Qualitative Phytochemical Screening of *Tithonia diversifolia*: A Gray Aqueous Root Extract

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ABSTRACT

This study investigated the phytochemical composition of *Tithonia diversifolia*, commonly known as the Mexican sunflower, a plant renowned for its diverse pharmacological properties. Fresh root bark was collected from mature plants in Migori County, Kenya, and processed through cleaning, peeling, shade drying, and grinding into fine powder. The aqueous extract was obtained by macerating 50g of the powdered root bark in 200 mL of distilled water for 24 hours, followed by filtration that yielded 19.4%w/w crude. Qualitative phytochemical analysis was conducted on the extract to identify the presence of various bioactive compounds. The results indicated the presence of alkaloids, flavonoids, tannins, saponins, terpenoids, phenols, carbohydrates, anthocyanins, and coumarins. However, glycosides, steroids, sterols, proteins, amino acids, and lignin were not detected. In conclusion, the phytochemical analysis of *Tithonia diversifolia* root extract has revealed a diverse array of bioactive compounds, providing valuable insights into its potential medicinal applications. The presence of these phytochemicals suggests that *T. diversifolia* possess broad therapeutic potential, particularly in the management of oxidative stress, inflammation, infections, and metabolic disorders. These findings underscore the plant's promise as a source of natural remedies and highlight the need for further research to explore its application in drug development and integrative medicine.

Keywords: *Tithonia diversifolia, Alkaloids, Anthocyanins, Carbohydrates, Coumarins, Flavonoids, Phenols, Phytochemical Analysis, Saponins, Tannins, Terpenoids*

INTRODUCTION

Tithonia diversifolia, a member of the Asteraceae family (Rai *et al.*, 2023), is a perennial shrub native to Mexico and Central America (Acevedo-Rodríguez & Strong, 2022). The plant is widely distributed in tropical and subtropical regions and is renowned for its various medicinal properties. Traditionally, different parts of *T. diversifolia*, including leaves, stems, and roots, have been used in folk medicine to treat ailments such as malaria, wounds, inflammation, and gastrointestinal disorders (Tagne *et al.*, 2012). However, while the leaves and stems have been studied to some extent, the root bark's phytochemical composition remains largely unknown. Given the importance of phytochemicals—naturally occurring compounds in plants with bioactive properties—in contributing to medicinal effects (Hasler & Blumberg, 1999), it is essential to investigate the root bark of *T. diversifolia.*

Despite the widespread traditional use of *Tithonia diversifolia* for treating various ailments, the specific phytochemicals present in the root bark remain largely unexplored. Understanding the chemical composition of this part of the plant is crucial, as the root bark may contain unique bioactive compounds that contribute to its medicinal properties. However, the phytochemical profile of the root bark of *T. diversifolia* has not been thoroughly studied, leaving a gap in the knowledge of its potential therapeutic applications.

This study aimed to perform a qualitative phytochemical analysis of the aqueous root extract of *Tithonia diversifolia* to identify its phytochemical constituents. Hence, fill the knowledge gap regarding the root bark's chemical composition, which could help explore its potential medicinal applications and elucidate its mechanism of action.

METHODOLOGY

Preparation of Aqueous Root Extract

The preparation of the aqueous root extract followed a method by Alli *et al.* (2011). The fresh root bark of mature *Tithonia diversifolia* plants was collected from Migori County, Kenya. The collected root bark was thoroughly cleaned with fresh water, peeled, and then shade-dried for seven days. After drying, the root bark was ground into a fine powder using an electric grinder. The powdered root material was then taken to the University of Eldoret, where the plant specimen was identified by a taxonomist from the Department of Biological Sciences. The specimen was assigned a voucher number, M.U.H/MD/0020/21, for reference.

To extract the phytochemicals from the root bark, a maceration process was employed. This involved soaking 100 grams of the *Tithonia diversifolia* root powder in 1000 ml of distilled water. The mixture was then placed in an electric shaker and agitated for 12 hours at room temperature to ensure thorough extraction of the phytochemicals. Following the agitation, the mixture was filtered through muslin cloth and Whatman No. 1 filter paper to obtain the filtrate.

The obtained filtrate was subjected to a drying process using a rotary evaporator, with the water bath maintained at 50°C. This process continued until dark green syrup, representing the crude extract, was obtained. The crude extract was then transferred into a pre-weighed crucible and left exposed to air overnight to allow any remaining water to evaporate, yielding the final concentrate. This concentrate was carefully stored in an airtight container at 4ºC until further use. The percentage yield of the aqueous extract was determined to be 19.4%, indicating the efficiency of

the extraction process. This concentrated extract was later used for qualitative phytochemical analysis to identify its chemical constituents and explore its potential medicinal applications.

Qualitative Phytochemical Analysis

The aqueous root extract of *T. diversifolia* was subjected to qualitative phytochemical analysis using standard chemical tests to detect the presence of various phytoconstituents. The tests conducted and their observations were summarized in Table 1.

Test for alkaloids

To detect the presence of alkaloids, four distinct tests were carried out using both precipitant and colorimetric methods. In Mayer's test, a few drops of Mayer's reagent, which is a potassium mercuric iodide solution, were added to the extract, and the formation of a creamy white precipitate indicated the presence of alkaloids (Harborne, 1973). For Dragendorff's test, three drops of freshly prepared Dragendorff's reagent, a potassium bismuth iodide solution, were added to 3 mL of the extract, and the appearance of an orange-red precipitate confirmed the presence of alkaloids (Wagner, 1993). In Wagner's test, three drops of Wagner's reagent, an iodine in potassium iodide solution, were introduced to 3 mL of the extract, and a brown or reddish-brown precipitate signaled the presence of alkaloids (Wagner & Bladt, 1996). Finally, in Hager's test, three drops of a saturated picric acid solution were added to 3 mL of the extract, with the formation of a yellow precipitate indicating the presence of alkaloids (Hager, 1890).

Test for flavonoids

To detect various flavonoid classes, several tests were performed to cover a wider range of these compounds. In the Shinoda test, three drops of concentrated hydrochloric acid were added to the extract, followed by a small piece of magnesium ribbon, with the appearance of a pink or red color indicating the presence of flavonoids (Evans, 2002). The alkaline reagent test involved adding three drops of 10% sodium hydroxide to 5 mL of the extract, and the development of a yellow color, which became colorless upon adding dilute acid, confirmed the presence of flavonoids (Garg & Garg, 2019). In the lead acetate test, three drops of 10% lead acetate solution were added to the extract, and the formation of a yellow precipitate signaled the presence of flavonoid glycosides (Shah & Hossain, 2014).

Test for tannins

In the ferric chloride test, three drops of 1% ferric chloride solution were added to 5 mL of a pre-boiled and filtered extract in a test tube. The appearance of a blue-black or greenish-black color in the solution indicated the presence of tannins (Harborne, 1998).

Test for saponins

To detect the presence of saponins, two tests were conducted. In the froth test, 5 mL of the extract was placed in a test tube, vigorously shaken, and observed for the formation of stable froth that persisted for several minutes, indicating the presence of saponins (Sofowora, 1993). In the hemolytic test, a 2% red blood cell suspension was prepared using rabbit blood diluted in isotonic saline, while the plant extract was dissolved in distilled water to a concentration of approximately 1 mg/mL. Equal volumes of the red blood cell suspension and the plant extract solution were mixed in a test tube and incubated at room temperature for one hour with gentle agitation. Hemolysis was observed, with the appearance of a clear or pinkish solution confirming the presence of saponins (Obadoni & Ochuko 2001).

Test for glycosides

To comprehensively test for glycosides, several specific assays were employed:

Cardiac Glycosides

To detect the presence of cardiac glycosides, four tests were conducted. In the Kedde test, 1 mL of Keddes reagent (a solution of 2,4-dinitrophenylhydrazine) was added to 1 mL of the plant extract in a test tube, followed by 1 mL of sodium hydroxide solution. The appearance of a yellow or orange color indicated the presence of cardiac glycosides (Kedde, 1931). For the Keller-Killiani test, 1 mL of the plant extract was mixed with 2 mL of Keller-Killiani reagent, consisting of glacial acetic acid, concentrated sulfuric acid, and water. After adding 1 mL of concentrated sulfuric acid, the formation of a reddish-brown ring at the interface of the two layers confirmed the presence of cardiac glycosides (Keller & Killiani, 1886). In the Baljet test, 1 mL of Baljet reagent (a sodium picrate solution) was added to 1 mL of the plant extract, and the development of an orange or yellow color signaled the presence of cardiac glycosides (Baljet, 1947). Finally, in Legal's test, 1 mL of the plant extract was treated with 1 mL of Legal's reagent, a mixture of sodium nitroprusside and sodium hydroxide, with the formation of a pink or red color indicating the presence of cardiac glycosides (Legal, 1891).

Cyanogenic Glycosides

In the sodium picrate test, a small amount of the plant extract was mixed with a sodium picrate solution. The development of a pink or red color in the solution indicated the presence of cyanogenic glycosides (Oppenheimer, 1932).

Anthraquinone Glycosides

In the Borntrager's test, 1 mL of 10% sulfuric acid was added to 1 mL of the plant extract in a test tube, which was then boiled, cooled, and extracted with benzene. The benzene layer was treated with ammonia, and the appearance of a pink or red color indicated the presence of anthraquinone glycosides (Bornträger, 1897). In the modified Borntrager's test, the procedure was similar, but the benzene extract was first treated with sodium carbonate before the addition of ammonia. The formation of a pink or red color after ammonia treatment confirmed the presence of anthraquinone glycosides (Harborne, 1973).

Test for terpenoids

In the Keller-Killiani test, 5 mL of glacial acetic acid was mixed with 5 mL of the plant extract in a test tube. A drop of 5% ferric chloride solution was added, followed by three drops of concentrated sulfuric acid. The formation of a reddish-brown ring at the interface of the two layers indicated the presence of terpenoids (Keller & Killiani, 1886).

Test for phenols

In the ferric chloride test, three drops of 1% ferric chloride solution were added to 5 mL of the plant extract in a test tube. The solution was observed for the appearance of green, blue, or violet shades, indicating the presence of phenolic compounds (Harborne, 1998).

Test for carbohydrates

To t test for carbohydrates, including various classes of sugars, several specific assays were conducted. In Molisch's test, 5 mL of the plant extract was mixed with three drops of 1% alcoholic α-naphthol solution. Concentrated sulfuric acid was then added slowly along the sides of the test tube. The presence of carbohydrates, especially sugars, was indicated by the formation of a violet ring at the interface (Molisch, 1886). Fehling's test involved adding 1 mL each of Fehling's

solution A and Fehling's solution B to 1 mL of the plant extract, followed by heating in a boiling water bath. The appearance of a red or brick-red precipitate confirmed the presence of reducing sugars, such as glucose and fructose (Fehling, 1849). In Tollens' test, 1 mL of Tollens' reagent was mixed with 1 mL of the plant extract and heated in a water bath. The formation of a silver mirror or a black precipitate on the test tube walls indicated the presence of reducing sugars, particularly aldoses (Tollens, 1883). Seliwanoff's test involved mixing 1 mL of the plant extract with 1 mL of Seliwanoff's reagent, a solution of resorcinol in hydrochloric acid, and heating the mixture in a boiling water bath. A red or pink color indicated the presence of ketoses, such as fructose (Seliwanoff, 1887). Finally, Barfoed's test required adding 1 mL of Barfoed's reagent, a solution of copper acetate in acetic acid, to 1 mL of the plant extract, followed by heating in a boiling water bath. The development of a red or orange precipitate indicated the presence of monosaccharides (Barfoed, 1864).

Test for steroids and sterols

In the Liebermann-Burchard test, 5 mL of the extract was placed in a test tube, to which three drops of acetic anhydride were added. Concentrated sulfuric acid was then slowly introduced. The solution was observed for a color change, starting with green, transitioning to blue, and finally turning red, which indicated the presence of steroids and sterols (Nath *et al.*, 1949).

Test for proteins and amino acids

To test for proteins and amino acids, two specific assays were performed. In the Biuret test for proteins, 5 mL of the plant extract was placed in a clean test tube, and three drops of freshly prepared 2% copper sulfate solution were added, followed by three drops of freshly prepared 10% sodium hydroxide solution. The mixture was gently mixed and observed for any color change. The presence of proteins or amino acids was indicated by the formation of a violet or lavender color (Gornall *et al.*, 1949). In the Ninhydrin test for amino acids, three drops of freshly prepared 0.2% ninhydrin solution in acetone were added to 5 mL of the plant extract in a boiling tube. The mixture was then gently heated in a boiling water bath for 10 minutes. The appearance of a purple color after heating signaled the presence of amino acids in the plant extract.

Test for anthocyanins

To test for anthocyanins, an acid test was performed by adding three drops of dilute hydrochloric acid (HCl) to 5 mL of the plant extract in a test tube. The solution was observed for a color change to red or violet, which indicated the presence of anthocyanins (Fossen & Andersen, 2003).

Test for lignin

To test for lignin, the phloroglucinol test was conducted by adding three drops of phloroglucinol reagent to 5 mL of the plant extract, followed by three drops of concentrated hydrochloric acid. The solution was then observed for the development of a red color, indicating the presence of lignin (Lin & Dence, 2012).

Test for coumarins

To test for coumarins, three drops of 1M sodium hydroxide (NaOH) solution were added to 5 mL of the plant extract in a test tube. The solution was observed for the development of a yellow color, which indicated the presence of coumarins (Harborne, 1998).

RESULTS

The qualitative phytochemical analysis of the aqueous root extract of *T. diversifolia* revealed the presence of alkaloids, flavonoids, tannins, saponins, terpenoids, phenols, carbohydrates, anthocyanins, and coumarins. Conversely, glycosides, steroids and sterols, proteins and amino acids, and lignin were not detected in the aqueous root extract of *T. diversifolia* (Table 1).

Table 1:

Phytochemical Constituents of the Aqueous Root Extract of Tithonia diversifolia

	Phytochemical	Test	Observation	Indication
1	Alkaloids	Mayer's Test	Creamy white precipitate	+
		Dragendorff's Test	Orange-red colouration	+
		Wagner's Test	Reddish-brown precipitate	+
		Hager's Test	Yellow precipitate	+
2	Flavonoids	Shinoda Test	Reddish colouration	+
		Alkaline Reagent Test	Yellow coloration, which turned colorless upon the addition of dilute acid	+
		Lead Acetate Test	Yellow precipitate	+
3	Tannins	Ferric Chloride Test	Blue-black colouration	+
4	Saponins	Froth Test	Froth that persists for a few minutes	+
		Hemolytic Test	Pinkish solution	+
5	Terpenoids	Salkowski Test	Reddish-brown coloration at the interface	+
6	Cardiac Glycosides	Kedde Test	Yellow or orange color	-
		Keller-Killiani Test	Darkening of the solution	-
		Baljet Test	Orange or yellow colour	-
		Legal's Test	Pink or red color	-
7	Cyanogenic Glycosides	Sodium Picrate Test	pink or red color	-
8	Anthraquinone Glycosides	Borntrager's Test	pink or red color	-
		Modified Borntrager's Test	pink or red color	-
9	Terpenoids	Keller-Killiani Test	Reddish-brown ring at the interface of the two layers	+
10	Phenols	Ferric Chloride Test	Blue-green coloration	+
11	Carbohydrates	Molisch's Test	Dark Violet ring at the interface	+
		Fehling's Test	Red or brick-red precipitate	+
		Tollens' Test	Silver mirror or a black precipitate	+
		Seliwanoff's Test	Red or pink color	-
		Barfoed's Test	A red or orange precipitate	-
12	Steroids and Sterols	Liebermann-Burchard Test	The solution turned dark brown	-
13	Proteins and Amino Acids	Biuret Test	Bluish colouration	-
		Ninhydrin Test	purple color after heating	-
14	Anthocyanins	Acid Test	Reddish colouration	+
15	Lignin	Phloroglucinol Test	Browning of the solution	-
16	Coumarins	NaOH Test	Yellowing of the solution	+

***(+)** Present; **(-)** Absent*

DISCUSSION

Phytochemicals exhibit diverse pharmacological and biochemical actions, playing a crucial role in treating and managing various illnesses. They are responsible for plants' characteristic odor and color and can contribute to their toxicity and medicinal properties. The availability of these bioactive compounds, which may exhibit activities akin to conventional synthetic drugs, can be employed to predict the potential toxicity and side effects associated with medicinal plants. Furthermore, studying these phytochemicals holds promise for developing novel medicinal agents. Many herbs contain potent phytochemical compounds that can enhance overall health and protect against various diseases. Phytochemicals, being bioactive natural plant compounds, are predominantly employed for their medicinal properties due to their therapeutic potency (Juliani, 2017). These phytochemicals warrant in-depth investigation for the potential development of novel therapeutic agents (Dasgupta, 2021). Various herbal sources have demonstrated the presence of potent phytochemical compounds capable of enhancing overall health and conferring protection against a spectrum of diseases. Phytochemicals, as bioactive constituents derived from plants, are primarily harnessed for their therapeutic efficacy due to their medicinal potential (Okarter, & Liu, 2010).

In this study, the qualitative phytochemical analysis of the aqueous root extract of *Tithonia diversifolia* revealed the presence of several bioactive compounds (Table 1), including alkaloids, flavonoids, tannins, saponins, terpenoids, phenols, carbohydrates, coumarins, and anthocyanins. These findings provide insight into the potential medicinal value of this plant part, highlighting its diverse therapeutic potential.

The studies by Obayomi (2021) and Omolola (2020), which focused on the leaf extract of *Tithonia diversifolia*, did not detect anthocyanins, coumarins, or carbohydrates. In contrast, the current analysis shows these compounds are present in the root extract. This indicates that different parts of the plant may contain varying concentrations of bioactive compounds, which could influence their therapeutic properties and effectiveness.

The findings of Olayinka et al. (2015), which reported the presence of alkaloids, flavonoids, tannins, saponins, terpenoids, and phenols in the stem extract but not carbohydrates, coumarins, or anthocyanins, further underscore the differences between plant parts. The root extract's broader spectrum of phytochemicals suggests that it may offer a more comprehensive range of bioactive properties compared to the stem extract.

The phytochemical profile of the aqueous root extract of *Tithonia diversifolia* reveals several compounds with significant pharmacological implications. Alkaloids, identified through tests such as Mayer’s, Dragendorff's, Wagner’s, and Hager’s, are known for their diverse therapeutic activities. These compounds exhibit potent analgesic, antimalarial, and antimicrobial properties, making them valuable in treating various diseases (Venkatesan *et al.,* 2019). For instance, alkaloids like quinine and morphine are well-documented for their effectiveness against malaria and pain, respectively (Ouma, 2020).

Flavonoids, detected by Shinoda, Alkaline Reagent, and Lead Acetate tests, contribute significantly to the antioxidant, anti-inflammatory, and anticancer activities of the extract (Kopustinskiene et al., 2020). These compounds play a crucial role in reducing oxidative stress and inflammation, which are linked to chronic diseases such as cardiovascular conditions and cancer. Research has highlighted the health benefits of flavonoids, including their ability to mitigate oxidative damage and enhance overall health (Li et al., 2020).

Tannins, revealed by the Ferric Chloride test, are noted for their antioxidant and antimicrobial properties. They are involved in wound healing processes and contribute to gastrointestinal health. The presence of tannins in the extract may provide protective effects against oxidative stress and microbial infections, thus supporting their traditional use in medicinal applications (Sweidan et al., 2023).

Saponins, identified by the Froth and Hemolytic tests, have been associated with immunostimulant, antifungal, and cholesterol-lowering effects. These compounds enhance immune responses and may be beneficial in managing hypercholesterolemia. Their diverse pharmacological activities underline their potential therapeutic applications (Timilsena et al., 2023).

Terpenoids, detected by the Salkowski and Keller-Killiani tests, are known for their anti-inflammatory, antioxidant, and anticancer activities. These compounds are valuable in treating inflammatory diseases and cancer, with their effectiveness supported by recent studies (Gil-Martínez et al., 2023).

Phenols, identified through the Ferric Chloride test, possess potent antioxidant properties (Soares et al., 2024) that can protect against oxidative stress and related diseases. They are crucial for cardiovascular health and cancer prevention, highlighting their role in mitigating disease risks (Marrero et al., 2024).

The presence of carbohydrates, detected by Molisch's, Fehling's, and Tollens' tests, indicates their importance in providing energy and supporting metabolic functions. Carbohydrates may also have prebiotic effects that promote gut health, further enhancing the therapeutic potential of the extract (Li et al., 2024).

Coumarins were identified by the NaOH test. Coumarins' outstanding compatibility with biological systems (Akumu et al., 2021) promote their anticoagulant, anti-inflammatory, and antimicrobial properties. These compounds are useful in managing blood clotting disorders and infections, adding to the extract's therapeutic value (Zeki & Mustafa, 2024).

Finally, anthocyanins, detected by the Acid test, are associated with antioxidant activity and protection against oxidative stress (Zaa et al., 2023). They support cardiovascular health and cognitive function, demonstrating the wide-ranging benefits of these compounds in promoting overall health (Ellis et al., 2024). The diverse array of phytochemicals present in the aqueous root extract of *Tithonia diversifolia* suggests a broad spectrum of therapeutic potentials. These compounds collectively offer significant health benefits and underscore the potential of the root extract in developing novel medicinal agents.

Conclusion

The qualitative phytochemical analysis of the aqueous root extract of *T. diversifolia* revealed the presence of alkaloids, flavonoids, tannins, saponins, terpenoids, phenols, carbohydrates, anthocyanins, and coumarins, indicating its potential medicinal value. These phytochemical constituents contribute to the diverse pharmacological properties of *T. diversifolia* and warrant further investigation for their therapeutic potential.

Recommendations

Given the comprehensive phytochemical profile of the aqueous root extract of *Tithonia diversifolia*, it is recommended that further in-depth studies be conducted to explore and validate the therapeutic potentials of this plant. The presence of various bioactive compounds, such as alkaloids, flavonoids, tannins, saponins, terpenoids, phenols, carbohydrates, coumarins, and anthocyanins, suggests that this extract could serve as a rich source of natural medicinal agents.

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Conflict of Interest

The authors declare no conflict of interest.

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