

# Phytochemical Constituents of *Cissampelos pareira* L. with Different Solvents

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## Abstract

*Cissampelos pareira* belonging to Menispermaceae family contain various phytoconstituents with specific value which is significant in the field of health care system. Qualitative phytochemicals compounds such as alkaloids, coumarins, flavonoids, phenols, protein, quinones, saponins, steroids, tannin and terpenoids were found to be recorded in the acetone and aqueous extracts solvents of leaf of *C. pareira* respectively. Were as flower extract of *C. pareira* maximum phytochemical constituents like acetone extracts of alkaloids, coumarins, flavonoids, phenols, protein, saponine and steroids were represented respectively. In the fruit extracts of *C. pareira* phytochemical compounds of alkaloids, coumarins, flavonoids, phenols, proteins, quinines, steroids, tannins and terpenoids was maximum quality recognized in aqueous and ethanolic extracts in the respective fruit part. Determination of maximum quantitative phytochemicals constitutions of leaf extract of *C. pareira* in alkaloids content at methanolic extraction than that of other solvents extract respectively. Whereas *C. pareira* flowers with methanolic extract was voluminous quantity represented when compared to other solvents respectively. In the same way of flavonoids phytochemical compounds was maximum quantity found to be recorded in the *C. pareira* plant. However, the *C. pareira* leaf, flower and fruit with phytochemicals were generally maximum represented. These phytochemicals are indispensable for pharmaceutical field of biological system.

**Key words:** *Cissampelos pareira*, Phytoconstituents, Qualitative, Quantitative, Pharmaceutical field

*Cissampelos pareira* Linn Manispermaceae family is perennial climbing herbs with small greenish-yellow flowers. The genus *Cissampelos*, of which thirty to forty species are distributed in the tropical and subtropical region including Rajasthan, Himachal Pradesh, Bihar [1]. *C. pareira* is a medicinal plant which traditionally used for treatment of many diseases like inflammation, pain, haemorrhage, gastrototoxicity, cancer, diarrhoea, diabetes, cardiotoxicity, sores and used in hepatoprotective [1-2]. Extractions has been performed on dried leaves of *Cissampelos pareira* using various solvents such as water, ethanol, methanol, petroleum ether and chloroform (in order of increasing polarity of the solvents). Thereupon, phytochemical screening was carried out on the various extracts to identify the phytoconstituents. Such studies have revealed the presence of Alkaloids (hayatine, hayatinine), flavonoids, steroid tri-terpenoids, saponins, tannins & some essential oils etc. Various pharmacological activities of *C. pareira*. Traditionally *C. pareira* is a tropical medicinal plant which is claimed for treatment of various ailments. Therefore, many

scientific studies have been performed for validations the traditional claims.

*Cissampelos pareira* 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 [3]. 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 [4].

Medicinal plants have been discovered and used in traditional medicine practices since prehistoric times [5]. 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

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different medicinal plants and traditional therapies for incurable diseases [6].

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 leaves of plants [7]. *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 [8].

## MATERIALS AND METHODS

### Collection of plant

The fresh, healthy plant was collected from the Mannargudi, Thiruvavur District, Tamil Nadu, India. The plant leaves, 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 leaves 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 leaves, flower and fruit of *Cissampelos pareira* were taken separately in labelled airtight bottles and 50 ml of each solvent such as acetone, aqueous, methanol, and ethanol were individually added.

### Qualitative phytochemical analysis [9]

Phytochemical test was 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 [9]. 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 terpenoids. General reactions in this 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 indicated the presence of alkaloids.

### Test for coumarins

Extract solution is concentrated to yield a residue. Dissolve residue in hot water. After cooling divide solution in two test tubes. To one test tube added 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 indicated 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  $\text{FeCl}_3$  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  $\text{H}_2\text{SO}_4$ . A grey color produced and indicated the entity of terpenoids.

### Quantitative phytochemical analysis [9]

#### Estimation of alkaloids [9]

Alkaloid determination by using Harborne [9] 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  $\text{NH}_4\text{OH}$  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  $\text{NH}_4\text{OH}$  and then filtered. The residue is the alkaloids which was dried and weighed.

#### Estimation of coumarins [9]

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  $37^\circ\text{C}$  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 [10]

One gram 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 [9]

The fat free samples were 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  $\text{NH}_4\text{OH}$  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 [11]

The total proteins content was determined by using Bradford's method. 100  $\mu\text{l}$  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 [12]

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 concentrated samples were 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 butanol 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 [9]

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 [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 hr 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  $\text{FeCl}_3$  in 0.1 N HCL and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 mm.

#### Estimation of terpenoids [9]

Dried *Cissampelos pareira* leaf flower and fruit extract of 100mg was taken and soaked in 9ml of methanol and aqueous solvent for 24 hours. 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 ( $\text{wi-wf/wi} \times 100$ ).

## RESULTS AND DISCUSSION

Estimation of qualitative phytochemical constituents of *Cissampelos pareira* of leaf, flower and fruit extraction with different solvents like acetone, aqueous, ethanol and methanol were performed. The maximum phytochemicals such as alkaloids, coumarins, flavonoids, Phenols, protein, quinones, saponin, steroids, tannin and terpenoids were estimated in leaf of *Cissampelos pareira* with acetone and aqueous solvents respectively and minimum qualitative phytochemical constituents such as alkaloids, flavonoids, phenols, saponin and terpenoids in ethanolic extract were analysed whereas mehtanolic extract was alkaloids, coumarins, flavonoids, quinones and terpenoids compounds profuse from *C. pareira* leaf extracts respectively.

According to the flower extract of *C. pareira* with aqueous extraction in maximum compounds such as alkaloids, coumarins, flavonoids, Phenols, protein, saponin and steroids were experimentally determined. Whereas minimum at methanolic extract of flower of *C. pareira* phyto constituents of alkaloids, flavonoids, Phenols and protein were screened respectively.

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

Phytochemical compounds	Leaf				Flower				Fruit			
	Acetone	Aqueous	Ethanol	Methanol	Acetone	Aqueous	Ethanol	Methanol	Acetone	Aqueous	Ethanol	Methanol
Alkaloids	+	+	+	+	+	+	+	+	+	+	+	+
Coumarins	-	+	-	+	+	-	+	-	-	-	+	-
Flavonoids	+	+	+	+	+	+	+	+	+	+	+	-
Phenols	+	-	+	-	+	-	-	+	+	-	-	+
Proteins	+	+	-	-	+	+	+	+	+	+	+	+
Quinones	+	-	-	+	-	-	-	-	-	+	-	-
Saponin	+	-	+	-	+	-	-	-	-	-	+	-
Steroids	-	+	-	-	+	-	+	-	+	-	-	+
Tannins	-	+	-	-	-	+	-	-	-	+	-	-
Terpenoids	+	+	+	+	-	+	-	-	+	-	+	+

(+) Present, (-) absent

Analysis of active phytochemical constituents of fruit of *Cissampelos pareira* with aqueous solvents can extracted in five compounds such as alkaloids, flavonoids, protein, quinones and tannin found to be recorded at maximum levels respectively. Moreover, the ethanol extract of fruit extract of *Cissampelos pareira* phyto constituents like alkaloids, coumarins, flavonoids, protein, saponin and terpenoids found to

be performed respectively and minimum quality of active compounds at methanol solvent. It was alkaloids, Phenols, protein, steroids and terpenoids and acetone extract of alkaloids, flavonoids, Phenols, protein, quinones, saponin, steroids, tannin and terpenoids can extracted from fruit sample of *Cissampelos pareira* plant (Table 1). Differential analytical determination of *Cissampelos pareira* leaf phytochemicals at maximum level of

methanolic extract was (1.81±0.07 mg/g) alkaloids founds to be recorded respectively whereas in minimum level of coumarin

(1.00±0.75 mg/g) can be registered in the quantity level of compounds respectively (Table 2).

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	-	1.02 ±0.02	-	1.00±0.75
Flavonoids	1.20±0.00	1.30±0.01	1.48±0.09	1.24±0.01
Phenols	1.02±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	1.08±0.00	1.09±0.04	1.09±0.05	1.00±0.04
Coumarins	1.20 ±0.00	-	1.20±0.03	-
Flavonoids	1.00±0.06	1.08±0.03	1.50±0.02	1.70±0.08
Phenols	1.08±0.03	-	-	1.50±0.06
Protein	1.04±0.08	1.75±0.07	1.06±0.04	1.90±0.06
Saponin	1.09±0.07	-	1.40±0.02	-
Steroids	1.08±0.03	1.20±0.00	1.09±0.02	-
Tannin	-	1.10±0.05	-	-
Terpenoids	-	1.08±0.09	-	-

Values are expressed in mean ± S.D

Recording quantitative phytochemicals of flower of *Cissampelos pareira* with methanolic extract was flavonoids (1.70±0.08 mg/g) and minimum phytochemical of quantity of alkaloids (1.00±0.04 mg/g) revealed with respective plant (Table 3).

The aim of the present study was to investigate the presence of phytochemicals at maximum quantity in ethanolic extract of fruit extract with alkaloids compounds in (1.70±0.03

mg/g) and minimum at the level of flavonoids in (1.00±0.08 mg/g) found to be tested in the plant contain medicinally important bioactive compounds and it justified thesis uses in the traditional medicines for the treatment of plant diseases.

Obviously the plant part of *Cissampelos pareira* leaf phytochemicals of qualitative and quantitative determination were excellent production when compared with flower and fruit extract.

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	-	-	1.02±0.01	-
Flavonoids	2.50±0.01	1.84±0.07	1.00±0.09	-
Phenols	-	2.00±0.05	-	1.36±0.63
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

The various phytochemicals with protein binding properties, such as flavonoids, polyphenols, saponins, tannins, and alkaloids bind with toxic venom proteins, thereby inactivating them [14]. Phytochemical screening revealed the presence of alkaloids, phenols, and tannins in all the aqueous and methanolic extracts of the four plants were studied [15] 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 [7]. The results showed that almost all extracts

were found rich in flavonoid content as compared to standard quercetin. *A. bidentata* exhibited the highest total flavonoid contents (TFC) 5.93 mg QE/g, whereas *Malvaviscus arboreus*, *Cleistocalyx operculatus*, *C. pareira*, *T. coadunata*, and *Sphenomeris chinensis* are moderate in flavonoid content. The plant extract of *A. indica* exhibited the lowest TFC content. The results of the present study were found comparable to the previously reported results [16]. Plants have enormous potential to synthesize secondary metabolites and play an important role in plant defense mechanism against prey, microorganisms, insect, herbivores and stress as well as interspecies protection. These secondary metabolites have been used as a drug from the

time immemorial, hence screening of phytochemicals serve as the initial steps in predicting the potential active compounds in the plant extracts [17]. Arumugam *et al.* [18] reported that maximum phytochemicals were extracted in methanolic extract of leaf and leaf derived callus of *Centella asiatica*. Whereas, Jhonson *et al.* [19] found ethanol to be better solvent for extraction of phytochemicals in *Baliospermum montanum* [17].

Analysis of the phytochemical properties of the medicinal plants used to show and isolate the drug, lead compounds and components from the parts of the plant. The unique biological activity of the plants can be identified by their phytochemical's properties. Most parts of the plants used for the analysis of the phytochemical properties were leaves, roots, stem barks, and fruits. In this review, medicinal plants were investigated for phytochemical constituents of ethanol, methanol, chloroform, acetone, hexane, petroleum ether, ethyl acetate, and aqueous (water) extraction of different phytochemicals [20]. The tested medicinal plants showed significant variation in the percentage yield with 80% methanol extraction by maceration. The highest yield was observed in *C. englerianum* (38%), while the lowest yield was detected in *E. depauperata* (22%) [21].

In the current study preliminary phytochemical screening of leaf and callus extract of *C. pareira* revealed the

presence of major phytochemicals like are alkaloids, coumarins, flavonoids, phenols, protein, saponins, steroids, tannins and terpenoides. alkaloids are present in the all solvents.

## CONCLUSION

The goal of the current study was to identify phytochemicals in *Cissampelos pareira* leaf extracts from various solvents, which are traditionally important plants. All solvents contain phytochemicals like alkaloids presented in all solvents. Which is proof that plants have important medicinal effects. This study so demonstrated the value of using plants in conventional medical practices. Inferred from the study's findings that plants contain a variety of phytochemicals is the need for more research into these medicinal plants in order to identify, purify, and describe the active phytochemicals and prove their pharmacological and therapeutic efficacies.

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