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Extraction and screening of bioactive compounds of some common hydrophyticand wetland plants from East Singbhum,Jharkhand, India.

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Abstract:*Five commonly available hydrophytic and wetland plants of East Singbhum, Jharkhand, Indiawere assessed for its bioactive compounds namely alkaloids, carbohydrate, glycoside, flavonoids, phenols, amino acids and proteins, saponins, tannins, terpenoids, quinones, resins, coumarins in two different extracts i.e. Solvent extract and Soxhlet extract. The retardation factor using thin layer chromatography were also calculated in both the extracts for all the five samples. The specimens taken for screening, possessed remarkable ethnomedicinal valuesand most of them were edible by the local people. The result of phytochemical screening could be of immense importance in formulating new drugs from these hydrophytes.*
Keywords: *East Singbhum, Hydrophytes, Phytochemical Screening, Solvent Extraction, Soxhlet Extraction.*

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I. Introduction

Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs [1]. These days medicinal products derived from plants are gaining lot of importance.They usually contain a mixture of a number of medicinal plant metabolites. Such plants are considered to be the very basis of traditional medicinal knowledge. The phytochemical screening of such medicinal plants, exhibit a variety of bioactive constituents which well defines its medicinal property. Knowledge of these bioactive constituents is also very important for the synthesis of complex chemical substances. A number of such phytochemical surveys have been conducted and their results have been published in various journals, from time to time. The major bioactive constituents of interest are alkaloids and saponins because of its varied implications in the field of medicine. But other constituents like tannins, quinones, coumarins, amino acids etc. too have gained the attention of researchers and have also been analyzed forits varied utilities.

Hydrophytes and wetland plants play a very important role in our ecosystem. Though the potential of aquatic plants as food and feed has been emphasized by several authors [2], they often seem to be neglected by the masses. Huge anthropogenic pressure has led to the extinction of a number of natural water bodies, thereby also leading to the extinction of a number of hydrophytes and wetland plants with immense ethnomedicinal values.

The petiole and corm of *Colocasiaesculenta*(L.) Schott is used in the preparation of a number of medicines. The juice of the petiole is used as an astringent, rubefacient, stimulant and styptic while the juice of the corm is useful in alopecia. The leaves, petiole and also the corm are used as vegetable by the local tribes.

The whole plant of *Hydrilla verticillata* (L.f.) Royle is used for various medicinal purposes. It is used for proper digestion and gastrointestinal function, improves blood circulation and helps in detoxification. It is found to be good for neurological health, and cardiovascular function. It increases endurance, helps in blood sugar control, strengthens immunity and slow ageing[3].

The plant of *Ipomoea aquatica*Forsk. is considered to be a very nutritious diet for females suffering from genera and nervous debility. It is used in the treatment of liver diseases constipations, diabetes, abscesses, mental illness, intestinal problems, nose bleeds and high blood pressure, anthelmintic, central nervous system depression (CNS) depressant, antiepileptic, hypolipidemic effects, antimicrobial and anti-inflammatory as well as nootropic effect on rat hippocampus [4]. Its juice is used as an antidote to opium and arsenic poisoning. The whole plant is cooked and eaten by the local tribes.

*Peperomia pellucida*Kunth., a common wetland plant is used as a vegetable by the local tribal people of East Singbhum. The whole plant is crushed, mixed with water, heated and then orally administered to stop haemorrhage. It is also used in treating abdominal pain, abscesses, acne boils, colic, fatigue, gout, headache, renal disorders rheumatic pain, breast cancer, impotence, measles, mental disorder and small pox. The plant is used as a cough suppressant, diuretic, emollient and to treat cardiac arrhythmia. It is cooked and eaten locally.

*Nymphaeanouchali*Burm.f., a hydrophytic plant is well known for its medicinal uses. Its peduncles and rhizomes have nutritive values and used as vegetable. Decoction of flowers is refrigerant, cardiotonic in palpitation of heart, effective in combating thirst, fainting, vomiting and biliary disorders. Rhizomes are demulcent, prescribed in the form of powder in dyspepsia, diarrhea and piles. Filaments are astringent, cooling and useful in burning sensation of the body, bleeding piles and in menorrhagia. Seeds are used for cooling effects in cutaneous diseases [5].

With increasing interest in finding new drugs, the unutilized plants receive more attention which offers a good scope to meet the increasing demand for novel drug discovery.The present study was carried out on common and locally available hydrophytes and wetland plants of East Singbhum, Jharkhand, India. Knowledge of the phytochemical screening would not only help in the discovery of therapeutic agents but also in revealing new sources of economically important materials as tannins, oils, gums, resins, quinones etc. In the present work, of the five chosen plants, four are consumed as food by the local tribes and all of them possess immense ethnomedicinal values.

II. Materials and Methodology

2.1 Sample collection: Different hydrophytic samples were collected from waterbodies and marsh lands of East Singbhum, Jharkhand, India. These plant resources were authenticated by The Botany of Bihar and Orissa by H.H Haines.The different plants were *Colocasiaesculenta*(L.) Schott, *Hydrilla verticillata*(L.f.) Royle, *Ipomoea aquatica*Forssk, *Peperomia pellucida* Kunth, *Nymphaeanouchali*Burm.f.

2.2 Sample Processing: The leaves of different hydrophytes were collected and washed with distill water to remove dirt and other contaminants. Then they were washed with 10% saline solution and again with distill water. Then the leaves were shade dried until the moisture is reduced to 10%. The dried leaves were blended into powder form.

2.3 Crude Extract Preparation: The crude extract was prepared from the leaves of different hydrophytes by Solvent extraction [6] and Soxlet extraction[7]. In solvent extraction, the dried plant sample were taken and required solvent in the ratio (1gm: 20ml) respectively in an amber colored glass container with air tight lids for 4 days with frequent agitation. Then they were filtered and the filtrate is the crude extract. In soxlet extraction, the sample was loaded into the thimble and solvent in the bottom flask/boiling flask. The cycles were continued until all the extract has been exhausted from the plant sample (generally 10 cycles).

2.4 Qualitative Screening for secondary metabolites: The presence of alkaloids, flavonoids, glycosides, carbohydrate, saponins, tannins and terpenoids can tested qualitatively using the standard procedures to identify the constituents. [8-10].

2.5 Thin Layer Chromatography: In thin-layer chromatography, the stationary phase is a polar absorbent, usually finely ground alumina or silica particles. This absorbent is coated on a glass slide or plastic sheet creating a thin layer of the particular stationary phase. Almost all mixtures of solvents can be used as the mobile phase. By manipulating the mobile phase, organic compounds can be separated [11].

III. Results

The plant samples were coded as **A**: *Colocasiaesculenta*(L.) Schott, **B**: *Hydrilla verticillata*(L.f.) Royle**, C**: *Ipomoea aquatica*Forssk, **D:** *Peperomia pellucida* Kunth, **E1:** Flowers of *Nymphaeanouchali*Burm.f, **E2:**Leaves of *Nymphaeanouchali*Burm.f.

The Plant samples were dried and the solvent system used wasMethanol:Water (70:30), both for solvent extract and soxlet extract. Qualitative analysis was carried out for the solvent extract "TABLE-1" and soxlet extract "TABLE -2".

Table1: Qualitative screening for different metabolites of different samples (solvent extraction).

Sl.NO	**Metabolite**	**Observation**	**A**	**B**	**C**	**D**	**E1**	**E2**
1.	Alkaloid (Wagner's test)	Reddish brown ppt or colouration	Absent	Absent	Present	Present	Present	Present
			-	-	++	++	++	+
2.	Carbohydrate (Molisch'sTest)	Red/Dull violet	Absent	Absent	Present	Present	Present	Present

		colourat interface	-	-	++	++	++	++
3.	Cardiac Glycosides (Keller Kelliani Test)	Violet ring at interface or greenish ring	Present	Present	Present	Absent	Absent	Present
			++	++	++	-	-	++
4.	Flavonoids (Alkaline reagent test)	Yellow colouration becomes colourless on addition of HCl	Absent	Absent	Present	Present	Present	Absent
			-	-	++	++	++	-
5.	Phenols (Ferric Chloride Test)	Deep blue or Black colour	Absent	Present	Absent	Absent	Present	Absent
			-	++	-	-	++	-
6.	Amino acid and Protein (Ninhydrin Test)	Purple colouration after boiling	Absent	Absent	Absent	Absent	Absent	Absent
			-	-	-	-	-	-
7.	Saponin (Foam Test)	Formation of persistence foam	Absent	Absent	Present	Absent	Present	Absent
			-	-	++	-	++	-
8.	Tannins (Braymers Test)	Blue or greenish colour	Absent	Absent	Absent	Absent	Present Bluish	Present Greenish
			-	-	-	-	++	++
9.	Terpenoids (Salkowski test)	Reddish brown Precipitate	Present	Present	Absent	Absent	Absent	Absent
			++	++	-	-	-	-
10.	Quinones	Yellow precipitate or colour	Absent	Absent	Absent	Present	Present	Absent
			-	-	-	++	++	-
11.	Resins	Turbidity formation	Absent	Absent	Present	Present	Present	Present
			-	-	++	++	++	++
12.	Coumarins	Yellow colouration	Absent	Absent	Absent	Absent	Present	Absent
			-	-	-	-	++	-

Table2: Qualitative screening for different metabolites of different samples (soxlet extraction).

Sl.NO	**Metabolite**	**Observation**	**SA**	**SB**	**SC**	**SD**	**SE1**	**SE2**
1.	Alkaloid (Wagner's test)	Reddish brown ppt or colouration	Present	Absent	Present	Absent	Present	Present
			++	-	++	-	++	++
2.	Carbohydrate (Molisch's Test)	Red/Dull violet colour at interface	Present	Present	Present	Present	Present	Present
			++	++	++	++	++	++
3.	Cardiac Glycosides (Keller Kelliani Test)	Violet ring at interface or greenish ring	Absent	Present	Absent	Absent	Present	Present
			-	++	-	-	++	++
4.	Flavonoids (Alkaline reagent test)	Yellow colouration becomes colourless on addition of	Absent	Present	Present	Present	Absent	Absent
			-	++	++	++	-	-

		HCl						
5.	Phenols (Ferric Chloride Test)	Deep blue or Black colour	Absent	Absent	Present	Present	Present	Present
			-	-	++	++	++	++
6.	Amino acid and Protein (Ninhydrin Test)	Purple colouration after boiling	Absent	Absent	Absent	Absent	Absent	Absent
			-	-	-	-	-	-
7.	Saponin (Foam Test)	Formation of persistence foam	Present	Present	Present	Present	Present	Absent
			++	++	++	++	++	-
8.	Tannins (Braymers Test)	Blue or greenish colour	Absent	Present	Absent	Present	Present	Present
			-	++	-	++	++	++
9.	Terpenoids (Salkowski test)	Reddish brown Precipitate	Absent	Absent	Absent	Absent	Present	Present
			-	-	-	-	++	++
10.	Quinones	Yellow precipitate or colour	Absent	Absent	Absent	Present	Present	Absent
			-	-	-	++	++	-
11.	Resins	Turbidity formation	Absent	Absent	Absent	Absent	Present	Absent
			-	-	-	-	++	-
12.	Coumarins	Yellow colouration	Absent	Absent	Absent	Absent	Absent	Absent
			-	-	-	-	-	-

The Thin layer Chromatography was carried out for different plant extracts. The different plant extracts were analysed for the constituents by using mobile phase. (Chloroform:Glacial acetic acid: Methanol :Water = 16:8:3:2) "TABLE-3, TABLE-4".

Table 3: Retardation Factor for Solvent extraction Samples.

SAMPLE A - Chloroform:Glacial acetic acid: Methanol :Water = 16:8:3:2				
70 % Methanolic Extract	Visualization/Band	Solute Font	Solvent Font	Rf
	Light yellow band	2.0	2.5	0.80
	Light Brown	2.1	2.5	0.84
	UV at 254nm (Black quenching spot)	2.1	2.5	0.84
SAMPLE B - Chloroform:Glacial acetic acid: Methanol :Water = 16:8:3:2				
70 % Methanolic Extract	Visualization/Band	Solute Font	Solvent Font	Rf
	Light yellow	1.8	2.6	0.69
	Pale yellow	2.0	2.6	0.77
	Light Brown	2.5	2.6	0.96
	UV at 254nm (Orange fluorescence spot)	2.5	2.6	0.96
SAMPLE C - Chloroform:Glacial acetic acid: Methanol :Water = 16:8:3:2				
70 % Methanolic Extract	Visualization/Band	Solute Font	Solvent Font	Rf
	Light Green	2.1	2.9	0.72
	Light yellow	2.2	2.9	0.76
	Pale Yellow	2.7	2.9	0.93
	UV at 254nm (Orange Fluoroscence)	2.3	2.9	0.79
	UV at 254nm (Black quenching spot)	2.6	2.9	0.90
SAMPLE D - Chloroform:Glacial acetic acid: Methanol :Water = 16:8:3:2				
70 % Methanolic Extract	Visualization/Band	Solute Font	Solvent Font	Rf
	Light yellow	2.2	2.9	0.76

	UV at 254nm (Black quenching spot)	2.8	2.9	0.96
SAMPLE E1 - Chloroform:Glacial acetic acid: Methanol :Water = 16:8:3:2				
70 % Methanolic Extract	Visualization/Band	Solute Font	Solvent Font	Rf
	Pale Yellow	2.2	2.7	0.81
	Light Yellow	2.4	2.7	0.88
	Light Brown	2.6	2.7	0.96
	UV at 254nm (Orange Fluoroscence)	2.2	2.7	0.81
	UV at 254nm (Orange Fluoroscence)	2.4	2.7	0.88
SAMPLE E2 - Chloroform :Glacial acetic acid: Methanol :Water = 16:8:3:2				
70 % Methanolic Extract	Visualization/Band	Solute Font	Solvent Font	Rf
	Light Brown	2.7	2.9	0.93
	Pale Yellow	2.8	2.9	0.96
	UV at 254nm (Black quenching spot)	2.4	2.9	0.82

Table 4: Retardation Factor for Soxlet Extraction Samples.

SAMPLE SA - Chloroform :Glacial acetic acid: Methanol :Water = 16:8:3:2				
70 % Methanolic Extract	Visualization/Band	Solute Font	Solvent Font	Rf
	Light yellow	2.0	2.8	0.71
	Light Brown	2.3	2.8	0.82
	UV at 254nm (Black quenching spot)	2.4	2.8	0.86
SAMPLE SB - Chloroform :Glacial acetic acid: Methanol :Water = 16:8:3:2				
70 % Methanolic Extract	Visualization/Band	Solute Font	Solvent Font	Rf
	Blue Black Spot	2.7	3.2	0.84
	UV at 254nm (Orange Fluorescence)	2.4	3.2	0.75
	UV at 254nm (Black quenching spot)	2.6	3.2	0.81
SAMPLE SC - Chloroform :Glacial acetic acid: Methanol :Water = 16:8:3:2				
70 % Methanolic Extract	Visualization/Band	Solute Font	Solvent Font	Rf
	Brown	2.4	3.4	0.70
	Blackish Brown	2.8	3.4	0.82
	UV at 254nm (Orange Fluoroscence)	2.4	3.4	0.70
SAMPLE SD - Chloroform :Glacial acetic acid: Methanol :Water = 16:8:3:2				
70 % Methanolic Extract	Visualization/Band	Solute Font	Solvent Font	Rf
	Light green	2.7	2.8	0.96
SAMPLE SE1 - Chloroform :Glacial acetic acid: Methanol :Water = 16:8:3:2				
70 % Methanolic Extract	Visualization/Band	Solute Font	Solvent Font	Rf
	Light Green	2.2	3.0	0.73
	Yellow spot	2.8	3.0	0.93
	UV at 254nm (Black quenching spot)	2.7	3.0	0.90
SAMPLE SE2 - Chloroform :Glacial acetic acid: Methanol :Water = 16:8:3:2				
70 % Methanolic Extract	Visualization/Band	Solute Font	Solvent Font	Rf
	Light Green	2.3	2.9	0.79
	Light Brown	2.7	2.9	0.93
	Pale Yellow	2.8	2.9	0.96
	UV at 254nm (Black quenching spot)	2.3	2.9	0.79
	UV at 254nm (Black quenching spot)	2.8	2.9	0.96

IV. Discussion

Analysis of the plant extracts revealed the presence of phytochemicals such as phenols, tannins, flavonoids, saponins, glycosides, steroids, terpenoids and alkaloids.In the present study, the observed alkaloid content could be responsible for their much acclaimed, medicinal values though the exact mode of action is poorly understood. The biological function of alkaloids and their derivatives are very important and used in analgesic, antispasmodic and bactericidal activities. However, alkaloids are mainly observed in large number of the young twigs and are taken with common salt to amount, in flowering plants, and they have an important role in treating white discharge[12]. Flavonoids are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection and they have been found to be antimicrobial substances against wide array of microorganisms in vitro. They possess anti-inflammatory, antiallergic, antioxidant, antimicrobial, anticancer, antidiahorrealactivity. Flavonoids are found to be effective antimicrobial substances against a wide range of microorganisms, probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall[13]. Tannins (commonly referred to as tannic acid) are polyphenols present in this extract are known antimicrobial agents. They precipitate proteins and prevent the development and growth of microorganisms thereby making nutritional protein unavailable to the microbes [14]. The growth of many fungi, yeasts, bacteria and viruses was inhibited by tannins. Saponin are a special class of glycosides which have soapy characteristics [15]. It has the property of precipitating and coagulating red blood cells. Some of the characteristics of saponin include formation of forms in aqueous solution, haemolytic activity, cholesterol binding properties and bitterness.

V. Conclusion

The different hydrophytic plant samples included in this study are less explored in East Singbhum, Jharkhand. These hydrophytes are proposed to have ethnobotanical symmetry with the different bioactive compounds that have been established in this study. More biochemical, chemical and biological survey and assay must be carried out to achieve best and safe sources of nutritional and medicinal requirement. Such plants can be considered as an asset for the future medicinal purposes because of its bioactive components.

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