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Phytochemical screening and Biochemical assay of *Typha angustifolia* L.

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Abstract: *Typha angustifolia L. perennial aquatic plant belongs to the family Typhaceae. plant parts contain virtuous nutritive and Ethno-medicinal value. The leaf of Typha angustifolia was analysed for preliminary phytochemical studies. The leaf extract prepares in two different solvents such as methanol and hexene. Its results show great differences in phytochemical extraction in a polar and non-polar solvent and it's also proof in Thin Layer Chromatography. The biochemical tests are helpful for the quantification of particular secondary metabolites. This plant is highly observed in traditional medicine. The available research will help further the validation and standardization of the system.*

Key Words: *Typha angustifolia L., Phytochemical screening, Biochemical and TLC.*

1. INTRODUCTION:

We all know the importance of plants. The botanical kingdom is a treasure trove of potential medicines, and over the last years, there has been a growing awareness of the importance of medicinal plants. Plant-derived medicines are readily available, inexpensive, safe, efficient, and have few side effects. The plants selected for medicinal purposes for thousands of years are the most obvious option for investigating the current search for therapeutic new drugs such as anti-cancer drugs, antibacterial drugs, and anti-hepatotoxic compounds. World Health Organization (WHO) data shows medicinal plants useful source different of medicines. Approximately 80% of people in developed countries use traditional medicines containing compounds derived from medicinal plants.

However, such systems need to be investigated to better understand their characteristics, safety, and efficiency. Medicinal plants contain several organic compounds that have a definite physiological effect on the human body, and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids. These compounds are synthesized by the primary and secondary metabolism of the organism, respectively. It can be obtained from bark, leaves, flowers, roots, fruits, and seeds. Knowledge of the chemical composition of plants is desirable as it helps in the synthesis of complex chemicals.

Typha, locally known as Gha-bajariyu is one of the common weeds in all most every part of Gujarat state. Three common species of *Typha* are shown in India is T.latifolia, T. Angustifolia and T. elephantina. These are robust, 2-4 m tall, perennial grass-like plants, developing in shallow water. In India, the younger shoot, rhizome, marrow of immature inflorescence is eaten in diverse ways.

2. Material and Methodology:

The fresh leaves of *Typha angustifolia* L. were collected from two separate places in Gujarat kadi taluka of Mehsana district and department of botany, Gujarat University, Ahmedabad. (January 2022). The plant material was identified by Dr. Bharat B. Maitreya, professor, Department of Botany, Bioinformatics, Climate Change and Impacts Management, School of Science, Gujarat University.

3. Plant Extract Preparation Method:

The leaves of *Typha angustifolia* L. were air-dried for 7 days and crushed to form a dry plant material powder. After pulverization, the pulverized sample was collected, continuous extraction was performed with an organic solvent such as hexene and methanol.10 g of the weighed powder material of each sample was treated with various solvents

such as methanol and hexene. The material was incubated in a shaker for 24 hours. After 1 day, all samples were filtered through Whatman no. 1 filter paper. The filtered extract was kept at room temperature due to solvent evaporation. Two days later, a crude extract of each sample was obtained.

3.1 Qualitative Analysis of Secondary Metabolites:

Test for Alkaloids:
Mayer test: 2 ml of Mayer reagent was added to 1 ml of filtrate. The cream-coloured precipitate indicates the presence of alkaloids.
Wagner test: Wagner reagent was added to 1 ml of filtrate. Reddish-brown indicates the presence of alkaloids.
Dragendroff test: 2 ml of Dragendroff reagent was added to 1 ml of filtrate. Orange-Red Precipitation specifies the presence of alkaloids.

Test for Flavonoids:
Lead acetate test: 10% lead acetate solution was added to 1 ml of extract. The development of the yellow precipitate signals the manifestation of flavonoids.
H_2SO_4 test: A few drops H_2SO_4 of were added to 1 ml of extract. The orange precipitate indicates the presence of flavonoids.
Zinc Hydrochloride Reduction Test: 1 ml of extract was mixed with zinc powder and concentrate. HCl; Red indicates the presence of flavonoids.
Pew Test: 1 ml of extract was treated with a piece of metallic magnesium and a few drops of concentrate HCl was mixed. The brownish colour suggests the presence of flavonoids.

Test for Phenols:
Lead acetate test: few ml of lead-acetic acid was added to 1 ml of extract.
The development of the yellow precipitate suggests the presence of flavonoids.
Potassium dichromate test: few ml of Potassium dichromate treated with 2 ml extract. presence of precipitation shows phenolic compound.
Alkaline reagent test: Sodium hydroxide was added to 1 ml extract. The formation of yellowish-red precipitation displays the phenols.

Test for Saponins:
Frothing test: Approximately 0.5 mg of extract was shaken with 5 ml of distilled water. The formation of bubbles (the appearance of small creamy bubbles) indicates the presence of saponins.
Foam test: 1 ml extract is treated with 20 ml distilled water. It was Shaken vigorously. Foam development suggests the existence of saponins.

Test for Tannins:
Lead acetate test: 1 ml of 10% lead acetate solution was added to 1 ml of extract. Precipitation of white indicates the presence of tannins.
Ferric chloride test: A small amount of extract was mixed with water, heated in a water bath, the mixture was filtered and 0.1 µl of ferric chloride solution was added. Added to filtrate; dark green indicates the presence of tannins.
Potassium dichromate test: few ml of Potassium dichromate treated with 2 ml extract. presence of precipitation shows phenolic compound.

Test for Terpenoids:
Salkowski's test: A few mg of extract treated with 2 ml of chloroform and 3 ml of concentrate H_2SO_4 was Carefully added to the test tube. the reddish-brown ring shows the presence of terpenoids.
Copper Acetate Test: The extract was mixed in water and considered with a 5% copper acetate solution. The formation of emerald, green precipitates indicate the presence of terpenoids.
Chloroform test: 1 ml extract mixed with 2 ml chloroform and 3 ml conc. H_2SO_4. The appearance of the red-brown ring indicates the presence of terpenoids.

Test for Glycosides:
Bromine H_2O test: 1 ml of extract solution was dissolved in bromine H_2O. The formation of a yellow-coloured precipitate indicates the presence of glycosides.

Ferric chloride test: 1 ml filtrate is treated 1 ml glacial acetic and 1-2 ml of conc. H_2SO_4. Violet to blue-green colour shows glycosides.
Ammonia test: 1 ml filtrate is with 3 ml chloroform. Shake well and add 10% ammonia solution. The pink colour shows glycoside.

Test for fixed Oil & Fats:
The extract is applied to filter paper and oil stain presence shows oil and fat contents.

Test for Protein:
Millons test: Add a few drops of Milon reagent to the 2 ml filtrate. A white precipitate indicates the occurrence of protein.
Ninhydrin test: Add a few drops of ninhydrin solution to the 2 ml filtrate. A purple precipitate indicates the presence of protein.

Test for Carbohydrate:
Benedict test: Add 0.5 ml of Benedict reagent to 0.5 ml of filtrate. put the mixture in a water bath for 2 minutes. A characteristic-coloured precipitate shows the presence of sugar.
Fehling test: few ml extract treated with 1 ml Fehling A and Fehling B. brick red colour indicates carbohydrate.
Iodine test: 1 ml extract-treated with 3 drops of iodine solution. Blue colour shows sugar contain.

Test for Steroid:
Liebermann Burchard test: a few ml of extract treated with a few drops of acetic anhydride. then place in a water bath for some time. cool. Add concentration sulfuric acid. a brown ring is formed suggesting steroid.
Liberman sterol test: Concentration of a few ml of extract added to 1 ml of glacial acetic acid and 1 drop H_2SO_4. colours formation of red, purple, blue to green.
Salkowski test: Shake 2 ml of an extract with 1 ml of chloroform and add alongside dark H_2SO_4. The red colour suggests steroids.

3.2 Biochemical Test

Total sugars and reducing sugars in plants were estimated using the method of Nelson, 1944. The efficacy determination of starch was performed using the standard method of Chinoy, 1939. The total protein of the plant was estimated using the method of Bradford, 1976.

The total phenol in the plant is estimated using the method of Bray *et al.*, 1954, and chlorophyll was estimated by Arnon, 1949, the standard method.

4. Thin Layer Chromatography (TLC):

Thin-layer chromatography is a basic method for the separation of a different chemical compound. The leaf extract was analysed by TLC methods. Glass or aluminium plates coated with silica gel G-60 (1-1.5 mm thick layers) is used for this technique. Hexene plant extract was applied separately to each plate.
For elution 5 mobile phases were analysed but Ethyl acetate: Hexene (2:8) shows the best separation.

1. Ethyl acetate: Hexene (1:9)
2. Ethyl acetate: Hexene (2:8)
3. Ethyl acetate: Hexene (3:7)
4. Ethyl acetate: Hexene (5:5)
5. Ethyl acetate: Hexene (9:1)

5. RESULT:

Table-1 shows Qualitative analysis of secondary metabolites in methanolic, hexene

Phytochemicals	Test	Sample 1		Sample 2	
		Methanol	Hexene	Methanol	Hexene
Alkaloids	1) Mayer's test	+	+	+	+
	2)Dragendroff's test	+	+	+	+
	3) Wagner's test	+	+	+	+
Flavonoids	1) Pew test	+	-	+	-
	2) lead acetate test	+	-	+	-
	3) H_2SO_4 test	+	-	+	-

	4)Zinc hydrochloride reduction test	+	-	+	-
saponins	1) Frothing test	+	-	+	-
	2) Foam test	+	-	+	-
carbohydrates	1) Fehling's test	+	+	+	+
	2) Benedicts's test	+	+	+	+
	3) Iodine test	+	+	+	+
Protein	1) millon's test	+	-	+	-
	2) ninhydrin test	+	-	+	-
phenols	1) lead acetate	+	-	+	-
	2)Potassium dichromate test	-	-	-	-
	3) Alkaline reagent test	+	-	+	-
	4)Folin ciocalteu test	+	-	+	-
Tannins	1) Ferric chloride test-	+	-	+	-
	2) Lead acetate test	+	-	+	-
	3)Potassium dichromate Test	-	-	-	-
Glycosides	1) Bromine h_2o test	+	-	+	-
	2) Ferric chloride test	+	-	+	-
	3) Ammonia test	+	-	+	-
steroids	1) Liebermannn burchard test	-	+	-	+
	2) Liebermann sterol test	+	+	+	+
	3) Salkowski's test	+	+	+	+
terpenoids	1) Salkowki's test	+	-	+	-
	2) Copper acetate test	-	-	-	-
	3) Chloroform test	+	-	+	-
Fixed oil & fats	1) Oil stain check	-	-	-	-

Table-2 shows the result of the biochemical test

Phytochemicals	Concentration (mg/ml)
Protein	5.383 ± 0.049
Phenol	0.058±0.017
Starch	9.985±0.017
Sugar	14.875±0.004
chlorophyll a	0.241±0.140
chlorophyll b	0.114±0.052
Total chlorophyll	0.356±0.254

The table shows that plant contain more sugar and starch than other compounds.

TLC Result: Ethyl acetate: Hexene (2:8) solvent system shows the best separation. TLC plat displays 6 bands. The retention factor (rf) value of bands is 0.173,0.195,0.293,0.402 cm,0.434 and 0.510, respective

6. DISCUSSION:

Rahul Shukla. *et al*., 2013 accomplished a phytochemical analysis on Typha angustifolia, that shows shown the presence of Alkaloids, sterols, sugars, flavonoids, and tannins were present in the different extracts of Rhizome, leaf, shoot and pollen. Varghese *et al*.,2009 performed methanolic extract of the leaves of the plant displayed the presence of alkaloids, sterols, and flavonoids. Kasarkar *et al*.,2017 examined *Typha* leaves Aqueous extrawhichthat shows the presence of saponin, Coumarin, flavonoids, Tannins. As table-1 shows results of Secondary metabolites analysis of leaves; Methanolic leaves extracts showed the presence of Alkaloids, Flavonoids, Phenols, Tannins, carbohydrates,

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Protein, saponin, glycosides, steroids and Terpenoids. Hexene leaves extracts showed the presence of Alkaloids, steroids, carbohydrates.

PHOTO PLATES OF TLC

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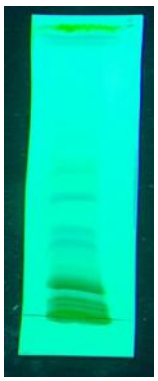
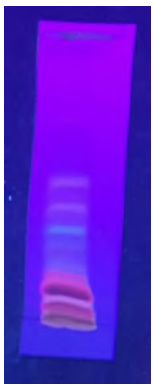
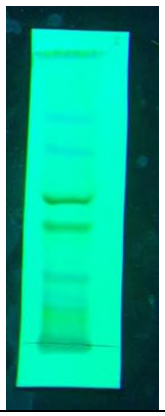
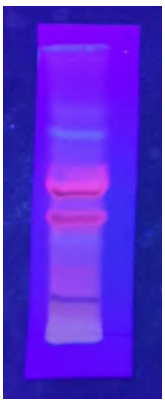
<tr><td>  </td><td>  </td><td>  </td><td>  </td><td>  </td><td>  </td></tr>
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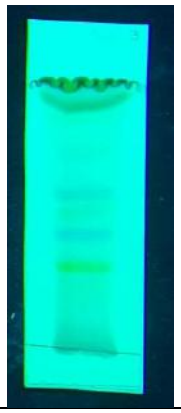
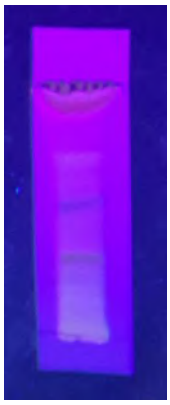
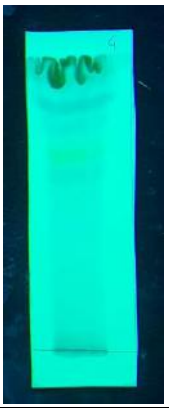
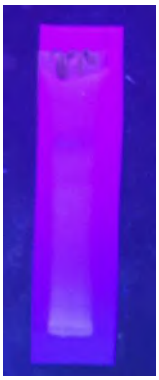
<tr><td>  </td><td>  </td><td>  </td><td>  </td><td>  </td><td>  </td></tr>
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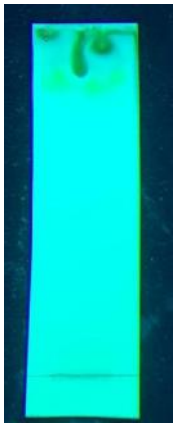
<tr><td>  </td><td>  </td><td>  </td></tr>
<tr><td>Daylight</td><td>Uv-256nm</td><td>Uv-360nm</td></tr>
<tr><td colspan="3">Ethyl acetate: Hexene (9:1)</td></tr>
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7. CONCLUSION:

The results showed that the examined plants had medically important components. Much evidence has been accumulated in previous studies, confirming that the identified phytochemicals are bioactive. Several studies have

confirmed that these phytochemicals provide both medicinal and physiological properties to the plant's understudy in the treatment of various diseases. Therefore, extracts from these plants can be considered an excellent source of useful medicines. Traditional medicine practices are strongly recommended for this plant, suggesting that further work needs to be undertaken to isolate, purify, and characterize the active ingredients involved in the activity of these plants. Additional studies are also encouraged to elucidate the possible mechanisms of action of these extracts.

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