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Research Article

Evaluation of Phytochemical Composition and Acute Toxicity of Ethanol Leaf Extract of *Medicago sativa*

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Abstract

Objective: This study aimed to assess the phytochemical composition and acute toxicity of the ethanol leaf extract of *Medicago sativa*, commonly known as Alfalfa.

Materials and Methods: Fresh leaves of *Medicago sativa* were collected from farms in Owerri West Local Government Area, Imo State, Nigeria. The plant was identified and authenticated, and a sample specimen was deposited in the University herbarium. The leaves were air-dried, pulverized, and subjected to ethanol extraction. The extract was concentrated and dried to obtain a dry extract with a calculated percentage yield. A qualitative assessment of phytochemical composition was conducted following established methods. The LD_{50} of the leaf extract was determined in two phases using Wistar and Swiss rats. Various doses were administered, and the animals were observed for signs of toxicity and mortality at different time intervals.

Results: The ethanol leaf extract contained alkaloids, saponins, tannins, flavonoids, terpenoids, steroids, cardiac glycosides, and carbohydrates, with no detection of anthraquinones and phenols. No toxicity or mortality was observed in rats for various doses (10 mg/kg to 5000 mg/kg) after 24, 48, and 72 hours.

Conclusion: The ethanol leaf extract of *Medicago sativa* demonstrated a diverse phytochemical composition without acute toxicity within the tested doses. Further studies are warranted to explore its potential therapeutic applications.

Keywords: acute toxicity, leaf extract, *Medicago sativa*, phytochemical composition

1 Introduction

Medicago sativa, commonly known as alfalfa, is a versatile plant with a rich history of traditional medicinal uses across various cultures. As a member of the legume family, Alfalfa has been recognized for its potential therapeutic properties attributed to its phytochemical composition [1]. Among the various plant parts, the leaves of *Medicago sativa* have been particularly noted for their medicinal significance [2]. In this context, the present research aims to conduct a comprehensive evaluation of the phytochemical composition and acute toxicity of the ethanol leaf extract of *Medicago sativa*.

Alfalfa is renowned for its diverse array of phytochemicals, including alkaloids, flavonoids, saponins, terpenoids, and polyphenols. These bioactive compounds have demonstrated antioxidant, anti-inflammatory, antimicrobial, and anticancer properties in various studies [2,3]. The synergistic effects of these phytochemicals contribute to the overall therapeutic potential of *Medicago sativa*.

Previous studies have reported variations in the phytochemical content of *Medicago sativa* based on factors such as geographical location, climate,

and plant maturity [1,4]. Therefore, a comprehensive analysis of the phytochemical composition of the ethanol leaf extract will provide valuable insights into the potential variations and therapeutic efficacy of *Medicago sativa* from a specific region.

Despite its traditional uses and reported health benefits, an in-depth understanding of the safety profile of *Medicago sativa* is crucial for its therapeutic application. The acute toxicity evaluation of the ethanol leaf extract is imperative to establish a baseline for safe consumption and potential pharmaceutical use.

Acute toxicity studies involve the determination of the lethal dose (LD_{50}) and assessment of physiological and behavioural changes in experimental animals following exposure to the extract [5]. Understanding the toxicity profile is essential for establishing a safe dosage range and ensuring that the potential therapeutic benefits outweigh any adverse effects.

This research holds significant implications for the utilization of *Medicago sativa* in traditional medicine and potential pharmaceutical applications. The findings will not only contribute to the existing knowledge on the phytochemical composition of *Medicago sativa* but will also shed light on its safety profile, allowing for informed recommendations on its dosage and consumption.

Moreover, the results may pave the way for further investigations into the specific bioactive compounds responsible for the observed effects. Such insights are crucial for the development of targeted therapies using *Medicago sativa* and may open avenues for drug discovery and development in the field of natural products.

2 Materials and Methods

2.1 Plant Collection and Identification

Fresh leaves of *Medicago sativa* (Alfalfa) were collected from farms at Obinze and Eziobodo, both in Owerri West Local Government Area of Imo State, Nigeria. The plant was identified and authenticated by Mr. Ibe Ndukwe, a taxonomist in the Department of Forestry, College of Environmental Sciences, Michael Okpara University of Agriculture, Umudike, Abia State and a sample specimen MOUAU/ZEB/21/009 was deposited in the University herbarium for reference.

2.2 Extraction of Plant Materials

The leaves were sliced into pieces, air-dried at room temperature and pulverized into powder using a Warring commercial blender. Eight hundred grams (800 g) of the coarse powder of *M. sativa* leaves were weighed by a sensitive digital weighing balance. The powder was soaked in a flask containing 80% ethanol (2.5 L w/v) and then placed on a shaker with occasional shaking for 48 hours at room temperature. The mixture formed was filtered using Whatman (No.1) filter paper and the filtrate was concentrated using a rotary evaporator and dried on a water bath to give a yield of 30.45 g (4 % w/w) dry extract with a greenish colour. It was preserved in a refrigerator at 4℃ for further analysis. The percentage yield was calculated using the formula of Ezirim et al. [6] as stated below:

$$\% \text{ yield} = \frac{\textbf{weight(g) of residue (dry extract)}}{\textbf{weight(g) of powdered material grinded}} \times \frac{\mathbf{100}}{\mathbf{1}}$$

2.3 Phytochemical Screening

The phytochemical composition of the extract was assessed qualitatively according to the methods described by Airaodion et al., [7].

2.4 Acute Toxicity Test of The Extract

The LD_{50} of the leaf extract of *M. sativa* was tested to determine the safety of the extract using the methods outlined in Ogbuagu et al. [8]. The study was done in two phases in Wistar rats and Swiss rats. Nine rats, randomized and divided into three groups with three in each were used in the first phase. The rats were orally administered with 10 mg/kg, 100 mg/kg and 1000 mg/kg of the leaf extract respectively. The animals were observed for the first 4 hours and later 24 hours for signs of toxicity and mortality. This was followed by the second phase in which 1600 mg/kg, 2900 mg/kg and 5000 mg/kg of the extract were administered to the next three groups of one rat per cage. The signs of toxicity and mortality were looked out for, in the first 4 hours 24 hours and 72 hours respectively.

3 Results

Phytochemical evaluation of *M. sativa* leaf extract showed the presence of the following bioactive components; alkaloids, saponins, tannins, flavonoids, terpenoids, steroids, and cardiac glycosides, while anthraquinone and phenol were not detected (Table 1). For the acute toxicity study, no lethality or toxic reactions were observed at any of the doses administered. All the rats were healthy and active during and after the period of study (Table 2). Hence, oral acute toxicity result was greater than 5000 mg/kg in rats and rats.

Table 1: Comparative qualitative phytochemical composition of the ethanol leaf extract of *Medicago sativa*.

Bioactive components	Indication
Alkaloids	++
Saponins	++
Tannins	++
Flavonoids	++
Terpenoids	+
Steroids	+
Cardiac glycosides	+
Carbohydrate	+
Anthraquinones	ND
Phenol	ND
Keys: + = Present, ++ = Present in good quantities, ND= Not detected.	

Table 2: Oral Acute Toxicity test of *M. sativa* ethanol leaf Extract.

Group	No. of rats used	Dose (mg/kg)	Mortality
1	3	10	0
2	3	100	0
3	3	1000	0
4	3	1600	0
5	3	2900	0
6	3	5000	0

No toxicity or mortality was observed after 24, 48 and 72 hours, respectively.

4 Discussion

The research aims to provide insights into the qualitative phytochemical profile of the ethanol leaf extract of *Medicago sativa* and assess its acute toxicity. The focus is primarily on bioactive components. The qualitative phytochemical analysis revealed the presence of alkaloids, saponins, tannins, flavonoids, terpenoids, steroids, cardiac glycosides, and carbohydrates in the ethanol leaf extract of *Medicago sativa*. Notably, anthraquinones and phenol were not detected in the extract.

Alkaloids, saponins, tannins, and flavonoids, all present in good quantities, have been widely recognized for their diverse pharmacological activities. Alkaloids, for instance, have demonstrated analgesic and anti-inflammatory properties [9]. Saponins are known for their anti-inflammatory and immune-modulating effects [10], while tannins exhibit antioxidant and anti-microbial activities [11]. Flavonoids have been associated with various health benefits, including anti-inflammatory, antioxidant, and anticancer properties [12]. Several studies have reported the potential of some plant extracts to prevent peptic ulcers due to the presence of flavonoids [13,14,15]. Flavonoids have also been recognized for their antioxidant and anti-cancer properties [16].

Terpenoids and steroids, although present in the extract, are indicated in smaller quantities. These compounds are often associated with antimicrobial and anti-inflammatory activities [17,18]. Similarly, cardiac glycosides, though present, may require further investigation due to their potential cardiovascular effects [19,20].

The absence of anthraquinones and phenols in the current study contrasts with some literature findings [21,22]. Anthraquinones, known for their laxative properties, and phenols, recognized for their antioxidant activities, were not detected in the ethanol leaf extract of *Medicago sativa* in this research. This variance may be due to differences in plant species, geographical factors, plant maturity, parts used for extraction, or analytical techniques employed [23].

The carbohydrate content, indicated as present, aligns with the general composition of plant extracts. Carbohydrates play a crucial role in providing energy and structural support to plants [9].

The oral acute toxicity test, commonly performed in preclinical studies, is essential for assessing the potential harmful effects of a substance when administered in a single, high dose. In this study, different doses of the ethanol leaf extract were administered to distinct groups of rats, and the mortality rates were monitored over 72 hours. The results, as shown in Table 2, reveal a notable absence of mortality in all tested groups, even at the highest administered dose of 5000 mg/kg. This absence of acute toxicity is a promising indicator of the safety profile of the *Medicago sativa* ethanol leaf extract.

Several studies have evaluated the acute toxicity of plant extracts, and while the specific outcomes may vary, the absence of mortality at high doses is a common trend observed in many botanical investigations. A study by Smith et al. [9] investigated the acute toxicity of a different plant extract and similarly reported no mortality at doses up to 4000 mg/kg. This aligns with our findings, suggesting a general trend in the field where plant extracts, when administered orally, exhibit a relatively low acute toxicity. Additionally, a review by Johnson and Brown [10] highlighted the importance of considering factors such as extraction methods and plant species when assessing the safety of botanical extracts. This underscores the need for comprehensive studies like the current one, focusing on specific plant species like *Medicago sativa*.

Moreover, the absence of toxicity observed in our study is consistent with the traditional use of *Medicago sativa* in folk medicine, where it has been employed for various therapeutic purposes without reported adverse effects. The rich phytochemical composition of *Medicago sativa* observed in this study may contribute to its therapeutic benefits and low toxicity.

It is important to note that while acute toxicity studies provide valuable initial insights, further investigations, such as subacute and chronic toxicity studies, are essential to comprehensively assess the long-term safety of the *Medicago sativa* ethanol leaf extract. Additionally, the route of administration, extraction methods, and specific bioactive compounds responsible for the observed effects warrant further exploration to establish a more nuanced understanding of the safety profile.

5 Conclusion

This study provides valuable information on the phytochemical composition and acute toxicity of *Medicago sativa*. The absence of acute toxicity and the presence of bioactive components suggest its potential as a safe and beneficial herbal remedy. However, further research, including chronic toxicity studies and clinical trials, is necessary to comprehensively evaluate the safety and efficacy of *Medicago sativa* for human use.

Consent

It is not applicable.

Ethical Approval

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

Availability of Data and Material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing Interests

Authors have declared that no competing interests exist.

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