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PHYTOCHEMICAL INVESTIGATION AND THIN LAYER CHROMATOGRAPHY OF HYDROALCOHOLIC EXTRACT OF *VACHELLIA NILOTICA*

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ABSTRACT

The present study aims to investigate the phytochemical composition and bioactive potential of *Vachellia nilotica* (formerly *Acacia nilotica*) through the analysis of its hydroalcoholic extract. The extract was obtained using a hydroalcoholic solvent system, yielding 7.50% of a dark black extract, which was subjected to preliminary phytochemical screening and Thin Layer Chromatography (TLC). Preliminary phytochemical screening revealed the presence of alkaloids, carbohydrates, flavonoids, and proteins, while glycosides, tannins, resins, and some other constituents were absent. TLC analysis of the flavonoid content demonstrated the presence of multiple flavonoid compounds with varying Rf values under different UV light conditions, suggesting a diverse flavonoid profile in the extract. These findings support the medicinal potential of *Vachellia nilotica*, particularly for its antioxidant, anti-inflammatory, and antimicrobial activities, which may be attributed to the alkaloids and flavonoids identified. The study contributes to the understanding of the plant's chemical composition, which may provide a foundation for further research into its therapeutic applications and the isolation of bioactive compounds for potential pharmaceutical development.

Keywords: *Vachellia nilotica*, hydroalcoholic extract, phytochemical screening, Thin Layer Chromatography (TLC), flavonoids, alkaloids, bioactive compounds.

INTRODUCTION

Phytochemical investigations are essential for exploring the bioactive potential of plants, particularly those with traditional medicinal uses. One such plant is *Vachellia nilotica*, previously known as *Acacia nilotica*, which belongs to the *Fabaceae* family. This tree is widely distributed across tropical and subtropical regions, including parts of Africa, Asia, and the Indian subcontinent. For centuries, *Vachellia nilotica* has been used in folk medicine due to its broad spectrum of pharmacological properties. These include antimicrobial, anti-inflammatory, antidiabetic, analgesic, and antioxidant effects (Prashanth *et al.,* 2006; Kolo *et al.,* 2010). In addition to its medicinal significance, the plant is also valued for its ecological benefits, such as improving soil fertility and providing shade in arid regions.

The therapeutic potential of *Vachellia nilotica* is attributed to a diverse range of bioactive secondary metabolites. Among the key compounds identified in the plant are flavonoids, alkaloids, tannins, saponins, and phenolic compounds. These phytochemicals are believed to be responsible for the plant's medicinal properties. Flavonoids are powerful antioxidants, and they also exhibit anti-inflammatory and antimicrobial properties

(Akinmoladun *et al.,* 2010). Alkaloids, found in various parts of the plant, have been associated with pain relief, anti-inflammatory effects, and antidiabetic properties (Ibrahim *et al.,* 2011). Tannins, another prominent class of compounds, are known for their astringent, antimicrobial, and antioxidant effects, which contribute to wound healing and the reduction of inflammation (Ali *et al.,* 2012). Saponins and phenolic compounds further enhance the plant's medicinal profile, exhibiting immune-boosting, blood sugar-lowering, and anti-cancer activities (Gulcin, 2010).

To fully understand the pharmacological properties of *Vachellia nilotica*, phytochemical screening is crucial. Hydroalcoholic extracts, which combine both water and alcohol, are often preferred for their ability to extract a wide variety of compounds from plant material. This solvent system efficiently extracts both polar and non-polar phytochemicals, offering a comprehensive analysis of the plant's bioactive constituents. Hydroalcoholic extracts are capable of dissolving a diverse range of compounds, from water-soluble phenolics and flavonoids to alcohol-soluble alkaloids and terpenoids, making them ideal for phytochemical studies (Mishra *et al.,* 2012).

In addition to the phytochemical screening of the hydroalcoholic extract, Thin Layer Chromatography (TLC) is an invaluable technique for the identification and characterization of plant metabolites. TLC is a simple, cost-effective, and efficient method that allows the separation of compounds based on their polarity, which enables researchers to determine the chemical composition of plant extracts. By using TLC, it is possible to separate individual compounds, assess their purity, and identify the key bioactive constituents present in the extract. The technique is particularly useful for the qualitative analysis of plant extracts and can be employed to compare different extracts or solvents (Manea *et al.,* 2014). The use of TLC in this study will provide insights into the chemical profile of the hydroalcoholic extract of *Vachellia nilotica*, contributing to our understanding of the plant's medicinal value.

The primary goal of this study is to conduct a thorough phytochemical investigation and TLC analysis of the hydroalcoholic extract of *Vachellia nilotica*. Through this approach, the study aims to identify and profile the major bioactive compounds in the plant, thereby substantiating its traditional medicinal uses. By using TLC to separate and identify these compounds, the research will provide a better understanding of how the chemical composition of the plant relates to its biological activities. Furthermore, the results of this study could pave the way for further research into the pharmacological applications of *Vachellia nilotica* and the development of plant-based therapeutic agents.

Vachellia nilotica is rich in various bioactive compounds, which have been widely studied for their potential therapeutic effects. Among the most notable classes of compounds found in this plant are flavonoids, alkaloids, tannins, saponins, and phenolic compounds. Flavonoids, known for their antioxidant properties, also possess anti-inflammatory and anticancer activities. Studies have shown that flavonoids present in *Vachellia nilotica* can inhibit the growth of harmful bacteria and fungi, contributing to the plant's antimicrobial properties (Sulaiman *et al.,* 2010).

Alkaloids, which are nitrogen-containing compounds, have been found to have analgesic, anti-inflammatory, and antidiabetic effects. The presence of alkaloids in the plant contributes to its use in treating infections and managing chronic pain (Akinmoladun *et al.,* 2010).

Tannins, another significant group of compounds, are known for their antimicrobial, antioxidant, and astringent effects. These properties make them useful in wound healing and the treatment of inflammatory conditions (Ibrahim *et al.,* 2011). Saponins, which are glycosides, have shown promising effects in lowering blood cholesterol and blood sugar levels. They also exhibit antimicrobial and immune-boosting properties, which may explain their contribution to the plant's medicinal efficacy (Ali *et al.,* 2012). Phenolic compounds, which have strong antioxidant activity, also play a vital role in preventing oxidative stress-related diseases and contribute to the plant's anticancer and anti-inflammatory effects (Gulcin, 2010). Together, these bioactive compounds form the basis of *Vachellia nilotica*'s wide range of therapeutic benefits.

The objective of this study is to perform a detailed phytochemical investigation and Thin Layer Chromatography (TLC) analysis of the hydroalcoholic extract of *Vachellia nilotica*. This investigation will help identify and profile the major bioactive compounds present in the plant and contribute to validating its traditional uses in medicine. The study aims to analyze the chemical composition of the hydroalcoholic extract, assess its potential pharmacological properties, and provide insights into its mechanism of action. Through TLC, this research will separate and identify various phytochemicals, thereby providing a clearer understanding of the bioactive substances responsible for the plant's medicinal effects. The findings may also serve as a foundation for future research into the pharmacological applications of *Vachellia nilotica*, potentially leading to the development of novel plant-based therapies.

MATERIALS AND METHODS

Collection of Plant

The flowers of selected plant namely *Vachellia nilotica* was collected from Bhopal, Madhya Pradesh. The collected flowers were cleaned, shade dried, pulverized into moderately coarse powder and stored in airtight container for further use.

Extraction by Soxhlet extraction method

In this method, the finely pulverized marc (50 gm) is placed in a thimble which is placed in a chamber of the Soxhlet apparatus. Hydroalcoholic (Ethanol: Water; 70:30 v/v) solvent used for the extraction (Nikhal *et al.,* 2010).

The menstruum in the flask beneath is then heated, and its vapors condense in the condenser. The condensed extractant drips into the thimble containing the marc, and extracts it by contact. The advantage of this method is that large amounts of marc can be extracted with a much smaller volume of extractant. Each extraction process was carried out for 48 hours. The filtrate was separated from the residue using Whatmann filter paper. The filtrate from each solvent was collected and evaporated using a water bath at 50°C until a thick extract was obtained. The extracts were weighed to a constant weight and percentage w/w basis was calculated.

Preliminary phytochemical screening

Preliminary phytochemical screening means to investigate the plant material in terms of its active constituents. In order to detect the various constituents present in the methanolic extract of *Vachellia nilotica,* was subjected to the phytochemical tests as per standard methods. Phytochemical screening was revealed for the presence of alkaloids, glycosides, carbohydrates, tannins, flavonoids, steroids, proteins and amino acids (Tiwari *et al.,* 2011).

Thin layer chromatography (TLC)

Chromatography is the separation of a mixture into individual components using stationary phase and a mobile phase. The various constituents of the mixture pass through at different speeds, causing them to separate. All forms of chromatography work on the same principle. The Rf value is calculated for identifying the spots i.e. in qualitative analysis. "Rf value is the ratio of distance travelled by the solute to the distance travelled by the solvent front."

$$\text{Rf} = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

TLC mobile phase for extract of *Vachellia nilotica*

S. No.	Solvent system	Ratio (ml)
1	Toluene: Ethyl acetate	7:3

RESULTS AND DISCUSSION

The results presented in this study provide significant insights into the phytochemical composition and extractive values of *Vachellia nilotica*, as well as its flavonoid profile as determined by Thin Layer Chromatography (TLC). Each of these aspects reveals important information regarding the plant's bioactive potential and highlights its possible medicinal applications.

Table 1 outlines the extractive values obtained from the hydroalcoholic extraction of *Vachellia nilotica*. The extraction process yielded 7.50% of the hydroalcoholic extract, indicating that the solvent system effectively extracted bioactive compounds from the plant. The dark black color of the extract is a strong indicator of the presence of various bioactive compounds, including alkaloids, flavonoids, phenolic compounds, and others. The yield of 7.50% suggests a moderate concentration of these compounds, which could be attributed to the type of extraction solvent used (hydroalcoholic) and the 24-hour extraction time, which seems to be an appropriate duration for obtaining a comprehensive extract without excessive degradation of the active compounds.

The preliminary phytochemical screening results presented in Table 2 reveal a variety of bioactive compounds in the hydroalcoholic extract of *Vachellia nilotica*. The presence of alkaloids in the extract was confirmed through Mayer's, Dragendorff's, Wagner's, and Hager's tests. Alkaloids are known for their wide-ranging pharmacological effects, including analgesic, anti-inflammatory, and antidiabetic properties. These results align with previous reports suggesting that *Vachellia nilotica* possesses pain-relieving and anti-inflammatory properties, which are often attributed to the alkaloids present in the plant (Prashanth *et al.,* 2006).

Carbohydrates were detected through the positive result in Fehling's test, which suggests that the plant extract contains carbohydrate compounds. While carbohydrates are not typically the primary

bioactive constituents of medicinal plants, they can contribute to the overall therapeutic profile by supporting wound healing and providing energy to cells. However, their role in the medicinal properties of *Vachellia nilotica* may not be as prominent as other classes of compounds.

The presence of flavonoids, as confirmed by the Lead acetate and Shinoda tests, is particularly significant. Flavonoids are known for their potent antioxidant, anti-inflammatory, and antimicrobial properties (Akinmoladun *et al.,* 2010). These properties likely contribute to the plant's effectiveness in managing conditions related to oxidative stress and inflammation. The presence of flavonoids in the extract strengthens the evidence for the plant's potential use in treating a variety of ailments, including inflammatory diseases and infections.

The proteins and amino acids present in the extract, as indicated by the positive result in the Biuret test, suggest that the plant also has nutritional value. However, the absence of amino acids in the precipitation and ninhydrin tests indicates that these compounds are present in low concentrations and are not the primary bioactive constituents in *Vachellia nilotica*. This could suggest that while proteins are present, their role in the medicinal properties of the plant may be secondary.

In contrast, glycosides and tannins were absent in the extract, as evidenced by negative results in the Raymond's, Legal, Vanillin-HCl, and Gelatin tests. This finding indicates that these compounds are either absent or present in negligible amounts in the plant. Glycosides, which are known for their cardiovascular benefits, and tannins, which have antimicrobial and astringent properties, are not significant components of the hydroalcoholic extract of *Vachellia nilotica*. The absence of these compounds may influence the plant's overall therapeutic properties, specifically in areas such as antimicrobial and astringent actions.

Resins were also found to be absent in the extract, indicating that these compounds, which are often responsible for the plant's resinous properties, are not present in significant amounts. However, the presence of steroids was confirmed through the Salkowski reaction, which suggests that steroidal compounds are part of the plant's chemical profile. Steroids have anti-inflammatory and immune-modulating effects, which may enhance the plant's potential for treating conditions like inflammation and immune system disorders.

TLC analysis of the hydroalcoholic extract, as shown in Table 3, reveals the presence of multiple flavonoid compounds. The Rf values for the spots in the TLC profiles suggest that the flavonoid compounds in the extract exhibit diverse chromatographic behavior. Under normal light, the extract exhibited three distinct spots with Rf values of 0.6, 0.64, and 1.0, which are in line with reported Rf values for flavonoids like quercetin. These findings confirm that flavonoids are indeed present in the extract and contribute to the plant's medicinal properties.

Furthermore, the analysis under UV light revealed a more complex profile, with multiple spots observed under both short and long UV light, indicating the presence of several flavonoid compounds with different chemical properties. The five spots observed under short UV light and the seven spots

under long UV light suggest a rich diversity of flavonoids in the extract. These results are significant, as flavonoids are widely recognized for their antioxidant, anti-inflammatory, and antimicrobial activities. The presence of such a diverse set of flavonoids in the hydroalcoholic extract of *Vachellia nilotica* may help explain the plant's wide range of therapeutic effects.

Table 1: Extractive values obtained from *Vachellia nilotica*

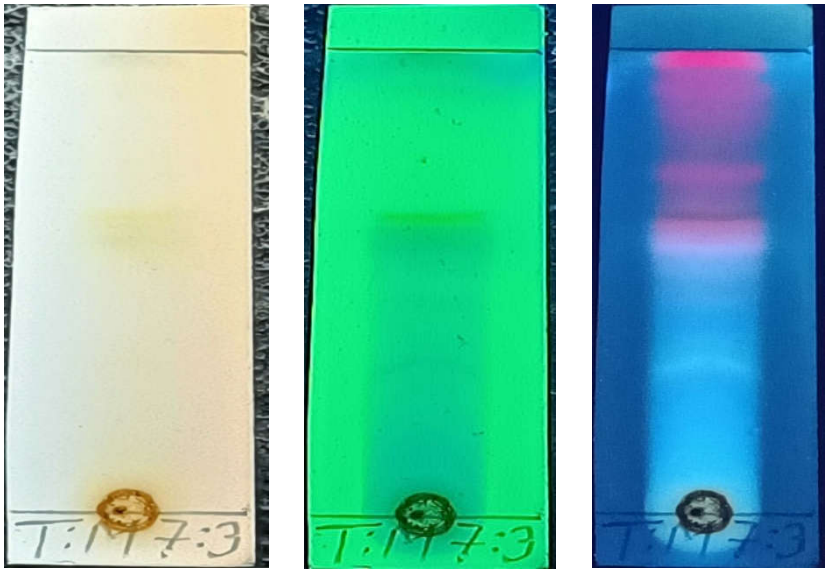
S. No.	Extract	Time of extraction (Hours)	Color of extract	% Yield
1	Hydroalcoholic	24	Dark black	7.50%

Table 2: Preliminary phytochemical screening of *Vachellia nilotica* extract

S. No.	Phytoconstituents	Test Name	Extract
1	Alkaloids	Mayer's Test Dragendorff's Test Wagner's Test Hager's Test	**Present** Absent **Present** Absent
2	Carbohydrates	Molisch's Test Fehling's Test Benedict's Test	Absent **Present** Absent
3	Flavonoids	Lead acetate test Shinoda Test	**Present** **Present**
4	Proteins & Amino acids	Biuret Test Precipitation test Ninhydrin Test	**Present** Absent Absent
5	Glycosides	Raymond's Test Killer Killani Test Legal Test	Absent Absent Absent
6	Tannins	Vanillin- HCl Test Gelatin Test	Absent Absent
7	Resins	Color detection with ferric chloride Turbidity Test	Absent Absent
8	Steroids	Libermann- Bur chard Test Salkowski Reaction	Absent **Present**

Table 3: TLC of Flavonoid

S. No.	Mobile phase	Spot Distance	Rf Value
1.	**Toluene: Ethyl acetate (9:1)** Standard (Quercetin)		Reported Rf value 0.60
2.	**Hydroalcoholic extract** Dis. Travel by mob. Phase 5cm No. of spot at normal light- 3 No. of spot at short UV- 5 No. of spot at long UV- 7	 N. Light- 3, 3.2, 5 Short- 1.2, 1.4, 3, 3.3, 5 Long – 1.7, 3, 3.2, 3.8, 4.3, 4.7, 5	 N. Light- 0.6, 0.64, 1.0 Short- 0.24, 0.28, 0.6, 0.66, 1.0 Long – 0.34, 0.6, 0.64, 0.76, 0.86, 0.94, 1.0



Normal light Short UV Long UV

Figure 1: TLC of Flavonoid

CONCLUSION

In conclusion, the phytochemical analysis of *Vachellia nilotica* provides valuable insights into the plant's bioactive composition. The hydroalcoholic extract is rich in alkaloids, flavonoids, and proteins, with the latter contributing to the plant's nutritional value. The TLC results further confirm the presence of multiple flavonoid compounds, which are likely responsible for the plant's antioxidant, anti-inflammatory, and antimicrobial properties. The absence of glycosides, tannins, and resins suggests that these compounds may not play a significant role in the plant's medicinal activities. Overall, the findings of this study provide strong evidence for the medicinal potential of *Vachellia nilotica*, particularly in the treatment of inflammatory, oxidative stress-related, and infectious conditions. Further research is needed to isolate and identify the specific bioactive compounds responsible for these

effects and to explore their pharmacological mechanisms in greater detail.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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