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Determination of phytochemical constituents of *Cissampelos pareira*, L with differend solvents

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Abstract
The plant *Cissamplelos pareira* belonging to Menispermaceae family contain various phytoconstituents with specific value which is significant in the field of health care system.The evaluation of Phytochemical of plant's leaf, flower and fruit of this plant. The phytochemical screening of the extract of *Cissampelos pareira* leaf extracet was maximum exhibited the presence of several phytochemical compounds including alkaloids, coumarins, flavonoids, phenols, protein, saponins, steroids, tannin and terpenoids. The preliminary phytochemical analysis established in the present study with different solvents in identifying the drug.Among the four solvents extraction, the aqueous solvent was excellent extraction protocol when campared with other solvents like acetone, ethanol and methanol.

Keywords: *Cissampelos pareira,* different solvents, phytoconstituents

Introduction
Cissampelos pareira is a woody climbing vine with leafs up to one foot long and it belongs to the family Menispermaceae and genus *Cissampelos*. It is found throughout the tropical region of India and Bangladesh. The parts of the plant used for medicinal effect are whole vine, seed, bark and leaf (Amresh *et al* 2007) [1]. The ethanol extract of *C. pareira* was found to have gum and carbohydrates, alkaloids, reducing sugars and terpenoids. The extract produced the dose dependent increase in latency time compared to control. (Reza *et al* 2014) [9]
Medicinal plants have been discovered and used in traditional medicine practices since prehistoric times. (Uthpala and Raveesha 2019) [12] Today a significant number of drugs have been developed from medicinal plants. The herbal medicines are considered to have great importance among different rural or indigenous communities in many developing countries. Traditional medicine system includes the knowledge, skills and practices based on the theories, beliefs and experiences of the folks communities to maintain their health problems. The indigenous communities have their own traditional medicine system with different medicinal plants and traditional therapies for incurable diseases (Gosh, 2003) [5].
The screenings of the leaf extracts of *Carica papaya* and *Cissampelos pareira* for phytochemicals. Different solvents such as acetone, methanol, ethanol and aqueous were used to screen phytochemicals using standard methods. Phytochemical screening confirmed the presence of alkaloids, carbohydrates, amino acids, glycosides, phenols and flavonoids in the test plants. The presence of these phytochemicals is an indicator of the pharmacological property as well as the nutritive value of the leafs of plants. (Divya *et al* 2018) [4]. *C. pareira* contains many secondary metabolites such as alkaloids (bisbenzylisoquinoline, hayatine, hayatidine, berberine, cissampareine, dicentrine, insularine, cycleanine, curine and isomerubrine), flavanoids, tannins, volatile oils and glycosides (Kamal *et al*., 2017) [7].

Materials and Methods
Collection of Plant
The fresh, healthy plant was collected from the Mannargudi, Thiruvarur Dt, Tamilnadu, India. The plant leafs, flowers and fruits of were washed thoroughly under running tap water, then with distilled water and shade dried at room temperature for remove the moisture completely in individually.
The dried leafs are then homogenized into fine powder using a mixer grinder and stored in airtight containers for further study.

Sample preparation
Ten gm of the dried powder of leafs, flower and fruit of *Cissampelos pareira* were taken separately in labelled airtight bottles and 50 ml of each solvents such as acetone, aqueous, methanol, and ethanol were individually added.

Qualitative phytochemical analysis (Harbone *et al.*, 1973) [6]
Phytochemical test were carried out of the acetone, aqueous, ethanol and methanol extract on the powdered specimens were using standard procedures to identify the constituents are described by Harborne (1973) [6].
It was done to assess the qualitative chemical composition of crude extracts using commonly employed, precipitation and colorations reaction to identify the major natural chemical groups such as alkaloids, coumarins, flavonoids, phenols, protein, saponins, steroids, tannins and terpenoides. General reactions in these analysis revealed the presence or absence of these compounds in the plant extracts.

Test for alkaloids
One ml of HCL and Mayer's reagent (2ml of 5%) was added to 1ml of plant leaf, flower fruit individually extract of *Cissampelos pareira*. The formation of green precipitate indicates the presence of alkaloids.

Test for coumarins
Extract solution is concentrated to yield a residues. Dissolve residue in hot water. After cooling divide solution in two test tubes. To one test tube add 10% (w/v) Ammonium Hydroxide. Other test tube is used as control. Fluorescence color indicated the presence of coumarin.

Test for flavonoids
Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow color, which becomes colorless on addition of dilute acid, indicated the presence of flavonoids.

Test for phenols
Ferric chloride and few drops of ethanol was added to 1ml of plant extract of *Cissampelos pareira.* Formation of violet color indicated the presence of phenols.

Test for proteins
Two ml of filtrate was treated with 2ml of 10% sodium hydroxide solution in a test tube and heated for 10 minutes. A drop of 7% copper sulphate solution was added in the above mixture. Formation of purplish violet colour indicates the presence of proteins.

Test for saponins
Few drops of water and two drops of coconut oil were added to plant extract of *Cissampelos pareira* leaf formation of layer or foam, indicated the presence of saponins.

Test for steroids
Acetic acid (2ml) was added to 2ml of plant extract of *Cissampelos pareira* sample boiled then allowed to cool and added sulphuric acid the formation of upper green color layers is positive and presence of steroids.

Test for tannins
Five ml of the *Cissampelos pareira* extract was placed in a test tube and then 2 ml of 5 % of $FeCl_3$ solution was added. A greenish-black precipitate indicated the presence of tannins.

Test for terpenoids
Two milliliter of chloroform was mixed with the plant extract and evaporated on the water bath then boiled with 2 ml of concentrated H_2SO_4. A grey color produced and indicated the entity of terpenoids.

Quantitative phytochemical analysis (Harborne (1973) [6]
Estimation of alkaloids (Harborne (1973) [6]
Alkaloid determination by using Harborne (1973) [6] method. One gram of the *Cissampelos pareira* leaf, flower and fruit was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and its covered and allowed to stand for 4 hrs. It was filtered and the extract was concentrated on a water bath to one quarter of the original volume. Concentrated NH_4OH was added by drop wise to the extract until the precipitation was completed. The whole solution was allowed to settle and the precipitate was collected and washed with dilute NH_4OH and then filtered. The residue is the alkaloids which was dried and weighed.

Estimation of coumarins (Harborne (1973) [6]
Powdered material (2.5 g) was added to a beaker containing 25 ml of water, methanol, chloroform or n-hexane placed in a shaker water bath adjusted at 37°C for 24 hours. The extracts were filtered using Whatmann No.1 filter paper and the resulted solutions were concentrated under reduced pressure and weighed. Coumarins stored in amber tightly-closed containers apparently labeled and kept in the refrigerator until used for phytochemicals were analysed.

Estimation of Flavonoids: (Bohm and Kocipai-abyazan (1994) [2]
One grams of *Cissampelos pareira* sample leaf, flower and fruits was repeatedly extracted with 100ml of 80% acetone, aqueous, ethanol and methanol at room temperature. The mixture was filtered through a Whatmann No1 filter paper into a pre-weighed 250ml beaker. The filtrate was transferred into a water bath and allowed to evaporate to dryness and weighed.

Estimation of Phenols: (Harborne (1973) [6]
The fat free samples was boiled with 50 ml of ether for the extraction of the phenolic compound for 15 min. 5 ml of the respective extract was pipetted out into a 50 ml flask, then 10 ml of distilled water was added. 2 ml of NH4OH solution and 5 ml of concentrated amyl alcohol were also added. The plant leaf, flower and fruits samples were made up to mark and left to react for 30 min for colour was developed. This was read at 550nm.

Estimation of protein: (Bradford (1976) [3]
The total proteins content was determined by using Bradford's method. 100 µl of the respective samples extract added 3 ml of Bradford's reagent and incubated in dark for 5 minutes. The absorbance was measured at 595nm. Bovine serum albumin dilutions (0.1mg/ml 0.5mg/ml) are used as standard solutions.

Estimation of saponins: (Obadoni and Ochuko (2001) [8]**.**
The *Cissampelos pareira* samples leaf, flower and fruits individually were ground. 20g of each plant samples respective were dispersed in 200 ml of 20% ethanol. The suspension was heated over a hot water bath for 4 hours with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrateds samples was transferred into a 250 ml separator funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added.
The combined nbutanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath.
After evaporation, the respective samples were dried in the oven to a constant weight. The percentage of saponins content was calculated.

Estimation of total steroids: (Harborne (1973) [6]
The extract (1 g) was macerated with 20 ml of ethanol and filtered. Two ml of chromagen solution was added and the

solution left to stand for 30 min. The absorbance was read at 550nm.

Estimation of tannins (Van-burden and Robinson, 1981) [13]

Five hundred mg of the *Cissampelos pareira* was weighed into a 50 ml plastic bottle. 50ml of methanol and aqueous solvent was added and shaken for 1 hrs in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtrate was pipette out into a test tube and mixed with 2 ml of 0.1M $FeCl_3$ in 0.1 N HCL and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm with in 10 mm.

Estimation of terpenoids: (Harborne (1973) [6]

Dried *Cissampelos pareira* leaf flower and fruit extract of 100mg was taken and soaked in 9ml of methanol and aqueous for 24 hour. The extract after filtration was extracted with 10mL of petroleum ether using separating funnel. The plant ether extract was separated in pre-weighed glass vials and waited for its complete drying (wf) was evaporated and the yield (%) of total terpenoids contents was measured by the following formula (wi-wf/wi×100).

Results and Discussion

The phytochemical analysis of the leafs, flower and fruit of *Cissampelos pareira* reveals the presence of secondary metabolites were analysed. The phytochemical analysis of *Cissampelos pareira* leaf extract of alkaloids and flavonoids are present in each solvents such as Phenols, protein, saponin and steroids recorded in the acetone solvent. Coumarins, protein, tannin and terpenoids are recorded in the aqueous extracts. Ethanolic extract were recorded in the phenols and steroids.Coumarins and saponin are estimated in the methanolic extract. (Table- 1, 2)

The qualitative phytochemical analysis was carried out with different solvents such as acetone, aqueous, ethanol and methanol are *Cissampleos pareira* flower extract. Alkaloids, flavonoids and protein are present in all solvents extracts. Coumarins and steroids are present in acetone and aqueous extracts. Saponins are present in acetone extract only. Methanolic extract only phenol compounds respectively. (Table-3)

In phytochemical analysis were carried at four solvents used and preparation of *Cissampelos pareira* fruit extract. Alkaloid and protein are present in the all solvents. Phenol, steroids and terpenoids are estimated in the acetone extract.Coumarins, flavonoids, saponin and terpenoids represent are in the aqueous and ethanolic solvent extract coumarins, flavonoids, saponin and terpenoids. Phenols, steroids, tannin and terpenoids are estimated in the methanolic extract. (Table- 4)

The various phytochemicals with protein binding properties, such as flavonoids, polyphenols, saponins, tannins, and alkaloids bind with toxic venom proteins, thereby inactivating them. (Sani *et al.,* 2020) [10] phytochemical screening revealed the presence of alkaloids, phenols, and tannins in all the aqueous and methanolic extracts of the four plants were studied (Stella *et al* 2021) [11]. designed to screen phytochemicals present in the leaf extracts of various solvents of traditionally significant plants *Cissampelos pareira*. The presence of phytochemicals such as alkaloids, amino acids, phenols, glycosides and flavonoids, they, evidenced that these plants possess significant properties to cure disease (Divya *et al*., 2018) [4]. The phytocompounds are alkaloids, flavonoids and protein are present in all solvents and some samples. mostly absent.

Table 1: Analysis of qualitative phytochemical compounds of *Cissampelos pareira*, L. extract with different solvents

Phytochemical compounds	Leaf				Flower				Fruit			
	A	Aq	E	M	A	Aq	E	M	A	Aq	E	M
Alkaloids	+	+	+	+	+	+	+	+	+	+	+	+
Coumarins	-	+	-	+	+	-	+	-	-	+	+	-
Flavonoids	+	+	+	+	+	+	+	+	+	+	+	-
Phenols	+	-	+	-	+	-	-	+	+	-	-	+
Proteins	+	+	-	-	+	+	+	+	+	+	+	+
Quinones	+	-	-	+	-	-	-	-	-	+	-	-
Saponin	+	-	+	-	+	-	-	-	-	-	+	-
Steroids	-	+	-	-	+	-	+	-	+	-	-	+
Tannins	-	+	-	-	-	+	-	-	-	+	-	+
Terpenoids	+	+	+	+	-	+	-	-	+	-	+	+

A-Acetone Aq-Aqueous E-Ethanol M-Methanol (+) Present, (-) absent

Table 2: Analysis of quantitative phytochemical compounds of *Cissampelos pareira*, L. leaf extract

Phytochemical compounds	Quantity (mg/g)			
	Acetone	Aqueous	Ethanol	Methanol
Alkaloids	1.79±0.01	1.78±0.01	1.78±0.09	1.81±0.07
Coumarins	-	08.2 ±0.02	-	1.00±0.75
Flavonoids	1.20±0.00	7.30±0.01	1.48±0.09	1.24±0.01
Phenols	1.2±0.01	-	1.20±0.46	-
Protein	1.23±0.07	1.69±0.05	-	-
Saponnin	1.96±0.02	-	-	1.07±0.02
Steroids	1.05±0.05	-	1.05±0.02	-
Tannin	-	1.18±0.08	-	-
Terpenoids	-	1.78±0.03	-	-

Values are expressed in mean ± S.D

Table 3: Analysis of quantitative phytochemical compounds of *Cissampelos pareira*,L. flower extract

Phytochemical compounds	Quantity (mg/g)			
	Acetone	Aqueous	Ethanol	Methanol
Alkaloids	1.8±0.00	1.9±0.04	1.9±0.05	1.00±0.04
Coumarins	1.2 ±0.00	-	1.2±0.03	-
Flavonoids	1.0±0.06	1.8±0.03	1.5±0.02	1.70±0.08
Phenols	1.8±0.03	-	-	1.50±0.06
Protein	1.4±0.08	1.75±0.07	1.6±0.04	1.90±0.06
Saponin	1.9±0.07	-	1.4±0.02	-
Steroids	1.8±0.03	1.20±0.00	1.9±0.02	-
Tannin	-	1.1±0.05	-	-
Terpenoids	-	1.8±0.09	-	-

Values are expressed in mean ± S.D

Table 4: Analysis of quantitative phytochemical compounds of *Cissampelos pareira*,L. fruit extract

Phytochemical compounds	Quantity (mg/g)			
	Acetone	Aqueous	Ethanol	Methanol
Alkaloids	2.20±0.02	2.00±0.04	1.70±0.03	1.30±0.04
Coumarins	-	1.00±0.11	1.02±0.01	-
Flavonoids	2.50±0.01	1.84±0.07	1.00±0.09	-
Phenols	-	2.00±0.05	-	-
Protein	2.60±0.00	1.20±0.09	1.60±0.88	1.39±0.09
Quinones	-	2.08±0.21	-	-
Saponin	-	-	-	1.60±0.04
Steroids	1.06±0.03	-	1.05±0.06	-
Terpenoids	1.20±0.02	-	1.30±0.00	1.50±0.06

Values are expressed in mean ± S.D

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