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Phytochemical Composition and Proximate Analysis of the Medicinal Plant *Eichhornia crassipes* L.

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Abstract: The plant *Eichhornia crassipes* L. is a very promising plant which belongs to Family Euphorbiaceae. Proximate, qualitative and quantitative phytochemical analysis of this plant showed variable phytochemical groups. The shade dried leaf powder was subjected to proximate and elemental analysis. Different extractions of *Eichhornia crassipes* L. using ethanol, methanol, hexane, ethyl acetate and chloroform were done and also qualitative, proximate composition and quantitative were performed. *Eichhornia crassipes* L. showed high level of total ash (7.90w/w) water soluble ash (6.40w/w), acid soluble ash (1.11w/w) and loss of drying (0.735w/w). The extractive value of *Eichhornia crassipes* L. was found high in methanol (8.64%) followed by ethanol (8.07%), chloroform (7.41%), ethyl acetate (6.91%) and hexane (1.45%). From the preliminary phytochemical analysis we found that the secondary metabolites such as flavanoids, steroid, sugar, alkaloids, quinine, phenolics, saponins and coumarin were present. Finally, the quantitative estimation of this plant have total alkaloids contents (TAC) 16.75 µg, total flavanoids contents (TFC) 15 µg and total phenolics contents (TPC) 20.5 µg were obtained. The quantitative determination of *Eichhornia crassipes* L. has revealed the presence of high phenolics content among all the other phytocompounds. The methanolic extract of *Eichhornia crassipes* L. plant contains pharmacological properties and they can be explored for biological potentials.

Keywords: *Eichhornia crassipes* L., Pharmacological, Proximate composition, Elemental analysis

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Introduction

Traditional medicines are described by World Health organization as information, skills, and practices which depends on way of life, speculative aspects and native experiences which vary among diverse cultures in deterrence or

mental illness (Mariah *et al.*, 2021). Plant materials are still used as an important resource for the treatment of combating illnesses, together with infectious diseases, development of novel drugs and also used as an agent for agrochemicals,

food additives and industrial chemicals (Durai *et al.*, 2016) which is easily existing, secure and competent and infrequently have side effects which enhances its acceptability to consuming people (Santosh *et al.*, 2019; Kaleeswaran *et al.*, 2019).

Phytochemical is natural bioactive composite(s) in plants which execute as a protection against several diseases due to their medicinal activity present in it. Based on the activity of plant metabolism, phytochemicals are of two forms-- primary and secondary compounds (Durai *et al.*, 2016). Amino acids, lipids, carbohydrates, proteins and chlorophylls comes under primary metabolites whereas secondary metabolites contain alkaloids, steroids, saponins, flavanoids, phenol and tannins (Bhardwaj and Dubey, 2019; Sundar *et al.*, 2020). At present, the researchers are interested in the plant based treatment due to the lower risk factor, excellent pharmacological properties and cost effective (Ramadevi *et al.*, 2018). It is necessary to discover bioactive compounds of medicinal plants for the treatment of various diseases.

Water hyacinth (*Eichhornia crassipes* L.) comes under family Pontederiaceae which is a free floating water macrophyte and represent as multi-ethnic persistent which means it can stand for a broad range of ecological circumstances e.g. temperature, lighting, moisture, salinity, pH, storm, current and drought (Firoj *et al.*, 2020). It has spikes of light blue flowers and leaves is in green color with overstated bladder like petioles which rapidly proliferate and can reduce the penetration of dissolved oxygen in water body, change in water chemistry, disruption of aquatic flora and increased rate of water loss due to evapotranspiration. In tropical countries it acts as a mediator for expand of severe diseases because the latex is toxic in some species and it is considered as a serious threat to biodiversity (Awaad *et al.*, 2017). But, in recent time's immense attention has been given to its harvesting for use as plant protein source for farm animals as a substitute and the taking out of rubber from *Hevea*

sapium species which belong to this family. It contains anthraquinones, fatty acids, triterpenoids, epoxides and anti-tumor agents. Alkaloids are present as quinoline, apmorphine, indole, pyridine and tropane form (Andrea *et al.*, 2014). It also plays a significant role in dropping the levels of the heavy metals in aqueous environment. It has high cellulose content and act as an alternative fuel. It also serves as raw material for paper manufacturing, vermicompost, potash, biogas production and fish feed formulation (Suleiman *et al.*, 2020). It has the capacity of natural antioxidants for retarding lipids peroxidation (Firoj *et al.*, 2020). In the present study we performed the qualitative and quantitative preliminary phytochemical analysis of different solvent extracts of leaves of *Eichhornia crassipes* L. plant.

Materials and Methods

Collection and identification of *Eichhornia crassipes*:

Fresh leaves of *Eichhornia crassipes* L. (Euphorpiaceae) were collected locally. The plants were authenticated by Senior Scientist and Head, ICAR- Krishi Vigyan Kendra, Hans Rover Campus, Valikandapuram, India.

Preparation of extracts:

The leaves of *Eichhornia crassipes* were washed thoroughly twice with sterile water. It was desiccated under shadow at room temperature for about 15 days and it was powdered by mixer grinder and sieved to provide particle size 40- 100 mm. 25 g of *Eichhornia crassipes* L. leaves powder and 250 ml of ethanol, methanol, hexane and chloroform solvents (separately) were used for extraction by using Soxhlet apparatus. Finally, the filtrates obtained were dried at temperature of $40\pm 2^{\circ}\text{C}$. It was stored in chill and dry situation for further use.

Physico-chemical analysis:

Fluorescence behaviour of powder:

To study the fluorescence behaviour, leaf powder was treated with different chemical reagents viz.

1 N sodium hydroxide, 1 N sodium hydroxide in methanol, 1 N hydrochloric acid, picric acid, acetic acid, 1 N nitric acid, acetone, nitric acid, 50% sulphuric acid in ammonia solution and observed under daytime light, long UV (365 nm) and short UV light (254 nm). The fluorescence analysis was carried out by the technique of Chase and Pratt (1949) and Singh *et al.* (2013) when cut surface or powder is exposed to UV light.

Proximate Analysis (Vidita et al., 2013; Willy et al., 2021):

The parameters determined for proximate analysis include ash value, extractive value and loss of drying and it was determined to guidelines guidelines of WHO (2002).

Loss on Drying:

The plant material of 2 g was taken in an evaporating plate and then desiccated in an oven at 105°C till stable weight was obtained. After drying, the weight was noted and then loss on drying was illustrated. Finally, the percentage was obtained (Santosh *et al.*, 2019).

Loss of drying (%) = Loss in weight $\times$ 100/w

Where W=Weight of drug in g.

Determination of Ash values:

Total ash:

3 g of powdered material was placed in a tarred silica crucible which was ignited and weighed in advance. The plant material was spread over the bottom of the crucible as a fine even layer. The crucible was incinerated slowly by rising the temperature to create it dull red hot still free from carbon. Then it was cooled and measured. The total ash content was estimated with reference to the air dried plant material.

Total ash (% w/w) = (Weight of ash/Weight of sample) $\times$ 100

Acid-insoluble ash:

The total ash content was boiled with 25 ml of dilute HCl for 5 min. After that, the insoluble ash was collected on an ashless sieve paper and rinsed with warm water. This insoluble ash was placed into a silica crucible and ignited, chilled and

measured. The process was repeated till to get stable weight. Finally, percentage was calculated with reference to the amount of air dried crude plant material.

Acid insoluble ash (% w/w) = (Weight of ash/ Weight of sample) $\times$ 100

Water soluble ash:

With 25 ml of water the total ash obtained was heated for 5 min and its impenetrable matter was collected in filter paper and thoroughly rinsed with hot water. Then it was transferred into silica crucible and was lightened, chilled and measured until constant weight was obtained. The water soluble ash was calculated by the difference between the weight of insoluble matter and weight of the total ash and its percentage was calculated with indication to the air dried drug.

Water soluble ash (% w/w) = (Weight of ash- weight of insoluble ash/ Weight of sample) $\times$ 100

Extractive Value:

Extractive value is used as an evaluating crude drug which is not readily estimated by other means. It is employed for that material for which no suitable chemical or biological assay method exist.

Alcohol extractive:

The dry powdered plant material was extracted with ethanol, methanol, hexane, ethyl acetate, and chloroform using a maceration method. Subsequently, 2 g of the coarsely plant powder was measured and then transferred into a desiccated 250 ml conical flask. Then it was filled with various solvents (30 ml) separately and tightly closed then kept for 24 h at room temperature, trembling repeatedly. The mixed solutions were filtered into a 50 ml measuring cylinder through Whatmann No. 1 filter paper, then the filtrate was transferred into a weighed petriplates. The obtained extracts were determined to dryness by stay filtrate for complete loss of solvent.

The percentage was calculated by using following formula:

$$\text{Extractive value (\%)} = \frac{\text{Weight of dried extract}}{\text{Weight of plant material}} \times 100$$

Phytochemical standardization analysis:

Qualitative phytochemical analysis:

The air dried plant materials were subjected under examination to phytochemical screening for the presence of different compounds based on the standard methods by Khan *et al.* (2011).

Quantitative phytochemical analysis:

Total phenolic content assay:

The total phenolic content of plant extracts were calculated with Folin- Ciocalteu reagent, with slight modification. Briefly, 2 ml of Folin–Ciocalteu reagent (1:9; Folin- Ciocalteu reagent: distilled water) and 1 ml of sample (5 mg/ml) was put into a 10 ml volumetric flask. The mixture was permitted to stand at room temperature and mixed gently with 3 ml of 7.5% (w/v) Na₂CO₃ was added to the mixture. The mixture was homogenized and a set of Gallic acid standard solutions (20, 40, 60, 80 and 100 µg/ml) was kept at room temperature for 90 min. Total polyphenol content absorbance was observed using a spectrophotometer at 760 nm. The total phenolic content was expressed as Gallic Acid Equivalents (µg GA/mg of dried extract) in mg/ml plant extract (Mariah *et al.*, 2021).

Estimation of total content of alkaloids:

Alkaloids content were determined using Harborne method. About 1 ml of 1 mg/ml of sample was transferred to a separating funnel whereby there was an addition of 5 ml of bromocresol green solution and 5 ml of 4.7 pH phosphate buffer. The mixture was shaken with 1, 2, 3 and 4 ml of chloroform by vigorous shaking, collected in a 10 ml volumetric flask and diluted to the volume with chloroform. The absorbance was determined on the reagent blank at 470 nm with an UV/Visible spectrophotometer for standard solutions and test solutions. A set of Atropine (20, 40, 60, 80 and 100 µg/ml) was prepared as test solution. The total content of alkaloids was calculated as Atropine equivalents (µg AE/mg of dried extract). Reagent blank was prepared in the

same manner but without extracts (Mariah *et al.*, 2021).

Total flavonoid content assay:

Total flavonoid content was calculated using the Dowd method as modified by Arvouet-Grand *et al.* (1994). For each extract, 1 ml of methanolic solution (100 µg ml⁻¹) was mixed with 1 ml of aluminium trichloride (AlCl₃) in methanol (2%). The absorbance was read at 415 nm after 10 min against a blank sample consisting of a 1 ml of methanol and 1 ml of plant extract without AlCl₃. The total content of flavonoid was expressed as Rutin equivalents (µg RE /mg dried extract) as standard graph. Reagent blank was prepared as test solutions but without extracts.

Results

Physicochemical Constants of *Eichhornia crassipes*:

The Physicochemical Constants of the plant are given in Table 1. The loss of drying, total ash content, acid insolubility ash and water-soluble ash was found to be 0.735, 7.90, 1.11 and 6.40, respectively.

Table 1: Physicochemical Constants *Eichhornia crassipes* L. plant

Parameters	Value W/W
Loss on Drying	0.735
Total Ash Content	7.90
Acid Insoluble Ash	1.11
Water Soluble Ash	6.40

Extractive values of *Eichhornia crassipes* L.:

The soluble extractive values of plant extract are depicted in Table 2, which shows 9.62 in Ethanol Extractive, 1.45 in Hexane Extractive, 7.14 in Chloroform Extractive, 6.91 in Ethyl acetate Extractive and 8.07 in Methanol Extractive values.

Preliminary phytochemical analysis of *Eichhornia crassipes* L.:

In the present study, presence of phytoconstituents such as Flavonoid, Steroid, Sugar, Alkaloids, Quinones, Phenols, Saponins and Coumarins were recorded (Table 3).

Table 2: Extractive Values of *Eichhornia crassipes* L. plant

Parameters	Value W / W
Ethanol Extractive Value	8.07
Hexane Extractive Value	1.45
Chloroform Extractive Value	7.14
Ethyl acetate Extractive Value	6.91
Methanol Extractive Value	9.62

Table 3: Phytochemical Screening

Test	Dry Powder of Selected Plant
Terpenoid	Absent
Flavonoid	Present
Steroid	Present
Glycosides	Absent
Sugar	Present
Alkaloids	Present
Quinones	Present
Phenols	Present
Tannin	Absent
Saponins	Present
Coumarins	Present
Anthroquinone	Absent

Table 4: Fluorescence analysis Dry Powder of Selected Plant

TREATMENT	DAY LIGHT (24 h)	UV LIGHT (24 h)
Dry powder	Green	Green
Dry powder + Hexane	Pale Green	Dark Orange
Dry powder + chloroform	Green	Orange
Dry powder + Benzene	Straw Yellow	Pale Blue
Dry powder + Ethyl acetate	Green	Dark Orange
Dry powder + Ethanol	Dark Green	Pale Green
Dry powder + Methanol	Yellow	Pale Orange
Dry powder + 50% H ₂ SO ₄	Pale Brown	Pale Green
Dry powder + 1N HCL	Green	Brown
Dry powder + alc.1N NaoH	Dark Brown	Blue
Dry powder + water	Reddish Brown	Dark Brown

Fluorescence analysis of Dry Powder of Eichhornia crassipes L.:

In the present study, the fluorescence analysis of these leaf extracts was observed under ordinary visible light and also under UV light (245 nm) and recorded in Table 4.

Quantitative Analysis of Eichhornia crassipes L.:

This study revealed that the crude plant extract exhibited the presence of Phenol (20.50 mg/g),

Alkaloid (16.75 mg/g) and Flavonoid (15.00 mg/g) (Table 5).

Discussion

Plants are precious for the remedial of various ailments as they are direct sources of compounds which serve as models for novel drug and also used as taxonomic markers. Still, only a limited number of the plants in the world has been studied. Based on their structures and genetic

Table 5: Quantitative analysis of *Eichhornia crassipes* L.

Parameters	Value mg/g
Phenol	20.50
Alkaloid	16.75
Flavonoid	15.00

functions of bioactive compounds it was represented as secondary metabolites which are non-dietary plant derivatives which contain various properties with antiviral, antimicrobial, antioxidants and numerous activities and act against variety of pathogens. Phytochemical screening method involves qualitative and quantitative analysis of the chemical constituents. In general, mostly traditional methods are used for the preparation of plant extracts, still throughout the research work various methods were involved for the extraction process in turn to establish the most efficient method that can lead to an isolation and purification of bioactive compounds. In this, compounds in various medicinal plants is now becoming more essential today in view of the commercialization based on traditional medicine. Consequently, it is necessary to develop systematic and clinical investigation to inspect the protection, quality and efficiency of these herbal therapies (Trishala and Lakshmi, 2018).

In this investigation, we performed the preliminary and physicochemical analysis and make possible to recognize the formulations in scheduled industrial production. The percentage of moisture content (loss on drying) validates both water and volatile matter. In the plant *Eichhornia crassipes* L. has 0.73 W/W moisture content and it does not have excess moisture which indicates that the mold and bacterial growth was less which reduces the spoilage of the drug. If the rate of drying is too slow, the rate of ingredients dried was good, therefore the drying limit was also proper. The amount of total ash is determined by the amount of materials residual such as minerals and earthy materials after ignition and its acid insoluble ash dealings by the quantity of silica which is obtained in the form of sand and siliceous matter. It is an important test for the determination of the drug quality and its

composition because it may cause alteration in the extractive values. Hence, it helps in the determination of adulterated drugs.

To evaluate the consistency of nature, the presence of acids, sugar and inorganic compounds, the amount of chemical constituents present in drugs, and also the extractive values are used. More or less extractive value represents the accumulation of exhausted material, adulteration during drying or storage purpose or formulating. Considering the significance of these physicochemical parameters, *Eichhornia crassipes* L. was characterised by validating the different soluble extractive, total ash content, water soluble ash and acid insoluble ash (Monisha and Ragavan, 2015; Trishala and Lakshmi, 2018). The fluorescent analysis of powder represents the character of phytochemicals present or adulterant. In this work, there was no such fluorescent substance found in the formulation of plant powder and it is crucial for indicating the drug's quality and purity (Snehalatha and Rasmi, 2021).

Plant products including phenolic, quinones, saponins, sugar, flavanoids, coumarins, steroids and alkaloids are found in the ethanolic plant extract and terpenoids, glycosides, tannin and anthraquinone was lacking in this extract. Therefore, the presence of these phytochemical substances support rapid healing and the development of new tissues. Each phytoconstituents showed effectiveness towards some biological behavior which may increase the probability to discover the new substance like antibiotics against pathogens. Flavonoids are hydroxylated phenolic substances which belongs to a class of polyphenols, which act as antiallergic, anti-inflammatory, antiosteoporotic, anticarcinogenic, antihepatotoxic, antimutagenic, antiulcerogenic, antiviral, antioxidant and antimicrobial, and even antitumor activity (Letiele *et al.*, 2020). They also play an active role in the quenching of free radicals. Steroids compound formulate the lipid-bilayer crust rupture and liberate liposome and it also release the enzymatic protein in microbes. It is involved in the stimulation of bone marrow and

growth (Amir *et al.*, 2020). Phenolics compounds are dominant donors of hydrogen, which formulate them as good antioxidants property and are major compounds which is widely dispersed as phytochemical of plants substances.

Alkaloids are known to be antioxidant potential which are secondary nitrogenous compounds used in folk medicine. It has anticancer, antiviral, antifungal and antimalarial activities. Saponin acts as a natural antioxidant and moreover it stimulates apoptosis process in tumor cells. Coumarins are endowed with a lot of biological activities like antioxidant, antimicrobial, anticancer and anti-inflammatory (Mansoori *et al.*, 2020).

It was suggested that several hydroxyl functional group present in flavonoids and phenolics are accountable for their biological and antioxidant behavior. It has anti-inflammatory, Molluscicidal, antiallergic, anthelmintic, anti-diarrheal, antiulcer, antihepatotoxic, vasodilatory action. It is because of the occurrence of hydroxyl groups, which play a significant role in their scavenging ability. Therefore, they have the ability to react with vigorous oxygen radicals such as hydroxyl radicals. Total phenolics content present in this plant is in the range of 20.5 and Total flavanoid content range was 15.00. Alkaloids are a diverse group of secondary metabolites that have the shield against the chronic diseases and it has capability of reducing headaches connected with hypertension. It has antimicrobial activity through inhibiting DNA topoisomerase. There is no previous report on the total phenolics, alkaloids and flavonoids content of *Eichhornia crassipes* L.

Plants are a source of large amount of secondary metabolites which are claimed to possess the bioactive compounds which are responsible for their antibacterial, antifungal, antifeedant, repellent, and pesticidal properties. Medicinal plants are one of the best alternatives to minimize the use of chemical-based fungicides.

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