Phytochemical and Pharmacognostic Study of Hydrocotyle Verticillata Thunb

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Abstract
Hydrocotyle verticillata Thunb. Is a shady creeping medicinal herb and commonly used for fever, wounds, colds, cough, sore throat, headaches, and urinary tracts problems. So the present study is focused on phytochemical and pharmacognostic study of Hydrocotyle verticillata. The whole plant subjected to the phytochemical analysis by using standard protocol. Phytochemical analysis revealed the presence of protein, carbohydrates, lipid, tannin, saponin, alkaloids, phenols, terpenoids, flavonoids, and streroids. In this study to evaluate the nutraceutical properties and pharamacognostic studies of Hydrocotyle verticillata. Parameters such as organoleptic study, extractive value, ash value, fluorescence analysis, macro and microscopic characters, were carried out by using the standard method. The present work may surve a base for further pharmacological activity and molecular work on Hydrocotyle verticillata

Keywords: Hydrocotyle verticillata, phytochemical composition, primary and secondary metabolites, Nutraceutical properties, Pharmacognostic study.

Introduction

Figure 1: Hydrocotyle verticillata

CLASSIFICATION
Division: Magnoliophyta
Class: Magnoliopsida
Order: Apiales
Family: Araliaceae
Genus: Hydrocotyle
Species: H.verticillata

Hydrocotyle verticillata is exotic and aquatic plant. It belongs to the family Araliaceae. It is also called as water penny wort, whorled penny wort, Indian penny wort, Marshy penny wort and thick leaved penny wort. The generic name is derived from the Greek hydro, water and kotyle, small cup, probably referring to the watery habitat and peltate leaves of certain species. The specific epithet is derived from the Latin verticillatus, meaning whorled, probably referring to the inflorescence (Reuben C.J.Lim et al., 2014). It is native to North and South America and distributed in temperate and tropical regions, Canada, Denmark, France, Purto, Rico and Hawaii, USA, California, Northern Aurgentina (Reuben C.J.Lim et al., 2014).Hydrocotyle verticillata is a perennial prostrate herb. It is about 10-15 cm long. The stem is glabrous, succulent, creeping and rooting at the nodes with long stolen. The leaves are simple, alternate, orbicular, deiform, palmate lobed, shiny, blackish green above, pale beneath, smooth, prominently veined, petiole is 8-25 cm long. The leaves are 1.5-3.5 cm long and 5-60 mm in

diameter and 8-14 nerved. The inflorescence is axillary and 5-25 cm long arising from the axil of the leaves. Peduncle is about 3-13 cm long with at least 2-6 verticillate flowers cultured at 1-3 cm apart and each cluster consisting of 6-12 flowers. Pedicle is very short. The flowers are greenish to creamy white in color and star shaped and small. Each flower is subtended by a small bract and each flower is 2 mm across. The fruit is about 1-3 mm long and 2-4 mm broad and fruit is ellipsoid, acute with distinct ribs. Fruit is sub-orbicular, 1-1.5 mm. Ovary is orbicular, glabrous, style is filiform, divaricated. Flowering and fruiting is during November to January and also between March to May (B.m.Rezia Khatun et al., 2010). Hydrocotyle verticillata the plant juice is used for fever. It is used for wounds and boils. Decoction of the plant is used for colds, coughs, pruritus, sore throat. It is also used for headaches and urinary problems (Lee Suan Chua et al.,2022). It will help to increase the memory power and it is used for diuretic. It is also used for leprosy. It is used for rheumatism and ulcers.It is mixed with sugar and cassia bark and given to children for treatment of coughs. It is used in the treatment of hepatitis. Juice of the plant is mixed with honey and used for typhoid fever. It is also used for antitumor, antioxidant, antiproliferative, it is used as background in aquarium, garden pot, it is used as condiment and it is aromatic plant used as stimulatant, it consists of phytochemical such as saponin used for liver (Prashant Rameshrao Umate and Meena S. Deogade 2020). Used as background in aquarium, garden pond, sometimes used as indoor plant in America (B.m.Rezia Khatun et al., 2010).

Nutraceutical properties:

Nutrition is a basic requirement and any imbalance in the requirement will leads to various risk factor leads to various risk factor leading to diseases/disorders such as cancer and diabetes. In India, clearly 20% of the total population and 44% of young childens are under-nourished conditions and are also underweight (Pandey et al., 2013). Nutritional deficiency has thrown up a major challenge in the form of “Lifestyle diseases”. Nutraceutical can play an important role in preventing these diseases and increasing healthy society. About 2000 years ago, Hippocrates correctly emphasized “Let food be your medicine and medicine be your food” (Rajasekaran et al., 2008).

In this present work was attempted to evaluate the nutraceutical properties of whole plant powder of Hydrocotyle verticillata.

Pharmacognostic studies:

Pharmacognosy is the study of crude drugs obtained from medicinal plants, animals, fungi, and other natural sources. Pharmacognosy is derived from Greek words Pharmakon means a drug and Gignosco meaning to acquire the knowledge. Pharmacognosy term is coined by German scientist Seydler in 1815 in the title of his work Analecta pharmacognostica. In the nineteenth century the term Materia medica was used for the subject Pharmacognosy. Pharmacognosy may be defined as a branch of bioscience which treats in details medicinal and related products of crude or primary type obtained from plant, animal and mineral originals (Kokate, 2009). Pharmacognosy is a simple and reliable tool by which complete informations of the crude drugs can be obtained Pharmacnostical studies give the authentication and also ensures the quality and purity standards of the plant. Pharmacognosy is an important link between pharmacology and medicinal industry, as a result of rapid development of phytochemistry and pharmacological testing methods in recent years, new plant drugs are finding their way into medicine as purified phytochemicals, rather than in the form of traditional galenical preparation (Kokate et al., 2009)

The present study includes pharamacognostic studies of Hydrocotyle verticillata. Parameters such as organoleptic study, extractive value, ash value, fluorescence analysis, macro and microscopic characters, stomatal index were carried out by using the standard method as follow.

Material and Methods

Plant material

Extraction of plant material: - Take 50 gm of total dried plant sample. The plant material should be sufficient to fill the thimble and kept in the extractor and next will be added 350ml of solvent like Methanol and aqueous separately were used for the extraction of 50 gm in the Soxhlet apparatus followed by the standard procedure. The plant material was loaded in the extractor of the Soxhlet apparatus and then filled into a round bottom flask containing methanol and water separately. The solvents were boiled gently at 58ºC over a heating mantle using the adjustable rheostat. The extraction was continued for 8-10 hours. After 8 hours removed the plant material from the extractor and again we have to empty run plant extraction for 7-10 min, after we collected from the round bottom flask and then extracted poured in the evaporating dish for evaporating. After evaporated, the extracted will be taken in the eppendorf tube.

Phytochemical screening Hydrocotyle verticillata.

The different phytochemical tests were performed for establishing the profile of plant extract of its phytochemical constituents.

1. Test for Tannins: -

a. Ferric chloride test: - 1 ml of the filtrate was diluted with distilled water and add 2 drops of ferric chloride. A transient greenish to black color indicates the presence of tannins

b. Lead acetate test: - 1 ml of solution was added to 3 drops of the leas sub-acetate solution. A cream gelatinous precipitate indicates the presence of tannins. (Pius O. Ukoha et al., 2011).

2. Test for Flavonloids :
a. Con. H2SO4 test: - when treated with con. H2SO4 appearance of the following colour like yellow colour (anthrocyanins), yellow colour change to orange(flavones) orange colour change to crimson (flavones) respectively.
b. Shinoda test: - take 1ml of solvent extract diluted with 3 ml of ethanol followed by 2 ml of diluted Hcl and pinch mg in test tube, shaken gently. Appearance of pink or brown precipitate indicates that occurrence of flavonoids. (Samuel Tadesse et al., 2016).

3. Test for Glycosides: - To 2 ml of extract, 3ml of chloroform and 10% ammonia solution was added formation of pink colour indicates the presence of glycosides.
a. Keller-killiani test: - 0.5 ml of extract, 2 ml of glacial acetic acid and few drops of 5% ferric chloride were added. This was under layered with 1ml of con. sulphuric acid. Formation of brown ring at the interface indicates presence of cardiac glycosides. b. Borntrager's test: - To 3ml extract dil. sulfuric acid was added. The solution was then filtered the equal volume of chloroform was added to the filtrate. After shaking organic solvent was separated. Finally equal volume of ammonia solution was added. No bright pink, red or violet colour was developed in the upper layer which indicates the absence of anthraquinones. (Rashmi Saxena pal and Yogendra pal., 2016)

4. Test for saponins: -
a. Foam test: - To 1 ml of the extracts and 5 ml of distilled water was added and shaken vigorously. Formation of foam indicates presence of saponins. (Samuel Tadesse et al., 2016).
b. Emulsion test: - 1ml of the filtrate was added to drops of olive oil. The mixture was added to another two drops of olive. The mixture was shaken and observed for the formation of emulsion (Pius O. Ukoha et al., 2011).

5. Test for Terpenoids: -
a. Chloroform test: - To 5ml of the extract few drops of chloroform and conc H2SO4 was added carefully along the sides of the tube. Formation of brown colour at interface was a positive indicator.

6. Test for alkaloids: - solvent free extract was stirred with 10ml of dilute Hcl and filtered. The filtered was tested with following alkaloidal reagent as follows.
a. Mayer's test: - To 3ml filtered two drops of mayer's reagent were added by the sides of the test tube. A white creamy precipitate observed. It indicates positive test
b. Dragendroff's test: - To 3ml of filtered, few drops of dragendroff's reagent were added by the sides of the test tube. (Samuel Tadesse et al., 2016).

7. Test for Phenols: -
a. Lead acetate test: - 3drops of lead acetate solution (5%) were added to the extract yellow precipitate is produced, which shows the presence of phenolic compound.
b. Gelatin test: - 2ml of 1% of gelatin solution was added to extract. Precipitate/Turbidity is produced which confirms the presence of phenolic compounds. (Rashmi Saxena pal and Yogendra pal., 2016)

8.Test for sterols:- To 1ml of plant extract equal volume of chloroform is added and subjected with few drops of conc sulphuric acid appearance of brown ring indicates the presence of steroids.
a. Leibermann-Burchard reaction: - To 3ml of extract 10ml chloroform was added followed by 2ml of acetic anhydride. Then 2drops of con sulphuric acid were added from the side of the test tube. The blue green colour appearance indicates the presence of steroids.
c.Salkowski reaction: - Add 2ml of solvent extract diluted with chloroform was mixed with 2ml of conc sulphuric acid appearance of bluish brown ring indicates the presence of phytosteroids. (Samuel Tadesse et al., 2016

Quantitative analysis of Hydrocotyle verticillata
Quantitative analysis for the detection of protein, carbohydrates, lipids, phenol, flavonoids, tannins, sterol, saponin, terpenoids, alkaloids were done after qualitative assessment by using standardized methods mentioned below.
1)Protein (Lowry's Method (Mahammad Azhar et al., 2012) :- 0.1ml of the sample and standard were pipette out into a series of test tubes. Distilled water was added to make it 1ml. A tube with 1ml of distilled water was put as blank. 5ml of 2%sodium hydroxide was added to each tube. It was mixed well and was allowed to stand for 10minutes. Then 0.5ml of folin reagent was added, mixed well and incubated at room temperature in dark for 30 minutes. The blue cololur was developed. The final readings were taken at 660nm on a spectrophotometer.
2)Carbohydrates (anthrone reagent method (Yemn and Willis, 1954):- The plant material is hydrolysed by keeping it in a boiling water bath for 3 hours with 5ml of 2.5N of HCL and cooled to room temperature and neutralized with solid sodium carbonate till the effervescence ceased. Further the solution was used for the estimation of total carbohydrates. 0.1ml of the supernatant is taken in a clean test tube and made upto 1ml by adding distilled water. Then 4ml of anthrone was added and incubated for 8 minutes in a boling water bath. This solution is further cooled rapidly and the absorbance is measured at 630nm. The amount of carbohydrates was calculated by using the standard curve of glucose.

3)Lipids (Barned and Blackstock, 1973) Method.: - The plant material is homogenized in 5ml of chloroform and methanol mixture (2:1). The homogenate is filterd using Whatmann filter paper NO 1. Then, 0.1ml of the filtrate is taken and kept aside for evaporation. After complete evaporation, 1ml of H_2SO_4 was added to the tube and boiled for 10min. To 0.2ml of this solution 5ml of vanillin reagent is added and shaken vigorously. The colour thus obtained is measured at 520nm against a reagent blank. The content of lipids was calculated using the calibration curve of almond oil

4) Phenol (Ainsworth and Gillespie, 2007):- The sample was crushed in a pestle and mortar with 100 of 80% ethanol. Centrifuge the homogenate at 1000rpm for 20 minutes further the residue was re-extracted with 5ml of 80% ethanol and centrifuged, the supernatant was evaporated to dryness. Dissolve the residue in a 5ml of distilled water. 0.1 ml of aliquot is taken test tube and add a water to make upthe volume to 3ml, add 0.5ml Folin-Ciocalteau reagent and incubated at room temperature for 3 min. after 3minutes add 2ml of 20%Na_2CO_3 solution was mixed thoroughly and incubated for 1minute on a boiling water bath further, the solution was cooled and the Absorbance of resulting blue color was measured at 650nm using a colorimeter. Amount of total phenolic was estimated from standard graphs of catechol. Amounts of total phenolics were expressed as GAE (mg/g).

5) Tannin (Vanillin Hydrochloride method):-. Extract the sample in 50 ml methanol. Mix occasionally by swirling. After 20-28hours, centrifuge and collect the supernatant. A 0.1 ml of aliquot of each extract was mixed with 5 ml of vanillin hydrochloride reagent (4% vanillin in methanol and then 8% concentrated HCl in methanol) was added. The solution was shaken vigorously and left to stand at room temperature for 20 min in darkness. Absorbance against blank was read at 500nm. Tannic acid was used to prepare the standard curve and results were expressed as mg catechin equivalents (CE)/g extracts.

6) Flavonoids (Swain and Hillis, 1959):- Total flavonoids content of plant material used to get its extract using 85%alcohol. The extract was centrifuge at 2000rpm for about 20min supernatant was collected and evaporated to dryness. The residue was dissolved in 5ml of water. 0.1ml of the extract was taken in a test tube and the final volume was made to 2ml with distilled water to this volume of vanilline reagent was rapidly. Exactly after 15min the absorbance was recorded at 500nm. Amount of total flavonoids was estimated from standard graphs of phloroglucinol.

7) Sterols (Libermann-Burchard method):- The sample were dissolved in chloroform to 10ml and further diluted to 10ml times (10,000rpm). Pipette out of diluted 0.1, 0.2 ml sample solution were mixed with 2ml of Liberman-Burchard reagent and 2ml of chloroform. The tubes were covered with black carbon paper and kept in ice bucket in dark place for 15min. Liberman-Burchard reagent react with sterol to produced characteristics green colour their absorbance were determined on spectrophotometer at 640nm. Amount of total sterol was estimated from standard graphs from diogenin.

8)Alkaloids (Harborne method):- The acetic acid (5%) extract of plant material was warmed upto 70ºc and PH 10 was made by adding NH_4OH and centrifuged at 5000rpm, precipitate is dissolved in ehanol(95%) and H_2SO_4(20%). The alkaloid solution is mixed woth 5ml of 60% H_2SO_4, after 5min add 5ml of formaldehyde in H_2SO_4, after 5min add 5ml of formaldehyde in H_2SO_4, after 15minutes absorbance solution is measured at 565nm. The amount of alkaloids was calculated using the standard curve of Brucine.

9)Terpenoids (Method of Ghorai et al., 2012) :- To 1ml of the plant extract to add 3ml of chloroform was added. The sample mixture was thoroughly vortexed and left for 3min and then 200µl of concentration sulfuric acid (H_2SO_4) was added. Then it was incubated at room temperature for 1.5-2hours in dark condition and during incubation a reddish brown precipitate was formed. Then carefully and gently all supernatant of reaction mixture was decanted without disturbing the precipitation. 3ml of 95% (v/v) methanol was added and vortex thoroughly until all the precipitation dissolve in methanol completely. The absorbance was read at 535nm using spectrophotometer. The total terpenoid content was calculated by calibration curve of linalool and result were expressed as linalool equivalent (mg/g).

10)Saponin (Method of Deepika jain and Dr. Savita shrivastav, 2017):- The sample was dissolved in aqueous methanol (80%). To the aliquots of each tube, vanillin reagent (8%, 0.25ml) was added and sulphuric acid (72% v/v,2.5ml) added slowly on the inner side of the wall. The solutions were mixed well and the tube was transferred to a 60ºc water bath. After 10mins incubations, the tubes were cooled in ice cold water bath for 3-4min. The absorbance was measured at 544nm against the reagent blank.

Nutritional values:

H.verticillata have been reported to be a source of several nutrients, such as VitaminA, Uranium, Lithium, Berelyium, Sodium, Magnesium, Aluminium, Pottasium, Calcium, Venedium, Chromium, Manganese, Iron, Cobalt, Nickel, Copper, Zinc, Gallium, Arsenic, Selenium, Rhubidium, Strontium, Silver, Cadmium, Indium, Cesium, Barium, Thallium, Lead, Bismuth by using standard method.

Organoleptic studies

Organoleptic character such as colour, odour, nature and taste of the extract were determined, plant powder was successively extracted in Soxhlet extractor using solvent i.e methanol and distilled water in the increasing order of polarity and obtained extracts were evaporated to dry at room temperature after evaporation of the solvent colour, nature and taste of the extract were recorded.

Extractive value

50g of plant powder was extracted in Soxhlet extractor with different solvents such as methanol and distilled water. The obtained extracts were evaporated to dry at room temperature. After complete evaporation of the

solvent, weight of the extracts were recorded.
Extractive value (%) = Weight of the residue obtained/weight of the plant material taken ×100

Ash values
Determination of total ash content
Place clean crucible in muffle furnace at 600º for 1 hour. Transfer crucible from furnace to a desicator and cool to room temperature. Weigh as quickly as possible to prevent moisture absorption (use metal tongs to remove the crucible after they are ashed or dried). Take 2g of plant material into silica crucible. Place in a muffle furnance and adjust the temperature at 600ºc for 6hours. Transfer the crucible to a desicator and cool to room temperature. Then, crucible is weighed and results were recorded (Lalitha Sree. T and Vijayalakshmi. K.,2018).
Total ash content%= W3-W1 /W2-W1
Where, W1= Weight of the crucible
W2=Weight of the empty crucible with plant material
W3= Weight of the crucible with ash

Determination of Acid soluble and insoluble Ash
25ml of HCl was added to the porcelain crucible containing the total ash, covered with watch glass and boiled gently for 10 minutes. Then watch glass was rinsed with 5ml of hot water and this liquid was added to the crucible. The insoluble matter was collected on an ashless filter paper and washed with hot water until the filtrate was neutral. The filter paper containing the insoluble matter was transferred to the original crucible, dried in an oven and ignited to constant weight obtained residue was allowed to cool in a desicator for 30 minutes and weighed without delay. (Lalitha Sree. T and Vijayalakshmi. K.,2018)
Acid insoluble ash% = Weight of crucible with ash – weight of crucible/weight of sample ×100

Determination of water-soluble ash content
25ml of water was added to the porcelain crucible containing the total ash and boiled for 10min. then the insoluble matter was collected on an ashless filter paper, washed with hot water and ignited in a crucible for 15min at temperature at temperature 450ºC then cooled and obtained residue is weighed. The percentage of water-soluble ash is calculated with reference to the air-dried plant powder. (Lalitha Sree. T and Vijayalakshmi. K.,2018)
Water soluble ash (%)=Loss of weight/weight of air-dried plant powder×100

Flourescence analysis
The dried powder of whole plant material were uses for the study. Powder plant material is sieved and obtained powder was used for the analysis. A pinch of plant powder was taken in a clean test tube and added 10ml of solvent, similarly plant powder is treated with different reagents such as acetone, benzene, chloroform, distilled water, ethanol, ethyle acetate, n-butanol, petroleum ether, toluene, hexane, acetic acid, hydrochloric acid, nitric acid, orthophosphoric acid, sulphuric acid. Test tubes were shaken well and incubated for 30min, after 30min the colour of the solutions were observed for their characteristics colour reaction under the visible light (fluorescent tube) and ultra violet (UV) light and obtained colours were recorded.
Macroscopic study: Macroscopic evaluation Fresh and healthy plants of Hydrocotyle verticillata were assessed for their external characteristics.
Microscopic character: Preparation of sections: Microscopic studies had been done by prepared by free hand sectioning with the help of sharp cutting edge of the blade, cleared with stained with freshly prepared dyes safranin and observed under microscope and photographed.
The distribution of stomata on the upper and lower surfaces of the leaf can be studied by removing the peels of the leaf from the upper and lower surfaces and observing the same under a microscope.
The count of the number of stomata and epidermal cells in the microscopic field is taken and the stomatal index of each surface of the leaf can be calculated using the following formula:
Stomatal index %= No. of stomata/No.of stomata+No.of epidermal cells ×10

Result
Tannins: - the result of the present study showed tannins were present in all the different days of dry powder. Recorded +ve results to the ferric chloride and lead acetate test. Ferric chloride test showed more concentration in 120,150,180,210 days of dry powder and also showed +ve result in methanol and water extraction. Its observed that tannins are present all the days of dry material and solvent extraction of Hydrocotyle verticillata.
Flavonoids:- the result of the flavonoids test shows that different days of dry powder and solvent extract of Hydrocotyle verticillata shows +ve response to shinoda test and concH2SO4 test. Shinoda test showed more +ve result in 180days, 210days and methanol extract. But in concH2SO4 test showed more +ve result in 90days, 180days, and 210days. Overall from the results showed that flavonoids present in all different days of dry plant and solvent extraction of Hydrocotyle verticillata.
Glycosides:- In Hydrocotyle verticillata only Borntrager's test of glycosides shows –ve result whereas remaining two test shows +ve result. All the different days of dry material and extract shows +ve response to the Keller-Killiani test and molisch test. But killer-killiani test shows more +ve response in aqueous extract. Its observed that only borntrager's test shows –ve result but Keller-Killiani and Molisch test shows +ve response in all the days of dry material as well as extraction of Hydrocotyle verticillata.
Saponin:- the different day of dry material and different extract of Hydrocotyle verticillata shows +ve result to

the foam test. Compared to all the different days and extraction of Hydrocotyle shows more +ve result in the 120,150,180,210 days and methanol extraction. In present study shows that saponin shows +ve response to the all the days of dry material and extraction of Hydrocotyle verticillata.

Terpenoids:- the results of the terpenoids test shows that all the days of dry material and extract of Hydrocotyle verticillata shows +ve response to the chloroform test. But compare to all chloroform test shows more +ve response in methanol extract. Overall result shows terpenoids present all the days of material and extract of Hydrocotyle verticillata.

Alkaloids:- the result of the present study shows alkaloids were present in all the different days of dry material and extracts of Hydrocotyle verticillata and recorded less +ve result to the mayer's test shows in 30, 60, 120,150,180 days and aqueous extract. In wagner's test also less +ve reponse in the 120 and 210 days. But equal response shows to the dragendroff's test in all days of dry material and extract of Hydrocotyle. From the result of the present study it is observed that presence of alkaloids in all days of dry material and extract of hydrocotryle verticillata.

Phenols: - The different extracts and different days of dry material of Hydrocotyle verticillata show +ve response to the lead acetate and gelatin test test. In 210 days and methanol extract shows more +ve response. Overall, it's observed that presence of phenol in both the extracts and all different days of Hydrocotyle verticillata.

Sterols: - the aqueous extract of Hydrocotyle verticillata shows –ve response to the Leibermann-Burchard reaction whereas, methanol extract and all days(30-210days) of dry material shows +ve response to test of salkowski and Leibermann-Burchard reaction. From the result it is found that steroids were present all the different days of dry material and methanol extract but except aqueous extract.

Table 1: - Phytochemical screening of Hydrocotyle verticillata

Sl no	Compounds	30 days	60 days	90 days	120 days	150 days	180 days	210 days	Methanol extract	Water extract
1	**Tannin**									
a.	Ferric chloride test	++	++	++	+++	+++	+++	+++	+++	++
b.	Lead acetate test	+	+	+	++	+	+	++	+	+
2	**Flavonoids**									
a.	Conc H2SO4	++	++	+++	++	++	+++	+++	++	++
b.	Shinoda test	++	++	++	++	++	+++	+++	++	++
3	**Glycosides**									
a.	Keller-killiani test	+	+	+	+	+	++	++	+++	+++
b.	Borntrager's test	–	–	–	–	–	–	–	–	–
4	**Saponin**									
a.	Foam test	++	++	++	+++	+++	+++	+++	++	++
b.	Emulsion test	++	++	++	++	+++	+++	+++	+++	+++
5	**Terpenoids**									
a.	Chloroform test	++	++	++	+	++	++	++	+++	++

6	**Alkaloids**									
a.	Mayer's test	+	+	++	+	+	+	++	++	++
b.	Wagner's test	++	++	++	+	++	+	++	++	++
c.	Dragendroff's test	++	++	++	++	++	++	++	++	++
7	**Phenols**									
a.	Gelatin test	++	++	++	++	++	+++	+++	+++	+++
b.	Lead acetate test	+	++	++	++	++	++	+++	+++	++
8	**Sterols**	**++**	**++**	**+++**	**++**	**++**	**+++**	**+++**	**++**	**++**
a.	Leiberman-Burchard reaction	++	++	++	++	++	++	++	++	–
b.	Salkowski reaction	++	++	++	++	++	++	++	++	++

Table 2:- Quantitative analysis of Hydrocotyle verticillata.

Sl.no	**Phyto chemical**		**30 days**	**60 days**	**90 days**	**120 days**	**150 days**	**180 day**	**210 day**
1	Protein	Dry plant	0.22mg	0.24mg	0.23mg	0.312mg	0.312mg	0.324mg	0.36mg
		Fresh plant	0.13mg	0.20mg	0.14mg	0.18mg	0.192mg	0.156mg	0.22mg
2	Carbohydrates	Dry plant	1.25mg	0.8mg	2.45mg	2.80mg	2.70mg	2.95mg	3.35mg
		Fresh plant	0.75mg	0.7mg	1.04mg	1.50mg	1.55mg	2.05mg	2.35mg
3	Lipids	Dry plants	0.81mg	0.87mg	1.17mg	0.60mg	0.93mg	1.11mg	1.26mg
		Fresh plant	0.18mg	0.63mg	1.32mg	0.51mg	0.81mg	0.93mg	1.08mg
4	Phenol	Dry plant	1.1mg	0.95mg	1.90mg	2.6mg	3.35mg	2.65mg	3.45mg
		Fresh plant	0.60mg	0.75mg	0.65mg	1.30mg	2.40mg	1.35mg	1.59mg
5	Flavonoids	Dry plant	0.55mg	0.5mg	2.15mg	2.65mg	2.85mg	2.7mg	3.15mg
		Fresh plant	0.35mg	0.4mg	0.85mg	0.9mg	0.95mg	1.0g	1.20mg
6	Tannin	Dry plant	0.93mg	0.84mg	1.24mg	1.60mg	1.84mg	2.04mg	2.12mg
		Fresh plant	0.44mg	0.76mg	0.92mg	1.24mg	1.36mg	1.52mg	1.72mg
7	Sterol	Dry plant	0.44mg	0.45mg	0.67mg	0.89mg	0.91mg	1.1mg	1.24mg

		Fresh plant	0.35mg	0.41mg	0.53m	0.68mg	0.73mg	0.98mg	1.5mg
8	Saponins	Dry plant	0.88mg	1.925mg	2.145mg	2.255mg	2.75mg	2.805 mg	2.365mg
		Fresh plant	0.99mg	0.44mg	0.99mg	1.21mg	1.76mg	1.815 mg	2.31mg
9	Terpenoids	Dry plant	0.84mg	1.62mg	1.8mg	1.71mg	1.86mg	2.01mg	2.07mg
		Fresh plant	0.51mg	1.14mg	1.38mg	1.56mg	1.59mg	1.8mg	1.74mg
10	Alkaloids	Dry plant	0.36mg	0.54mg	0.42mg	0.45mg	0.48mg	0.51mg	0.54mg
		Fresh plant	0.09mg	0.09mg	0.12mg	0.12mg	0.15mg	0.18mg	0.21mg

Table3:- Methanol and aqueous extract of Hydrocotyle verticillata.

Sl.no	**Phytochemicals**	**Content**	
		Methanol extract	Aquoeus extract
1.	Phenol	1.95mg	1.71mg
2.	Flavonoids	1.75mg	2.4mg
3.	Tannin	1.8mg	2.8mg
4.	Saponin	3.2mg	2.65mg
5.	Terpenoids	1.9mg	2.3mg
6.	Steroids	1.5mg	2.2mg
7.	Alkaloid	0.98mg	0.75mg

Result of quantitative analysis of Hydrocotyle verticillata are summarized in Table 2 and 3.

Proteins: the result of the Hydrocotyle verticillata showed highest amount of proteins present in 210 days of plant material (0.36mg/500mg in dry plant & 0.22mg/500mg in fresh plant) and least amount of terpenoids present in 30days of plant material (0.22mg/500mg in dry plant & 0.13mg/500mg in fresh plant). overall, the result shown compared to all the different days of H.verticillata shows highest number of proteins in 210 days of plant material but less concentration shows in 30 days of plant material.

Carbohydrates: The present investigation showed that very interesting results of carbohydrates in Hydrocotyle verticillata. It was observed that the maximum number of phenols were present in 210 days of plant material (3.35mg/500mg in dry plant & 2.35mg/500mg in fresh plant) and in 30 days of plant material shows (1.25mg/500mg in dry plant & 0.75mg /500mg in fresh plant). whereas the result of carbohydrates estimation of Hydrocotyle verticillata shows highest concentration in 210 days of plant material and least amount of carbohydrates shows in 60 days of plant material compared to other different days of Hydrocotyle verticillata.

Lipids: the result of lipids content in H.verticillata showed the highest number of flavonoids is present in 210 days of plant material (1.26mg/500mg in dry plant & 1.08mg/500mg in fresh plant) least amount of lipids present in 30 days of plant material (0.81mg/500mg in dry plant and 0.18mg/500mg in fresh plant. compared to all the different days plant material of shows more amount of lipids is present in 210 days of H.verticillata. less amount of lipids shows in 30 days of plant material.

Phenol: - The present investigation showed that very interesting results of phenols in Hydrocotyle verticillata. It was observed that the maximum number of phenols were present in 210 days of plant material (3.45mg/500mg in dry plant & 1.59mg/500mg in fresh plant) and in 30 days of plant material shows (1.1mg/500mg in dry plant & 0.60mg /500mg in fresh plant). Phenol content in methanol extract is 1.95mg/500mg and aqueous extract is 1.71mg/500mg. whereas the result of H.verticillata shows highest amount of phenol is present in maximum day i.e 210 days of plant material compared to other different days of plant material but least amount of phenol is present in 60 days of plant material. Whereas phenol content in methanol extract shows highest concentration compared to aqueous extract of H,verticillata.

Flavonoids: - The result of flavonoids content in H.verticillata showed the highest number of flavonoids is present in 210 days of plant material (3.15mg/500mg in dry plant & 1.20mg/500mg in fresh plant) and least number of flavonoids present in 30 days of plant material (0.5mg/500mg in dry plant and 0.35mg/500mg in fresh plant. Wheareas flavonoids content in methanol extract shows 1.75 mg/500 mg and aqueous extract shows 2.4mg/500mg. whereas the result of H.verticillata shows highest concentration in 210 days of plant material compared to other different days of plant material. Result of flavonoids content in aqueous extract showed highest concentraction compared to methanol extract.

Tannins: - From the results of the present study it is observed that in Hydrocotyle verticillata the maximum number of tannins is present in 210 days of plant material (2.12mg/500mg in dry plant and 1.72mg/500mg in fresh plant) and least amount of tannin present in 30 days of plant material (0.72mg/500mg in dry plant & 0.44mg/500mg in fresh plant). The result of tannin content in methanol extract shows 1.8mg/500mg and aqueous extract shows 2.8mg/500mg. whereas the result of tannin estimation of Hydrocotyle verticillata shows highest concentration in 210 days of plant material and least amount of tannins shows in 30 days of plant material compared to other different days of Hydrocotyle verticillata. But in different extract of Hydrocotyle verticillata shows highest concentration in aqueous extract compared to methanol extract of Hydrocotyle verticillata.

Sterols:- the result of sterols content in H.vericillata showed the highest amount of sterols is present in 210 days of plant material (0.44mg/500mg in dry plant & 0.35mg/500mg in fresh plant) and least amount of flavonoids present in 30 days of plant material (1.24mg/500mg in dry plant and 1.5mg/500mg in fresh plant. Wheareas sterols content in methanol extract shows 1.5mg/500mg and aqueous extract shows 2.2mg/500mg. whereas the result of H.verticillata shows highest concentration in 210 days of plant material compared to other different days of plant material. Result of sterols content in aqueous extract showed highest concentraction compared to methanol extract.

Saponins: - from the present investigation it is observed that maximum amount of saponins present in Hydrocotyle verticillata, the highest amount of saponins is present in 210 days of plant material (2.565mg/500mg in dry plant &2.31mg/500mg in fresh plant) and least amount of saponins present in 30 days of plant material (0.88mg/500mg & 0.99mg/500mg in fresh plant). whereas saponins content in methanol extract shows 3.25mg/500mg and aqueous extract shows 2.65mg/500mg. compared to all the different days plant material of shows more amount of saponin is present in 210 days of H.verticillata. less amount of saponins shows in 30 days of plant material. When the result of different extract of plant material shows more amount of saponins present in methanol compared to aqueous extract of Hydrocotyle verticillata.

Terpenoids:- the result of the Hydrocotyle verticillata showed highest amount of terpenoids present in 210 days of plant material (2,07mg/500mg in dry plant & 1.74mg/500mg in fresh plantand least amount of terpenoids present in 30days of plant material (0.84mg/500mg in dry plant & 0.51mg/500mg in fresh plant). wheareas result of terpenoids content in methanol extract shows 1.9mg/500mg and aqueous extract shows 2.3mg/500mg. overall the result shown compared to all the different days of H.verticillata shows highest amount of terpenoids in 210 days of plant material but less concentration shows in 30 days of plant material. In extraction of H.verticillata shows highest concentration in methanol extraction compared to aqueous extraction of Hydrocotyle verticillata.

Alkaloids:- the result of alkaloids content in H.verticillata shows highest concentration in 210 days & 60 days of plant material. 210days of plant material shows (0.54mg/500mg in dry plant & 0.21mg/500mg in fresh plant) .In the present study result of alkaloids content in methanol extract shows 0.85mg/500mg & ethanol extract shows 0.69mg/500mg. compared to both extraction of plant material methanol shows highest concentration compared to ethanol extract. In the present study shows compared to all different days of plant material showed highest concentration in 210 days & 60 days compared to other days of Hydrocotyle verticillata.

Nutritional value :-

Table4:- Nutritional value of Hydrocotyle verticillata.

Serial number	Nutritional Information	Concentration
1	Vitamin A	3.45
2	Uranium	0.08
3	Lithium	1.22
4	Berelyium	0.02
5	Sodium	23175.38
6	Magnesium	5521.77
7	Aluminium	1567.46
8	Pottasium	65564.86
9	Calcium	8041.88
10	Venedium	5.72
11	Chromium	4.86
12	Manganese	95.11
13	Iron	1568.87
14	Cobalt	1.19
15	Nickel	5.38
16	Copper	26.42
17	Zinc	49.27
18	Gallium	9.03
18	Arsenic	0.26
20	Selenium	1.03
21	Rhubidium	23.92
22	Strontium	339.69

23	Silver	1.28
24	Cadmium	0.38
25	Indium	0.00
26	Cesium	0.13
27	Barium	200.45
28	Thallium	0.00
29	Lead	1.60
30	Bismuth	0.01

Result of nutritional value of Hydrocotyle verticillata is depicated in the Table 4. The study have found that plant contain VitaminA(3.45%), Uranium(0.08%), Lithium(1.22%), Berelyium(0.02%), Sodium(23175.38%), Magnesium(5521.77%), Aluminium(1567.46%), Pottasium(65564.86%), Calcium(8041.88%), Venedium(5.72%), Chromium(4.86%), Manganese(95.11%), Iron(1568.87%), Cobalt(1.19%), Nickel(5.38%), Copper(26.42%), Zinc(49.27%), Gallium(9.03%), Arsenic(0.26%), Selenium(1.03%), Rhubidium(23.92%), Strontium(339.69%), Silver(1.28%), Cadmium(0.38), Indium(0.00%), Cesium(0.13%), Barium(200%), Thallium(0.00%), Lead(1.60%), Bismuth(0.01%). In the present study shows that comparatively more concentration shown in sodium, potassium, aluminium, calcium and iron. But very less concentration shown in indium, bismuth, thallium and berelyium respectively.

Organoleptic studies:

Results of the organoleptic study of Hydrocotyle verticillata were presented in Table-5. The two solvent extracts such as methanol and aqueous used. Methanol extract of H.verticillata showed dark green colour and aqueous extract of H.verticillata showed brown colour. Nature of the both methanol extract of H.verticillata is sticky whereas nature of the aqueous extract of H.verticillata is solid. Odour of the methanol extract were pungent and aqueous extract of H.verticillata were odourless. Tastes of the both solvent extract were bitter.

Table 5: Organolelptic study of Hydrocotyle verticillata.

Sl.no	Slovent	Colour	Nature	Odour	Taste
1	Methanol	Dark green	Sticky	Pungent	Bitter
2	Aqueous	Brown	Solid	Odourless	Bitter

Extractive values:

The results of the extractive values of two solvents such as methanol and aqueous extracts of Hydrocotyle verticillata were presented in Table-5. Extractive value determines the active constituents present in the drug and for the determination of exhausted or adulterated drug. The highest amount of extract of Hydrocotyle verticillata were obtained from methanol. The extractive value of methanolic extract is 14.93% followed by aqueous extract is 6.79%. The result of extractive value of Hydrocotyle verticillata were high in methanol bue least extractive value was observed in Aqueous.

Table 6:- Extractive values of Hydrocotyle verticillata

Sl. No	Solvent	% of extractive value
1	Methanol	14.93%
2	Aqueous	6.79%

Ash values: - The results of the ash values are mentioned in Table6. It is an important characteristic of a drug and with the help of this parameter we can detect the extent of adulteration which helps to determine the quality and purity of the drug. The total ash value of Hydrocotyle verticillata was 25.2%, water soluble ash was 16% and acid insoluble ash was 2%.

Table 7: Ash value of Hydrocotyle verticillata

Sl.no	Type of ash	% of ash value
1	Total ash value	25.2%
2	Acid soluble and insoluble Ash	2%
3	Water soluble	16%

Figure 2: Ash content of Hydrocotyle verticillata

Fluorescence analysis:

The result of the fluorescence analysis were represented in Table7. Powder of Hydrocotyle verticillata were treated with different solvents and acids which showed different colour reactions according to their nature of the constituent present in the plant material. Few plant extracts shows fluorescence in visible light and some of them shows fluorescence only when they are exposed to UV light.

Table 8:- Fluorescence analysis of Hydrocotyle verticillata

Sl. No	Solvent/Acid	Normal light	UV light
1	Acetone	Dark green	Cerise pink
2	Benzene	Brown	Hot pink
3	Chloroform	Light brown	Hot pink
4	Distilled water	Light green	Brown
5	Ethanol	Green	Magenta
6	Ethyle acetate	Green	Magenta
7	n-Butanol	Light green	French
8	Methanol	Brown	Ruby pink
9	Petroleum ether	Light yellow	Cerise
10	Toluene	Dark green	Magenta
11	Xylene	Light yellow	Creamy pink
12	Acetic acid	Light brown	Hot pink
13	Hydrochloric acid	Brown	Brown
14	Nitric acid	Yellow	Cedar
15	Orthophosphoric acid	Dark brown	Hiskory
16	Sulphuric acid	Dark brown	Chocolate

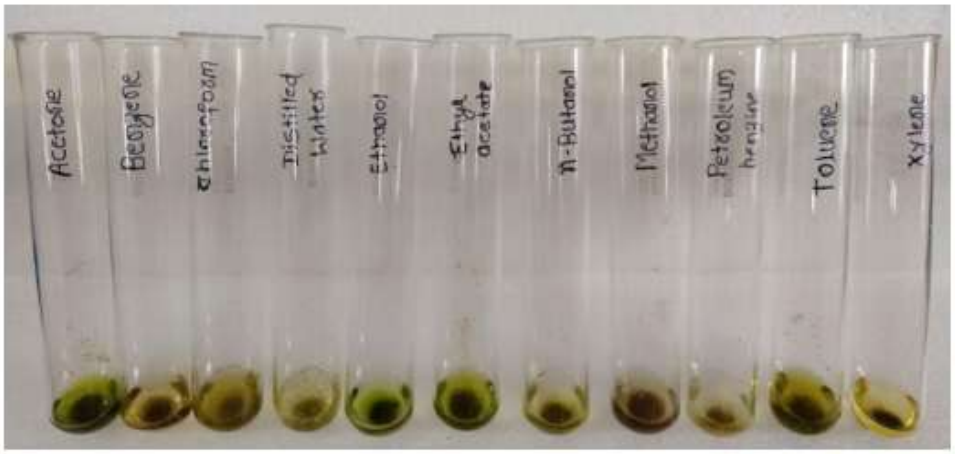

Figure 3: Under normal light 1)Acetone 2)Benzene 3)Chloroform 4)Distiled water 5)Ethanol 6)Ethyle acetate 7)n-Butanol 8)Methanol 9)Petroleum ether 10)Toluene 11)Xylene of Hydrocotyle vericillata

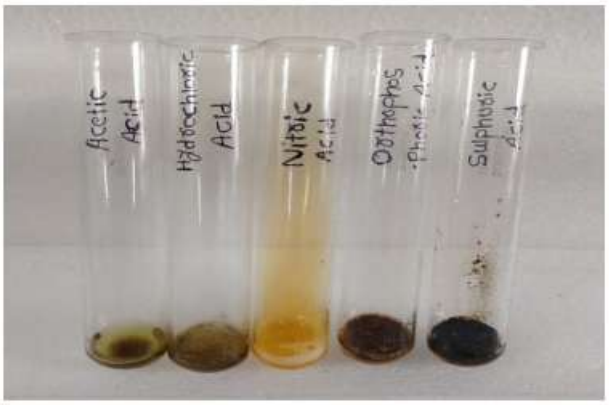

Figure 4: Under normal light 11)Acetic acid 12)Hydrochloric acid 13)Nitric acid 14)Orthophosphoric acid 15)Sulphuric acid of Hydrocotyle vericillata

Figure 5 : Under UV light 1)Acetone 2)Benzene 3)Chloroform 4)Distiled water 5)Ethanol 6)Ethyle acetate 7)n-Butanol of Hydrocotyle verticillata

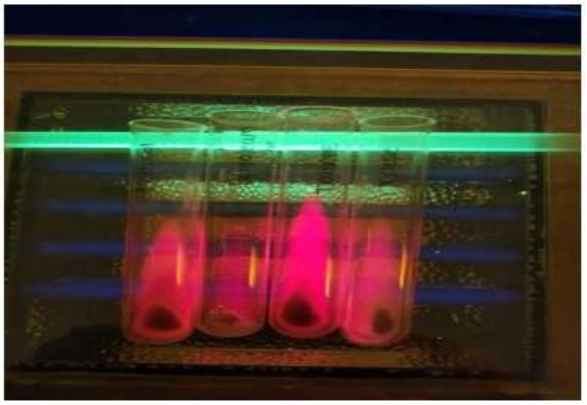

Figure 6 : Under UV light 8) Methanol 9) Petroleum ether 10) Toluene 11) Xylene of Hydrocotyle verticillata

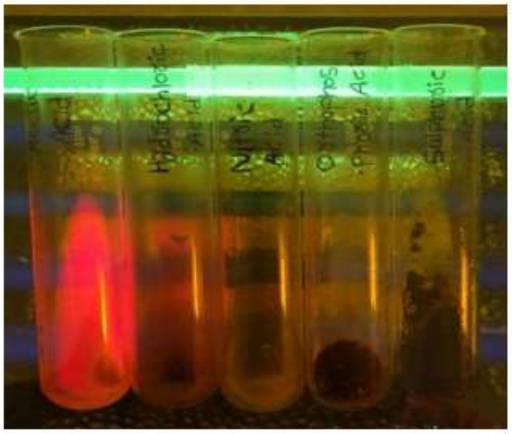

Figure 7: Under UV light 11) Acetic acid 12) Hydrochloric acid 13) Nitric acid 14) Orthophosphoric acid 15) Sulphuric acid of Hydrocotyle verticillata

Macroscopic character

Hydrocotyle verticillata is a perineal, glabrous, succulent prostate herb. 10–15cm long, stem creeping, rooting at the nodes, with long stolon. Leaf simple, alternate, leaflets are 2.0–5.0 × 2.5–6.0 cm orbicular-reniform and palmately lobed, margin very coarsely repand serrate, shiny, blackish green above and pale beneath, Sooth, prominently veined with long petiole 8–25cm long. Inflorescence is 5–25cm long arising from the axil of the leaves. Flowers in clusters, 6–12 in quantity of greenish to creamy white color, star shape, small with pedicel very short, 2–3 mm long in fruit; perianth 5, c. 1.5 × 1.0 mm, ovate-acute, each flower subtended by a minute bract at the base. Stamens usually 5. Ovary orbicular, glabrous; style filiform, divaricate. Fruits sub-orbicular, 1.0–1.5 × 2.0–2.5 mm, broader than long, laterally compressed with prominent ribs.

Microscopic chraracters

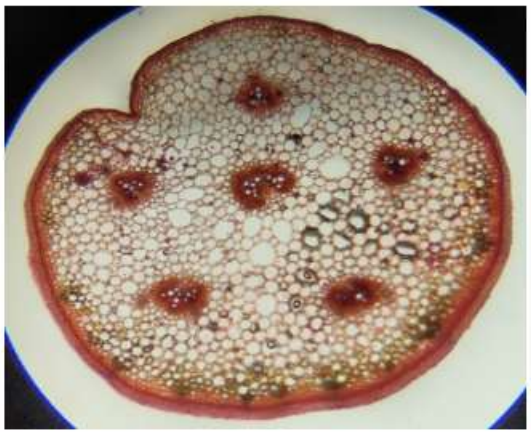

Figure 8: T.S. of Petiole of Hydrocotyle verticillata

Petiole: The transverse section shows that epidermis is followed by hypodermis and central ground tissue. The vascular bundles are abruptly distributed all over the ground tissue. Details of T.S. show that epidermis consists of compactly packed barrel-shaped epidermal cells rarely interrupted by stomata covered by cuticle, cuticle suberized.

Hypodermis: Hypodermis consists of two to three layers of collenchyma cells without any intercellular cell without supporting ground cells.

Ground tissue parenchymatous loosely arranged with some chlorophyll pigments toward the center, and some parenchyma cells consist of aerenchyma cells distributed all over the ground cells.

Vascular Bundle: The vascular bundle consists of outer phloem and inner xylem. Phloem consists of sieve elements and xylem consist of fibers and few parenchyma cells with a concentric system of the vascular bundle.

T. S. through midrib

T.S. through midrib shows upper epidermis and lower epidermis through nerve vascular bundle

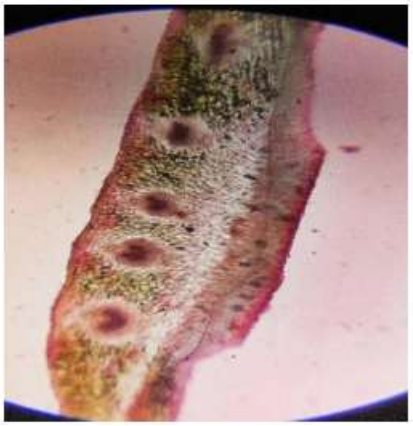

Figure 9: T.S. through midrib of Hydrocotyle verticillata

Lamina: Lamina is differentiated into upper and lower spongy parenchyma. Palisade parenchyma is compactly packed with chlorophyll pigment of two rows. Lower spongy parenchyma is 2–3 layered, oval-shaped surrounded by air chambers loaded with chlorophyll pigments. Upper and lower epidermis are covered with a thick cuticle, where the lower epidermis is interrupted by sunken stomata. The strong vein in the mesophyll shows that both below epidermis covered by several layers of collenchyma tissue supporting the bundle sheath. The vascular bundle consists of few elements of xylem toward the upper epidermis and phloem situated toward the lower epidermis.

Stomatal Index: Stomata index of Hydrocotyle verticillata is 28.57%

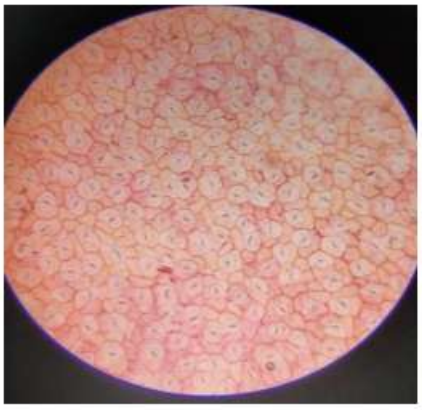

Figure 4: Stomatal index of Hydrocotyle verticillata

Discussion and conclusion

Phytochemical investigation reveals the presence of various important phytochemical in different days of dry material and solvent extracts of this plant. Different phytochemicals show their presence in different days and solvents of Hydrocotyle verticillata. Solvent of Methanol and Aqueous were use to get maximum important information about phytochemical. Many of the metabolites like Tannin, saponin, flavonoids, phenol, steroids were found more concentration in 210 days while some were also present in methanol and water extract. Comparison between different days of dry powder and methanol and water extract of Hydrocotyle verticillata showed more phytoconstituents were found in 210 days dry powder and methanol extract compared to other days of dry material and other extract viz., Water. But similar study (Sowndarya R et al., 2026) investigated that qualitative phytochemical analysis of Hydrocotyle verticillata revealed the presence of secondary metabolites such as phenols, flavonoids, tannins, glycosides, alkaloids, triterpenoids but steroids was absent in Hydrocotyle verticillata.

Based on the preliminary test quantitative determination phytoconstituents were carried out for the powdered plant material and solvent extract by various standard methods and found that presence of proteins, carbohydrates, lipids, tannins, flavonoids, phenols, saponins, terpenoids, steroids and alkaloids respectively. Metabolites like saponins, phenol and flavonoids shows high concentration compared to other metabolites. Study showed that comparision between different days plant powder and different solvent extract, primary and secondary metabolites were found in 210 days and methanol extract. But similar work (Sowndarya R et al., 2026) revealed that quantitative estimation of Hydrocotyle verticillata confirmed appreciable levels of flavonoids, tannins, phenols, and alkaloids in the hydroethanolic leaf extract, supporting its phytochemical richness.

Nutraceutical properties were found in dry powder of whole plant of H.verticillata followed by standard method. Nutrients such as sodium, potassium, aluminium, calcium and iron shows highest concentration compared to other elements. But similary (Sowndarya R et al., 2026) studied that plant nutrients such as carbohydrates and proteins present in the various extractive solvents Hydrocotyle verticillata.

Pharmacognostic study analysis will help to ensure identity, quality, purity and safety of drug for the human use (Shehla akbar et al., 2014). This study carried out for the organoleptic study, extractive value, ash value, macro

and microscopic nature and florescence analysis. Organoleptic study find out the taste, odour, nature and colour of the drug. Extractive value analysis the particular extractive value of methanol and aqueous extract of Hydrocotyle verticillata. Total ash is useful in detecting the crude drugs that are mixed with various mineral substances. Acid insoluble ash means the ash insoluble in dilute hydrochloric acid. The water soluble ash is used to detect the presence of material exhausted by water. Determination of fluorescence analysis of powdered of Hydrocotyle verticillata alone, and in combination with different solvents and acids was determined the under normal and UV light. Macroscopic and microscopic characters will helpful for the identification of particular part, cell and tissue of the Hydrocotyle verticillata. But similarly (Prashant Rameshrao Umate and Meena S. Deogade., 2020) studied that macro and microscopy study, organoleptic study and histochemical evaluation. Microscopic studied some characteristics of stomata, palisade, and surface, which is useful for the identification and standardization of Hydrocotyle verticillata.

Authors' Contributions
Each author actively contributed to the idea and design, collection of the data, or the analysis and interpretation of the findings. All authors also made substantial contributions in drafting the article and reviewing it.

Ethical Approval and Consent to Participate
Not applicable.

Availability Of Data and Materials
The data and supportive information is available within the article.

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Conflict Of Interest
The authors declare no conflict of interest, financial or otherwise.

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References

1. Ainsworth, Elizabeth A., and Kelly M. Gillespie. "Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent." Nature protocols 2, no. 4 (2007): 875-877.
2. Barnes, H., and J. Blackstock. "Estimation of lipids in marine animals and tissues: detailed investigation of the sulphophosphovanilun method for 'total'lipids." Journal of experimental marine biology and ecology 12, no. 1 (1973): 103-118.
3. Ghorai, Narayan, Sondipon Chakraborty, Shamik Gucchait, Samir Kumar Saha, and Suman Biswas. "Estimation of total Terpenoids concentration in plant tissues using a monoterpene, Linalool as standard reagent." (2012).
4. Khatun, BM Rezia, Md Oliur Rahman, and Syeda Sharmeen Sultana. "Hydrocotyle verticillata Thunb. (Apiaceae)-A new angiospermic record for Bangladesh." Bangladesh Journal of Plant Taxonomy 17, no. 1 (2010): 105.
5. Lim, Reuben CJ, Alex TK Yee, Xin Yi Ng, and Hugh TW Tan. "Whorled pennywort, Hydrocotyle verticillata Thunb. (Araliaceae), a new record of a casual aquatic macrophyte in Singapore." Nature in Singapore 7 (2014): 79-91.
6. Manikandaselvi, S., V. Vadivel, and P. Brindha. "Studies on nutraceutical properties of Caesalpinia bonducella L.: an Indian traditional medicinal plant." (2016): 127-139.
7. Pal, Rashmi Saxena, and Yogendra Pal. "Pharmacognostic review and phytochemical screening of Centella asiatica Linn." Journal of Medicinal Plants Studies 4, no. 4 (2016): 132-135.
8. Pandey, M.M., S. Rastogi and A.K.S. Rawat, 2013. Indian traditional ayurvedic system of medicine and nutritional supplementation. Evidence-Based Complement. Altern. Med. 10.1155/2013/376327
9. Rajasekaran, A., G. Sivagnanam and R. Xavier, 2008. Nutraceuticals as therapeutic agents: A review. Res. J. Pharm. Technol., 1: 328-340.
10. Sree, L., and K. Vijayalakshmi. "Proximate composition, nutritional evaluation and mineral analysis in the leaves of an indigenous medicinal plant Alternanthera sessilis." Int J Health Sci 8 (2018): 55-62.
11. Tadesse, Samuel, Kumar Ganesan, Suresh Kumar P. Nair, Neethu Letha, and Sharmila Banu Gani. "Preliminary Phytochemical Investigation of Different Solvent Extracts of Centella Asiatica L. (Family: Apiaceae), An Ethiopian Weed." International Journal of Pharmaceutical, Chemical & Biological Sciences 6, no. 1 (2016).
12. Ukoha, Pius O., Egbuonu AC Cemaluk, Obasi L. Nnamdi, and Ejikeme P. Madus. "Tannins and other phytochemical of the Samanaea saman pods and their antimicrobial activities." African Journal of Pure and Applied Chemistry 5, no. 8 (2011): 237-244.
13. Umate, Prashant Rameshrao, and Meena S. Deogade. "Pharmacognostic study of Hydrocotyl verticillata Thunb." Journal of Indian System of Medicine 8, no. 2 (2020): 122-129.
14. Yemn, E. W., and A. J. Willis. "The estimation off carbohydrate in plant extracts by antrone." Bioch. J 57 (1954): 504-514.

15. Swain, Tony, and W. E. Hillis. "The phenolic constituents of Prunus domestica. I. The quantitative analysis of phenolic constituents." Journal of the Science of Food and Agriculture 10, no. 1 (1959): 63-68.
16. Jain, D., and S. Shrivastava. "Estimation of total phenolic, flavonoid and saponin content in different extracts of butea monosperma bark." International Journal of Engineering Technology Science and Research 4, no. 2394-3386 (2017): 177-182.
17. Kozeko, Liudmyla, Yulia Ovcharenko, Sigita Jurkonienė, and Elizabeth Kordyum. "Understanding unique tolerance limits in Hydrocotyle verticillata: From submergence to water deficiency." Aquatic Botany 190 (2024): 103725.