grinding of dried plant

The grinding process reduces the particle size and increases the surface area which leads to enhancement in the efficiency of extraction. Thus, lesser amount of solvent required for extraction. The dried leaves were chosen from the dried plant samples of *Peperomiapellucida* and milled into coarse powder using the heavy duty blender. It was then kept in a clean closedcontainer. The blender was cleaned thoroughly before placing the dried leaves sample into it to prevent cross contamination with any remnants of previously grounded material or other foreign matter deposited in the blender.

Extraction with methanol, ethanol and aqueous by cold maceration process

Coarsely ground dried leaves sample of *Peperomiapellucida* were weighed and placed into 1000 ml conical beakers. The solvent methanol, ethanol and distilled water was added to conical beaker and stoppered with cotton wools followed by paraffin film and aluminum foil. The beakers with the contents were then allowed to stand at room temperature 25°C for a period of 7 days with frequent agitation on the mechanical shaker until the soluble matter dissolved.

Filtration of extract

After 7 days of cold maceration, the soaked coarse powdered material was passed through muslin cloth (double layered), in order to remove vegetative debris and the obtained filtrate was subsequently filtered through a Whatman-1 filter paper. The filtrate was stored in amber glass air-tight container.

Evaporation of filtrate

Evaporation had been performed to obtain concentrated crude extract of *Peperomiapellucida* leaves by removing the excess solvent. Rotary evaporator (Yamato RE300, Japan) attached with a vacuum pump and a recirculation chiller was used for evaporation of the filtrate, under reduced pressure at 37 °C. The dark green crude extract of methanol, ethanol and aqueous was lyophilized to remove moisture contents. At the end of the process, a sticky crude product of *Peperomiapellucida* leaves extract obtained in semisolid form. The product was then weighed and preserved in the desiccator to protect from humidity and prevent fungal growth. The product yield had been used for further conductance of the studies.

PHYTOCHEMICAL ANALYSIS

Test for alkaloids

Mayer's Test: Few drops of Mayer's reagent were added to 2ml of filtrate, by the side of the test tube and observed for creamy or white precipitate formation.

Wagner's Test: Few drops of Wagner's reagent were added to 2ml of filtrate and observed for reddish brown precipitate formation.

Dragendorff's Test: Few drops of Dragendorff's reagent were added to 2ml of filtrate and observed for orange brown precipitate formation.

Hager's Test: Few drops of Hager's reagent were added to 2ml of filtrate and observed for yellow precipitate formation.

Test for flavonoids

Alkaline Reagent Test: 5ml of dilute ammonia solution was added to a small portion of the extract (0.5g), followed by addition of concentrated H_2SO_4 and it was observed for appearance of yellow coloration.

Shinoda Test: 5ml of 95% ethanol was added to a small portion of the extract (0.5g), followed by addition of few drops of concentrated HCl and 0.5g of magnesium turnings. It was observed for appearance of pink coloration.

Test for Carbohydrate

Benedict's test: 0.5ml of Benedict's reagent was added to 0.5ml of filtrate and heated on boiling water bath for 2 minutes. It was then observed for formation of red colour precipitate.

Test for Phenols

Ferric Chloride Test: 0.5g of extract was diluted to 5ml with distilled water, followed by addition of 5% ferric chloride solution and observed for formation of dark green colour.

Test for Saponins

Foam Test: The extract was diluted with distilled water and made up to 20ml. It was then shaken in a graduated cylinder for 15 minutes and observed for formation of layer foam for about 2cm.

Test for Proteins and Amino Acids

Ninhydrin Test: 3ml of plant extract solution and 3 drops of 5% ninhydrin solution heated in boiling water bath for 10 minutes. It was observed for appearance of purple or bluish colour.

Test for Glycosides

Keller-Kelliani Test: 5ml of extract was mixed with 2ml of glacial acetic acid containing 1 drop of $FeCl_3$. The mixture was carefully added to prepare 1ml of concentrated sulphuric acid to form a lower layer. The presence of a brown ring at the interface indicated the presence of glycosides

Others Tests

Test for Tannins: 0.5g of dried extract powder was boiled in 20ml of water and filtered. A few drops of 0.1% ferric chloride added and observed for brownish green coloration.

Test for Mucilage: Small quantity of plant extract was diluted with distilled water, followed by addition few drops of ruthenium red solution. It was observed for appearance of pink coloration.

Test for Terpenoids (Salkowski Test): 5ml of aqueous extract was mixed with 2ml of chloroform and concentrated $H_2SO_4$to form a layer. It was observed for reddish brown coloration of the interface. (Appearance of golden yellow colour indicates presence of triterpenes)

Test for Diterpenes: Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green color indicates the presence of diterpenes.

Test for Coumarins: Small amount of extract were added in a test tube with 3ml of 10% sodium hydroxide. Yellow colour indicates the presence of coumarins.

Test for volatile oil: Few drops of alcoholic Sudan III solution were added into the extract. Formation of orange red colour indicated the presence of volatile oils

RESULTS AND DISCUSSION

The pharmacological effects of crude extracts are due to the presence of bioactive chemical constituents. Three extracts of all tested constituents as shown in Table 1. The phytochemical investigation of the *P. pellucida*extracts of methanol, ethanol and aqueous showed the presence of tannins, diterpenes, caumarins,saponins, proteins and glycosides.Tannins are well known for their antioxidant and antimicrobial properties as well as for soothing relief, skin regeneration, anti-inflammation and diuresis [14].Moreover the tannins act as free radical scavengers, astringent; promote restorative of wounds and peptic ulcers[15].The phytochemical compounds of *P. pellucida* significantly contributed to antioxidant, anticancer, and antimicrobial[16].*P. pellucida* has the phytochemical constituents like tannins.It supports the various pharmacological actions via antioxidant and antimicrobial actions.

According to Kusmic et al 2004[17] stated that terpenoids might have cardio protective and antioxidant property. *P.pellucida*has terpenoids as the chemical constituents it might have cardio protective and antioxidant property. Phenolic compounds are broadly distributed in all plants. The phenolic compounds of *P. pellucida*have multiple biological effects, including free radical scavenging abilities, antioxidant, anti-carcinogenic and anti-inflammatory actions[18]. Even, *P.pellucida*hasphenolic compounds as tannins and mucilage it might play role in the prevention of several diseases such as cardiovascular disorders, cancer and other vulnerable disease.

The phytochemical constituents of *i.e.,* saponinsare widely present in the many plants including *P. pellucid* and it is known to possess the lowering oflipid accumulation, anti-bacterial actions and anthelminticactions[19]. Further, the saponins of *P.pellucida* also have role in the prevention of bacterial infections (anti-bacterial activity). Moreover, glycosides, especially the cardiac glycosides act on the cardiac muscles and it

regulates the renal blood flow. The herbal preparation with cardiac glycosides is reported to treats the congestive cardiac failure and cardiac arrhythmia. The glycosidesof *P.pellucida*are also known to mitigate the cardiac functions from the disease state. The methanol extract of *P.pellucida* shows the carbohydrate whereas the ethanolic and aqueous extracts are not shows any carbohydrate moiety. Moreover, all three types of extracts are shown the absence of alkaloids and flavonoids. The details of phyto-contituents in the extracts of *P. pellucida*are shown in Table 1. Due to the presence of most pharmacological active molecules like tannins, diterpenes, caumarins, saponins, proteins and glycosides in *P. pellucida*may help to treats the various chronic disorders.

Table 1: Preliminary phytochemical analysis

Phytochemical Test	Aqueous Extract	Methanol Extract	Ethanol Extract
Test for Alkaloids			
Mayer Test	-	-	-
Wagner's Test	-	+	-
Dragendroff's Test	-	-	-
Hager's Test	-	-	-
Test for Flavonoids			
Alkaline Reagent Test	-	-	-
Shinoda Test	-	-	-
Test for Carbohydrate			
Benedict's Test	-	+	-
Test for Phenols			
Ferric Chloride Test	-	-	+
Test for Tannins	+	+	+
Test for Mucilage	+	-	+
Test for Terpenoids			
Salkowski Test	+	-	+
Test for Diterpenes	+	+	+
Test for Caumarins	+	+	+
Test for Saponins			
Foam Test	+	+	+
Test for Proteins			
Ninhydrin Test	+	+	+
Test for Glycosides			
Keller-kelliani Test	+	+	+
Test for Volatile Oil			
Sudan Red Test	+	+	-

CONCLUSION

In conclusion, the overall results of this study suggest that all the three (methanol, ethanol and aqueous) extracts of *P.pellucida* contains more than one pharmacologically active molecules. However, the chemical characterization and isolation of leads compounds are still need to evaluate. Based on the presence of bioactive lead molecules in *P. pellucida*; the extended animal studies will be useful for the exploration of molecular mechanism and specific disease management. Further works to be need in the future to correlate the lead compounds with its biological activity.

CONFLICT OF INTERESTS

Authors declared no competitive interests for the presented work.

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