Screening *Thevetia peruviana* (Pers.) K. Schum. for its Bioactive Phytochemicals

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ABSTRACT: *A plant cell produces two types of metabolites. They are primary metabolitesand secondary metabolites.The secondary metabolites are bio synthetically derived from primary metabolites and thus can also be termed as bioactive compounds. These bioactive compounds play crucial role in the lives ofthe plants, animals and human beings.Thevetia peruviana (Pers.) K. Schum. commonly known as yellow oleander is an ornamental plant with toxic properties. The study aims to find the presence of the secondary metabolites (bioactive compounds) present in the plants through the qualitative phytochemical analysis.*

Key Words: *Thevetia peruviana (Pers.) K. Schum., Soxhlet extraction, secondary metabolites, hydroalcoholic extracts, phytochemical tests.*

INTRODUCTION-

Thevetia peruviana (Pers.) K. Schum. commonly known as yellow oleander is an evergreen shrub belonging to the family Apocynaceae. This plant is native to Mexico, South and Central America. Nowadays it is frequently grown throughout the Tropical and Sub- Tropical regions of the world. In India it is widely cultivated as an ornamental shrub.The plant is also known for its cardiotoxic properties. In the study all the parts of the plant have been used for evaluating the presence of the different bioactive compounds in them. The extracts have been prepared in the hydroalcoholic solvent and different phytochemical tests have been done.

METHADOLOGY-

COLLETION OF SAMPLES FROM WILD

Different parts of the plant were collected these are listed as follows-
Leaves,Stems, Flowers,Fruits and Seeds

EXTRACT PREPARATION

SOXHLET EXTRACTION-

Plant parts were dried and finely powdered using mixer grinder then by using Soxhlet extraction procedure. The powdered leaves were placed in the thimble and the thimble was further placed in the Soxhlet extractor. The solvents system taken was in the ratio of 70:10, methanol: water, the solvents were boiled. Due to boiling the vapors rose up and were condensed by the condenser. The condensed solvent filled up the thimble completely and siphoned back down into the container of the organic solvent. This process took place over and over again until all the materials from the solid was extracted into the organic solvent. This procedure was followed for the other parts of the plant such as stem, fruits and flowers.

Fig:-1: Soxhlet apparatus

EXTRACT PREPARATION FOR PHYTOCHEMICAL ANALYSIS-

The solvents were poured in the petri plates from the round bottom flask of the Soxhlet extractor. The extracts were dried and 40 mg of the dried extract was weighed and dissolved in 20 ml of hydroalcoholic solvent system (methanol: water was taken in a ratio of 7:3).

Fig:-2: Liquid Extract of the different parts of the plant

PHYTOCHEMICAL ANALYSIS-

Phytochemical analysis was done using the standard protocol (P. Tiwari, *et.al.* 2011) and (K.S. Banu, *et.al*. 2015)**(Table:-1)**.

S. No.	PHYTOCHEMICAL	TEST	INFERENCE
1	Alkaloids	a) Dragendroff's Test- 1 ml extract + 2 ml of Dragendroff's reagent.	Orange-red precipitate
		b) Mayer's Test- 1 ml of extract + 2ml of Mayer's reagent.	White creamy precipitate
2	Phenolic Compounds and Tannins	a) Ferric Chloride Test- 1 ml extract + 3-4 drops of 5% Ferric chloride solution.	Dark green colour
		b) Lead Acetate Test- 1 ml of extract + 3 ml of 10% Lead acetate solution.	Bulky white precipitate
		c) Alkaline Reagent Test- 1 ml of extract + few drops of Sodium hydroxide solution.	Intense yellow colour
		d) Magnesium and Hydrochloric acid Reduction Test- 2 ml of extract + few fragments of Magnesium ribbon + drop wise addition of Hydrochloric acid.	Pink to crimson colour develops
3	Glycoside	a) Borntrager's Test- 2 ml of extract + 3 ml of Chloroform (shaking after addition) → separation of the chloroform layer + 10% Ammonia solution.	Pink to red color.
4	Cardiac Glycoside	a) Keller- Killiani Test- 2 ml of extract + 5ml of distilled water + Few drops of Glacial acetic acid + 1 drop of Ferric chloride solution + few drops of conc. Sulphuric acid.	Brown ring formation, below it green to violet ring formation.
5	Flavonoids	a) Alkaline Reagent Test- 1 ml of extract + few drops of Sodium hydroxide solution.	Intense yellow colour
		b) Lead Acetate Test- 1 ml of extract + 3 ml of 10% Lead acetate solution.	Bulky white precipitate
6	Diterpenes	a) Copper Acetate Test- 1 ml of extract + 3-4 drops of Copper acetate solution.	Emerald green colour
7	Steroids	a) Salkowski's Test- 1 ml of extract + 1 ml of chloroform + few drops of Conc. Sulphuric acid (shaken)	Golden yellow colour.

		b)	Liberman Burchard's test- 1 ml of extract + 2 ml Acetic anhydride + 2 drops of Sulphuric acid.	Formation of brown ring
8	Saponins	a)	Foam Test- 0.5 gm of extract + 2ml of water (shaken)	Formation of foam
9	Test for fixed oils and fats	a)	Spot Test- 10 mg of extract is pressed between two filter papers.	Oil stain on the paper.

Table:-1 ProceduresforPhytochemical Screening of*Thevetia peruviana* (Pers.)K.Schum. (Hydroalcoholic extracts).

QUALITATIVE ANALYSIS OF PHYTOCHEMICALS-

The hydroalcoholic extracts of the dried plant parts were prepared by the process of Soxhlet extraction. The qualitative screening of the *Thevetia peruviana* (Pers.) K. Schum.plants wereperformed according to the standard protocol of J. Rupesh, *et.al.* 2017; A. Kumar, *et.al.* 2018 and K. Pragati, *et.al.* 2012 **(Table:-2)**.

The qualitative screening of hydroalcoholic extracts of *Thevetia peruviana* (Pers.) K. Schum. (leaves, stems, flowers, fruits and seeds) was done for evaluating the presence of the secondary metabolites.Dragendroff's and Mayer's test was done for evaluating the presence of alkaloids. Alkaloids were found to be present in the extracts of the leaves, flowers, fruits and seeds. The presence of alkaloids was found to be highest in the extract of the seeds compared to the other extracts. In the extract of the stems absence of alkaloids was observed. Ferric Chloride Test, Lead Acetate Test, Alkaline Reagent Test and Magnesium and Hydrochloric acid Reduction Test were done to determine the presence of phenolic compounds and tannins. The Ferric Chloride Test showed positive result for the leaves, flowers and the seeds extract. The test displayed negative result for the stems and the fruits extract. The Lead Acetate Test showed extremely positive result for the presence of phenolic compounds and tannins in the extracts of the leaves. In the extracts of the stems and fruits the test exhibited negative result. The Alkaline Reagent Test showed positive result for the all the extracts. The Magnesium and Hydrochloric acid Reduction Test displayed positive result for the extracts of the leaves, flowers and seeds. It showed negative result for the extracts of the stems and the fruits.

Borntrager's Test was performed for determining the presence of glycosides. The test showed highly positive result for the extracts of the leaves, flowers and fruits while negative result was obtained for the extract of the stems. Keller- Killiani Test was performed for determining the presence of cardiac glycosides. In this test it was observed that all the extracts except the extract of the stems showed positive result. To determine the presence of flavonoids Alkaline Reagent Test and Lead Acetate Test was performed. All the extracts showed positive result in the alkaline reagent test. The extract of the stems and the fruits showed highly positive result as compared to others. In the Lead Acetate test it was observed that the extract of the leaves showed highly positive result while in the extracts of the stems and fruits the result was totally negative. Copper Acetate Test was done for evaluating the presence of diterpenes. This test showed positive result for all the extracts. Compared to the rest of the parts the leaves and the flowers extract showed extremely positive result. To determine the presence of steroids,Salkowski's Test and Liberman Burchard's test was performed. In Salkowski's Test it was found that all the extracts showed positive result while in the Liberman Burchard's test the extract of the leaves and the stems displayed negative result. In both the tests the extract of flowers showed more positive result as compared to the other extracts. Foam test was done for determining the presence of saponins. In this test all the extracts exhibited positive result. Spot test was done for determining the presence of fixed oils and fats. The result was found to be positive for the extracts of the fruits and seeds. In the extracts of the leaves, stems and flowers fixed oils and fats were found to be absent.

PHYTOCHEMICALS	TESTS	LEAVES	STEM	FLOWERS	FRUITS	SEEDS
Alkaloids	Dragendroff's Test	+	-	+	+	++
	Mayer's Test	+	-	+	+	++
Phenolic Compounds and Tannins	Ferric Chloride Test	++	-	+++	-	++
	Lead Acetate Test	+++	-	+	-	++
	Alkaline Reagent Test	+	+++	+	+++	++
	Magnesium and Hydrochloric acid Reduction	+	-	+++	-	+++
Glycoside	Borntrager's Test	+++	-	+++	+++	+
Cardiac Glycoside	Keller- Killiani Test	+++	-	+++	+++	+
Flavonoids	Alkaline Reagent Test	+	+++	+	+++	++

	Lead Acetate Test	+++	-	+	-	++
Diterpenes	Copper Acetate Test	+++	+	+++	++	+
Steroids	Salkowski's Test	++	++	+++	+	++
	Liberman Burchard's test	-	-	+++	+	++
Saponins	Foam Test	++	++	++	+	+
Test for fixed oils and fats	Spot Test	-	-	-	+	+

(**where** + =present and - = absent)

Table:-2 Results of Phytochemical Screening of *Thevetia peruviana* (Pers.) K.Schum. (Hydroalcoholic extracts).

DISCUSSION-

J. Rupesh, *et.al.*2017 and K. Pragati, *et.al.*2012 reported the presence of Alkaloids in the powder of the flowers of *Thevetia peruviana* (Pers.) K. Schum. A. Kumar, *et.al.*2018 reported the presence of alkaloids in the methanolic extract of the leaves of the plant. In the current experiment where hydroalcoholic extract of the plant parts had been used for the phytochemical screening it was observed that alkaloids were present in the extract of the leaves, flowers, fruits and seeds except in the extract of the stems. The absence of the phenolic compounds and tannins have been reported by A. Kumar, *et.al.*2018 in the methanolic extract of the leaves. In this experiment phenolic compounds and tannins have been found present in the extracts of all the parts of the plant. Total 4 tests had been done for finding the presence of the phenolic compounds and tannins. The alkaline reagent test showed positive result for all the parts. J. Rupesh, *et.al.*2017and K. Pragati, *et.al.* 2012reported the presence of glycosides and cardiac glycosides in the powder of the flowers A. Kumar, *et.al.*2018 reported the presence of the glycosides and cardiac glycosides in the methanolic extract of the leaves. In this experiment except the extracts of the stems all the extracts have shown the presence of the glycosides and cardiac glycosides. Diterpenes and saponins have been reported to be present in the methanolic extract of the leaves by A. Kumar, *et.al.*2018. In the current experiment diterpenes and saponins have been found to be present in all the extracts. J. Rupesh, *et.al.*2017 and K. Pragati, *et.al.*2012 reported the presence of fixed oils and fats in the powder of the flowers. In the present study only the extracts of the fruits and seeds have shown the presence of the fixed oils and fats. The steroids have been reported to be present in the extracts of the leaves by A. Kumar, *et.al.*2018. In the present study 2 tests have been done for evaluating the presence of the steroids. In the Salkowski's Test it was found that all the extracts showed positive result for the presence of the steroids. The presence of flavonoids had been reported in the powder of the flowers by J. Rupesh, *et.al.*2017 and K. Pragati, *et.al.*2018. The presence of the flavonoids has also been reported in the methanolic extract of the leaves byA. Kumar, *et.al.*2018. In the current experiment 2 tests had been conducted to evaluate the presence of the flavonoids. The Alkaline reagent test showed positive result for all the plant extracts whereas the lead acetate test showed the absence of flavonoids in the extracts of the stem and the fruits.

CONCLUSION-

In thehydroalcoholic extract (methanol and waterin the ratio 70:30) of the leaves, stems, flowers, fruits and seeds of *Thevetia peruviana* (Pers.) K. Schum. many phytochemicals have been found to be present. Thus, it can be concluded that *Thevetia peruviana* (Pers.) K. Schum. is rich in phytochemicals like alkaloids, phenolic compounds, tannins, glycosides, cardiac glycosides, flavonoids, diterpenes, steroids, saponins. It can be also concluded that the plant is also having a rich content of fixed oils.

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