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Phytochemical Screening and Extraction Procedure of Hydrocotyle Sibthorpioides

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Abstract- Hydrocotyle sibthorpioides Lam., a popular medicinal plant in Assam renowned for its ethnomedicinal values, belongs to the Araliaceae family. It is a short, edible herb extensively utilised in traditional medicinal systems. This study aims to investigate the phytochemical constituents present in the plant extract. Phytochemical analysis was conducted to identify therapeutic compounds within the extract. Results revealed the presence of various secondary metabolites in fresh plant parts, including flavonoids, phenols, tannins, saponins, alkaloids, carbohydrates, cardiac glycosides, terpenoids, quinones, and coumarins. These phytochemicals, with allelopathic properties, are crucial in formulating pharmacological and pharmaceutical drugs. The study's objective is to standardise the components of Hydrocotyle sibthorpioides Lam. Various extraction examinations demonstrated the presence of different phytochemical groups and their yields in each extract. This paper provides an overview of the phytochemical investigation conducted on the extract of Hydrocotyle sibthorpioides Lam.

Keywords: - Phytochemical studies, Extraction, Soxhlation, The biologically active compound, Hydrocotyle sibthorpioides Lam

INTRODUCTION

Plants-based Medicine has been used worldwide as traditional healthcare for hundreds of decades (Newman et al., 2000; Vadlapudi and Naidu KC. 2010). Since the prehistoric era, shrubs, plants and trees have played a very important role in the lives of human beings and all living organisms. The herbal remedial plants, i.e. Rasayana, are those plants whose each and every part, like trunk, stem, leaves, seeds, root, foliage, etc., have been widely used in the Ayurveda in the form of powder, latex, kadha, for the treatment of different types of diseases and infections (Kumar et al., 2011). For these important reasons, our ancestors or vaidya studied the medicinal value of plants and collected and transferred all the necessary knowledge to the next generation. It can heal different types of diseases without showing any adverse effects. The adverse, side or hazardous effects of herbal or ayurvedic drugs are negligible in human belief, but the collated knowledge on these effects remains very limited. (Anokwuru et al., 2012). The bioactive or phytochemical constituents show presence in different parts of plants like leaves, bark, roots, and stems that have defence procedures and provide protection for various ailments. These phytoconstituents include flavonoids, proteins, saponins, quinones, sugar, alkaloids, terpenoids, phenols, and tannins. Phytochemical constituents are described in two categories. They are primary

constituents like amino acids, proteins, sugar, chlorophyll, etc. and secondary constituents like phenols, flavonoids, alkaloids, terpenoids etc. These phytochemicals act as a natural defence system for plants. They are the cause behind their colour, aroma and flavour. More than 4,000 phytochemicals have been discovered in this respect (Singh et al., 2013). The healing capacity of Medicine produced by plants depends on these phytochemicals. The study of Ethno-pharmacology leads to the question of how people get Medicine or herbs from plants, shrubs, fungi, animals, and all other naturally present resources (T Rabe, and J.V. 1997). In the current scenario, mainly local or native people are continuously developing new drugs from plants or shrubs. People worldwide use plants as Medicine, but they have different names and processes, like allopathy, Unani, Siddha Ayurveda, etc. (Kirtikar KR, B.B. 1996). Hydrocotylesibthorpioides is native to southeastern Asia and is a small plant in size. It is also known as lawn marsh pennywort. It is a dicotyledon, placed in the family Apiaceae, but more recently suggested from the study that it belongs to the Araliaceae family (Chandler, G. T.; Plunkett, G. M. (2004-02-01). It grows in large amounts when the conditions are right. Hydrocotylesibthorpioides originated in southeastern Asia but slowly spread throughout the United States and other places worldwide. It can grow in a wide variety of habitats. This plant has been used medicinally in Asia and is common in the aquarium trade. Hydrocotylesibthorpioides Lam is a treasure house of phytochemicals. It is a constantly flourishing component of the vegetation of seed germination in all seasons of the year (S. R. Nikam and Dr. D. D. Namdas, 2022). It has been used in various ayurvedic Medicine as an antiaging, spermatogenic restorative tonic, and immune booster. It is highly recommended in the treatment of hepatosplenomegaly, sexual debility, menopausal syndrome, fertility disorders in women, cardiovascular diseases, and spermatorrhoea. The main objective of the current investigation was to study the qualitatively preliminary phytochemical screening of the plant Hydrocotylesibthorpioides Lam. The fresh leaves of the plant Hydrocotylesibthorpioides Lam were analysed to understand the phytochemical potential using four extracts: aqueous, acetone, methanol, and ethanol. These phytochemical constituents are important in formulating and preparing pharmacological and pharmaceutical drugs.

PLANT MATERIAL COLLECTION

Fresh leaves of Hydrocotyle sibthorpioides Lam. were gathered from natural habitats in suburban areas of the Bhopal district, Madhya Pradesh, India, in August 2021. The plant and its parts were authenticated at the Department of Botany, Sam Global University, Bhopal (Madhya Pradesh), and a herbarium was prepared and recorded for future reference. The collected plant parts intended for research were thoroughly washed with running water to remove impurities. Subsequently, these plant parts were dried at room temperature in a shaded area. The leaves were then cut into small pieces and dried for 8-10 days in a shaded area to prevent contamination, ensuring they were not exposed to direct sunlight. Macroscopic and microscopic examinations were conducted on the dried leaf pieces of Hydrocotyle sibthorpioides Lam. The dried materials were ground into a coarse powder using an electronic grinder, ensuring the powder was not too finely ground. Finally, the powdered

plant material was stored in an air-tight container in a shaded area at room temperature.

DEFATTING OF MATERIAL

The process of removing foreign materials such as dust, dirt, oil, and fat from plant material to obtain suitable material for further processing is known as defatting. To defeat the material, the coarse powder of the selected plant is placed in petroleum ether and left for 24 hours. After this duration, the sample material is filtered using a spatula, funnel, and filter paper (Harborne JB 1998), ensuring that impurities collected in petroleum ether are separated from the desired material. The filtered material is then dried and stored in a tightly sealed container. The materials from both plants are stored separately.

CHEMICAL REAGENT

Chemical reagents are essential substances or compounds mixed with a product to initiate a chemical reaction. In the context of phytochemical analysis, various chemical reagents were employed. These reagents included Hydrochloric acid (HCl), Picric acid (C6H3N3O7), Ferric chloride (FeCl3), Fehling solution A, Fehling solution B, Lead acetate (Pb(C2H3O2)2), Copper acetate (Cu(CH3COO)2), and Gelatin solution. These reagents are crucial in detecting and identifying specific chemical compounds in plant extracts. Through carefully conducted reactions with these reagents, researchers can isolate and analyse various phytochemical constituents, contributing to a comprehensive understanding of the chemical composition of the plant material under study.

EXTRACTION OF PLANT MATERIAL

The extraction of plant material involves isolating bioactive components from inert plant material using standardised extraction procedures and appropriate solvents. We employed the Soxhlation method for this purpose, utilising a Soxhlet apparatus. This extraction technique utilised three solvents based on their polarity: petroleum ether, ethanol (C2H5OH), and water (H2O). The extracted solution obtained from the Soxhlet apparatus underwent filtration, evaporation, and subsequent storage at 4°C in an air-tight container. This extract of plant material was then utilised for further study (Veena and Pracheta, 2013)—extraction of material in Soxhlet apparatus with solvents. Hydrocotylesibthorpioides Plant material was extracted by using the Soxhlation method. Solvents like ethyl acetate, petroleum ether, chloroform, ethanol, and water were used. Detailed phytochemical screening was performed to indicate the presence or absence of different phytoconstituents.

Presence of Alkaloid:- The extract of plant material was treated with four drops of hydrochloric acid (HCl) and shaken for some time as the extract was mixed completely with HCl, and then 5 to 6 drops of Picric acid were added. The yellow precipitate was formed. The formation of yellow precipitate shows the presence of alkaloids.

Presence of Carbohydrates:- The plant material extract was mixed with four drops of hydrochloric acid (HCl) and four drops of sodium hydroxide (NaOH) to detect the presence of carbohydrates and thoroughly shaken. The mixture was then heated over a medium flame. The filtrate obtained from this process was treated with 2-3 drops of alcoholic α-naphthol

solution. The formation of a violet ring at the junction confirmed the presence of carbohydrates.

Presence of Saponins:- The extract of both plant materials was separately treated with distilled water, shaken for at least 10 minutes, and then treated in a graduated cylinder for 15 min. The formation of foam indicates Saponin.

Presence of Phenols:- The extracts were treated with 3 to 4 drops of 2% Ferric chloride solution to form a blushing black colour, indicating the presence of phenols.

Presence of Flavonoids:- The extract was treated with a few drops of Lead Acetate solution. The formation of yellow precipitates may show the presence of flavonoids.

Presence of Tannins:- The extract was treated with a few drops of NACL and 1% Gelatin solution. The formation of a white precipitate indicates the presence of tannins.

Presence of Diterpene:- When extracts were treated with 3 to 4 drops of Copper Acetate solution, the formation of an emerald green colour indicated the presence of Diterpene.

Presence of Xenthoproteins:- The extract of plant material was treated with concentrated nitric acid. The formation of a yellow colour indicates the presence of Xenthoproteins.

In Table 1, the presence of phytoconstituents is indicated by the (+) sign, while the (-) sign indicates the absence. Phytoconstituents such as carbohydrates, saponins, phenols, flavonoids, diterpenes, alkaloids, xenthoproteins, glycosides, triterpenoids, and tannins were examined in this study through phytochemical analysis or qualitative examination of the extracts from the plant "Hydrocotyle sibthorpioides". The results revealed the presence of various phytoconstituents.

Table 1. Phytochemical analysis of the Hydrocotyle sibthorpioides Lam plant extract

Phytochemicals	Solvent		
	Petroleum ether	Ethanol	Water
Alkaloid Mayer's Test:	+	-	+
Carbohydrates Moli's Test:	+	+	-
Saponins Froth's Test:	+	+	+
Phenols Alkaline Reagent:	+	+	+
Tannins	+	+	+
Flavonoids Lead Acetate Test:	+	-	+
Flavonoids Alkaline Reagent:	+	+	+
Diterpene Salkowski Test:	+	+	-
Proteins Xenthoproteins Test	+	-	+
Glycosides Legal's test	-	-	-
Triterpenoid Salkowski Test:	+	+	-

CONCLUSION

The investigation into the phytochemical constituents of Hydrocotyle sibthorpioides has revealed a diverse array of bioactive compounds with significant medicinal properties. This study underscores the therapeutic importance of Hydrocotyle sibthorpioides as a valuable medicinal plant. Various phytoconstituents, including alkaloids, carbohydrates, saponins, phenols, flavonoids, diterpenes, proteins, triterpenoids, and tannins, suggest its potential in traditional Medicine and pharmacology. The findings highlight the need for further research to explore and elucidate these phytochemicals' specific medicinal benefits and mechanisms of action. Continued investigation into the pharmacological properties of Hydrocotyle sibthorpioides could lead to the development of novel therapeutic agents for various diseases. Overall, this study contributes to our understanding of the medicinal potential of

Hydrocotyle sibthorpioides and warrants further exploration in the field of natural Medicine.

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