



Research Article

Determination And Quantification of Alkaloids Content in Tridax Procumbens Leaves by Using Colorimetric Method

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ABSTRACT

Tridax procumbens, a widely distributed medicinal plant, is known for its diverse pharmacological properties, including antimicrobial, anti-cancer, anti-oxidant, anti-diabetic, anti-inflammatory, and wound healing effects. The presence of bioactive alkaloids contributes significantly to its therapeutic potential. This study aims to determine and quantify the alkaloid content in Tridax procumbens leaves using a colorimetric method. Alkaloids were extracted using soxhlet extraction techniques, and their concentration was measured colorimetrically. The absorbance was recorded at a specific wavelength of 450nm, and a calibration curve was established using a standard bismuth nitrate. The results indicated 8.5% of alkaloids in the leaf extract, demonstrating the potential of Tridax procumbens as a source of bioactive compounds. This study provides a simple, cost effective, and reliable method for alkaloid quantification, which could be useful for further pharmacological studies.

INTRODUCTION

COLORIMETRY:

A colorimeter is an analytical device that quantifies the concentration of a solute in a solution based on its ability to absorb light at a specific wavelength. It operates on Beer-Lambert's law, which says that light absorption varies directly with solution concentration and thickness. The device tracks the amount of light passing through the solution, enabling the

concentration of the solute to be calculated. Colorimetry is very commonly used in laboratories, industries, and environmental research due to its simplicity and reliability.

Plant standardization requires determination of the total content of various classes of secondary metabolites. Alkaloids have diverse pharmacological properties. Determination of total alkaloids contents in plants is important to determine the dosage of plants in the preparation.

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Colorimetric methods for alkaloids are reliable, accurate, and time-saving compared to chromatographic methods, which require advanced instrument and expertise, and are time-consuming, and expensive. Colorimetric methods are simple, rapid, and efficient methods for the routine determination of total alkaloids content. The amount of bismuth is estimated after alkaloids precipitation with Dragendorff reagent; bismuth forms a yellow complex with thiourea. The *T. procumbens* leaves have anti-inflammatory, antioxidant, wound healing, anti-microbial, anti-diabetic, anti-cancerous, hypotensive, anti-plasmodial (anti-malarial), and hepatoprotective properties.

Materials:

Tridax procumbens leaves, Ethanol, Bismuth nitrate pentahydrate, Thiourea, Potassium iodide, Acetic acid, Petroleum jelly, Distilled water.

Apparatus:

RBF, Condenser, Whatman filter paper, Soxhlet apparatus, Spatula, Funnel, Tripod stand, Measuring cylinder, Filter paper, Beaker, Volumetric flask (10ml, 100ml), Glass rod, Pipette, Test tubes, Cuvettes.

Instruments:

Digital balance, Colorimeter, Heating mantle

PLANT COLLECTION:

The collected fresh leaves of *Tridax procumbens* were washed and dried in shade. After drying plant leaves were finely powdered and kept in a well closed container, For further extraction procedure.

METHOD OF EXTRACTION:

Soxhlet method (Extract-1) :

25 grams of finely powdered *T. Procumbens* leaves are packed in a thimble and placed in the soxhlet apparatus. And pour some amount of

ethanol into it. Add 150 ml of ethanol to RBF. Finally, the solvent is boiled to its boiling point, while the condenser is kept cool with a chilled water recirculation. The extraction was carried out for 12 h at 80°C. Later on, the extract was filtered, and the filtrate was kept in the refrigerator for use.

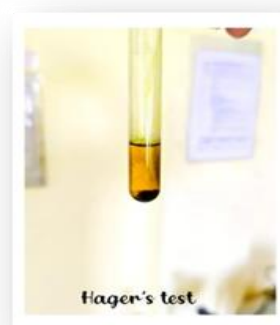


Cold maceration (Extract-2) :

25 grams of leaf powder was mixed with 120 ml of 99.9% ethanol in a conical flask. The mixture was stirred occasionally with a glass rod, covered with an aluminium foil and kept it at room temperature for 6 days. After maceration period, filter the extract by using filter paper. Repeat filtration if necessary to obtain a clear extract.

Phytochemical screening

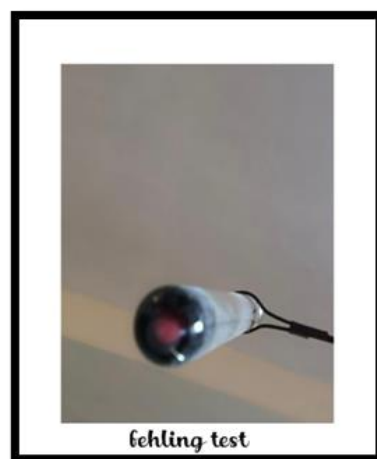
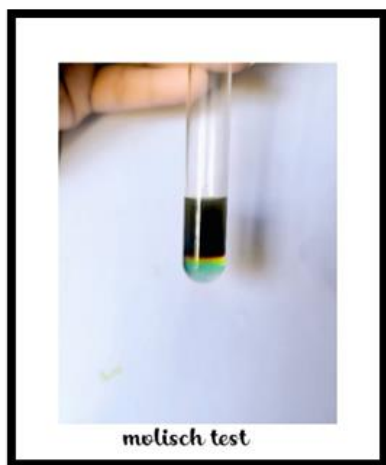
1)Identification tests for alkaloids:



TEST	PROCEDURE	OBSERVATION
1.Dragendorff's test	Take 2ml extract and add 2ml of Dragendorff's reagent to it.	A orange-red precipitate was formed. It indicates the presence of Alkaloids
2.Hager's test	Take 2ml extract and add two drops of aqueous picric acid solution [Hager's reagent].	A creamy white precipitate was formed. It indicates the presence of Alkaloids.
3.Wagner's test	Take 2ml extract and add one to two drops of wagner's reagent [along the sides of the test tube].	A reddish precipitate was formed. It indicates the presence of Alkaloids.
4.Mayer's test	Take 2ml of extract and add one to two drops of mayer's reagent [along the sides of test tube].	A yellow precipitate was formed.It indicates the presence of alkaloids.

2)Identification tests for Carbohydrates:

Test	Procedure	Observation
1.Molisch's test	Take 2ml extract and add α -naphthol and few drops of concentrated H_2SO_4	A violet ring was formed at the point of contact between the acidic which indicates the presence of carbohydrates
2.Fehling's test	Take 2ml of extract and add equal quantities of Fehling's A & B solutions and heat it.	A brick red precipitate was formed which indicates the presence of carbohydrates.



3.Identification tests for glycosides:

Test	Procedure	Observation
1.keller- kiliani test	Take 2ml extract and add 1ml of Glacial acetic acid, 5% $FeCl_3$ and 1ml concentrated H_2SO_4	Blue colour was formed which indicates the presence of glycosides.

4.Identification tests for Terpenoids:





DETERMINATION OF TOTAL ALKALOID CONTENT

METHOD OF PREPERATION:

- ❖ Preparation of bismuth nitrate solution:
 - By using an analytical balance, accurately weigh 200 mg of bismuth nitrate.
 - Now take a volumetric flask and add 30ml of distilled water to it.
 - Add 20 ml of nitric acid to it.
 - Gently shake the flask to form solution.
 - Add weighed bismuth nitrate into the volumetric flask.
 - Again shake the volumetric flask until it forms a clear solution.
 - Make up to 100ml with distilled water.
- ❖ Preparation of Thiourea solution:
 - By using analytical balance accurately weigh 3 gms of thiourea
 - Now take a beaker and add weighed thiourea into it.
 - Add few ml of water to it and dissolve thiourea by using stirrer.
 - After transfer the solution into the volumetric flask and make up to 100ml with distilled water.
- ❖ Dragendorff's reagent preparation:
 - For preparation of solution-1: Weigh 0.85g of bismuth and dissolve in 40ml of water. Add 10 ml of acetic acid to it.
 - For preparation of solution-2: 20g of potassium iodide is dissolved in 50ml of water.

- Equal quantities of solutions 1 and 2 are mixed together to form dragendorff's reagent

SELECTION OF WAVE LENGTH:

- For 1ml of bismuth nitrate solution, add 0.25ml of 3% thiourea in 10ml volumetric flask
- Make up the volume to 10ml

Measured the absorbance of the above solution at different nanometer

(450nm, 470nm, 510nm, 520nm, 540nm, 570nm, 600nm, 670nm) against blank (0.25ml of 3% thiourea + water make up to 10ml)

The maximum absorbance was at 450nm – λ_{max} -450nm.

S. No	Wavelength (Nm)	Concentration (Mg)	Absorbance
1.	450	2	1.56
2.	470	2	1.51
3.	510	2	0.47
4.	520	2	0.10
5.	540	2	0.03
6.	570	2	0.01
7.	600	2	-0.00
8.	670	2	-0.00

Preparation of calibration curve:

From bismuth nitrate solution take 5 different solutions (0.2ml, 0.4ml, 0.6ml, 0.8ml, 1ml) into 5 different 10 ml volumetric flasks. Now add 0.25ml of 3% thiourea solution to the above solutions and makeup to 10ml. The absorbance was measured at the 450nm against nitric acid and thiourea solution as blank

S. No	Wave Length (Nm)	Concentration (Mg)	Absorbance
1.	450	0.4	0.18
2.	450	0.8	0.42
3.	450	1.2	0.78
4.	450	1.6	1.24
5.	450	2	1.56

Procedure for the determination of extract-1 absorbance:

- For 0.25ml of extract, add 0.25 ml of dragendorff's reagent in a test tube.
- Leave the mixture for about 30 min, stir it occasionally.
- After 30 min there forms a precipitate in test tube which is filtered and filtrate is collected.
- Now take filtrate in a 10ml volumetric flask.
- Add 0.25 ml of thiourea to the collected filtrate.
- Make up the volume to 10ml

Measure the absorbance of the above solution at 450nm against blank (0.25ml of 3% thiourea+ water make up to 10ml).

❖ The absorbance of the solution was found to be 0.52

Procedure for determination of extract-2 absorbance:

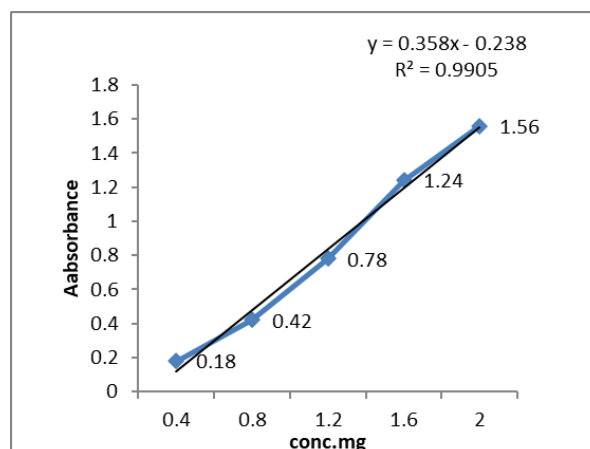
- For 0.25ml of extract, add 0.25ml of dragendorff's reagent in a test tube.
- Leave the mixture for about 30 min, stir it occasionally
- After 30 min there forms a precipitate in test tube which is filtered and filtrate is collected.
- Now take filtrate in a 10ml volumetric flask.
- Add 0.25ml of thiourea and make up the volume to 10ml.

Measure the absorbance of the above solution at 450nm against (0.25ml of 3% thiourea + water make up to 10ml)

❖ The absorbance of the solution was found to be 0.24.

RESULT AND DISCUSION

Calibration curve:



Calculation

S.NO	Wave length (nm)	Concentration (mg)	Absorbance
1	450	0.4	0.18
2	450	0.8	0.42
3	450	1.2	0.78
4	450	1.6	1.24
5	450	2	1.56

Extract-1:

- Concentration of alkaloid content in 25gm(100ml):

Formula:

$$\text{Total alkaloid content} = \frac{\text{concentration of alkaloid found} \times \text{Final volume of extract}}{\text{Quantity of extract}}$$

$$\text{TAC} = \frac{0.92 \times 100}{0.25} = 368 \text{ mg } (\because \text{Conversion of mg into gm}) = 0.368 \text{ gm}$$

$$\begin{aligned} \text{Percentage calculation: Total alkaloid \%} &= \frac{\text{TAC}}{M} \times 100 \\ &= \frac{0.368}{25} \times 100 \\ &= 1.472\% \end{aligned}$$

∴ The total alkaloid content (%) was found to be 1.472

Extract-2 :

- Concentration of alkaloid content in 25gm(100ml) of extract :



Formula:

$$\text{Total alkaloids content} = \frac{\text{concentration of alkaloid found} \times \text{Final volume of extract}}{\text{Quantity of extract}}$$

$$\text{TAC} = \frac{0.66 \times 100}{0.25} = 264 \text{mg } (\because \text{Conversion of mg into gm})$$

$$= 0.264 \text{ gm}$$

- Percentage calculation:

$$\begin{aligned} \text{Total alkaloid} &= \frac{TAC}{M} \times 100 \\ &= \frac{0.264}{25} \times 100 \\ &= 1.056\% \end{aligned}$$

$\therefore$ The total alkaloid content (%) was found to be 1.056%

SUMMARY

This study focuses on the determination and quantification of alkaloid content in *Tridax procumbens* leaves by using a colorimetric method. Alkaloids, known for their medicinal properties, were extracted using an soxhlet extraction and cold maceration techniques. The quantification was performed by forming a colored complex with dragendorff 's reagent, and the absorbance was measured colorimetrically at a specific wavelength of 450nm. A standard calibration curve of known standard solutions were used to determine the alkaloid content in the plant extract. The obtain results for soxhlet extraction and cold maceration are 1.472% and 1.056 % respectively This method provides a simple, cost effective, and reliable approach for alkaloid analysis, beneficial for quality control and further pharmacological research

CONCLUSION:

The colorimetric method used in this study successfully determined and quantified the alkaloid content in *Tridax procumbens* leaves. The extraction and complexation with dragendorff's reagent provided a reliable and cost-effective approach for alkaloid analysis. The results confirmed a significant presence of alkaloids,

highlighting the medicinal potential of *Tridax procumbens*. This method can serve as a valuable tool for quality control in herbal formulations and further pharmacological research. Further studies can focus on optimizing the method for higher sensitivity and exploring the biological activities of the quantified alkaloids.

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