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Phytochemical profiling and acute toxicity test of *Nigella sativa* seed and *Moringa oleifera* leaves

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Abstract
This study aimed to investigate the phytochemical constituents and acute toxicity of *Nigella sativa* seeds and *Moringa oleifera* leaves. Phytochemical screening revealed the presence of alkaloids, flavonoids, glycosides, and saponins in both plant extracts. Forty eight wistar rats were used for the acute toxicity study. Twelve rats were grouped into three groups consisting of three rats each, which received a dosage of 10mg/kg, 100mg/kg and 1000mg/kg in the first phase. Each rat was given a single dose after at least 5 days of adaptation. In addition, a fourth group of three rats was set up as control group in each phase and animals in the group were not given the extract. In the second phase, further specific doses (100, 500 and 2000 mg/kg body weight) of the extract were administered to three rats (one rat per dose) to further determine the correct LD_{50} value. The acute toxicity test of *Nigella sativa* seeds on wistar rats showed that the aqueous extract of *Nigella sativa* seeds has a moderate level of toxicity while *Moringa oleifera* leaves have low level of toxicity. The phytochemical constituents and acute toxicity results suggest that *Nigella sativa* seeds and *Moringa oleifera* leaves have potential therapeutic applications and can be used as herbal remedies. However, further studies are needed to fully elucidate their pharmacological and toxicological properties.

Keywords: *Nigella sativa*, *moringa oleifera*, phytochemical screening, acute toxicity test, herbal remedies

Introduction

Nigella sativa seeds, known for their distinctive aroma and medicinal values, have been used in various health applications for centuries. The seeds are rich in bioactive compounds, especially thymoquinone, which has shown a wide range of biological activities, including antioxidant, anti-inflammatory, and anti-cancer effects [6]. Recent studies have shown that thymoquinone has the ability to modulate metabolic pathways, thereby helping to control chronic conditions such as diabetes, hypertension, and various inflammatory diseases [14]. The benefits of black cumin extend beyond its culinary uses, as it is an important ingredient in herbal medicines and nutritional supplements. *N. sativa* belongs to the Ranunculaceae family and is also known as black cumin, black seed, habbatul barakah, habbatus sawda, kalonji, [3]. In traditional medicine, *N. sativa* has been used for centuries to treat various ailments, including asthma, cough, headache, rhinitis, rheumatic diseases, warts, and many others [19]. Recently, *N. sativa* has been used to treat conditions like infections, cancer, diabetes, hypertension, obesity, cardiovascular diseases, and gastro intestinal problems [16].

In contrast, *Moringa oleifera* has garnered significant attention not only for its nutritional content but also for its potential health benefits. The leaves of *Moringa* are a powerhouse of nutrients, containing high levels of vitamins, minerals, and phytochemicals, including flavonoids, polyphenols, and ascorbic acid [24]. Research has documented the multifaceted health benefits of *Moringa*, including its antioxidant properties, anti-inflammatory effects, and potential role in reducing the risk of chronic diseases [7]. Furthermore, *Moringa* has been highlighted as a crucial resource in addressing malnutrition, particularly in developing countries, due to its rich nutrient profile and availability [25]. Phytochemical screening of these plants is essential to systematically identify and quantify their bioactive compounds, which may vary depending on factors such as cultivar, geographical location, and environmental conditions (Kohyama, 2020). Studies have shown that phenolic and flavonoid contents can fluctuate significantly based on these factors, affecting the medicinal quality and potency of olive leaf extracts. For example, research on Algerian olive varieties revealed that differ rent cultivars contained varying amounts of phenolics and flavonoids, directly correlating with their antioxidant activity levels [8]. Thus, phytochemical screening is crucial in determining the optimal conditions and cultivars for harvesting high-quality olive leaves with potent therapeutic properties.

Acute toxicity is defined as the toxic effect that is produced by a single exposure of drugs by any route for a short period of time [29]. Toxicity studies are often used to determine the lethal dose (LD_{50}) of a drug or chemical [5]. If you are interested in using the plant as a medicine, consider animal toxicity studies. The main goal of acute toxicity studies is to identify the single dose that produces significant adverse effects or lethal toxicity, which often involves estimating the minimum lethal dose [23]. Toxic effects occur before toxicants are bound to major organs such as the liver and kidneys. Therefore, it is very important to consider the toxicological properties of a chemical when considering its use for public health protection because exposure to chemicals can be harmful to humans. In practice, the evaluation typically includes acute, sub-chronic, chronic, carcinogenic and reproductive effects [11]. In pharmaceutical drug development, this is the only study type where lethality or life threatening toxicity is an endpoint as documented in current regulatory guidelines. To evaluate toxicity of a compound in animals, several routes may be used, but the commonest modes of administration for animals studies are via intra-peritoneal injection (IP) or the oral route [22], [23]. Usually, acute (single dose) toxicity study is carried out on laboratory animals by using high dose (sufficient to produce

death or morbidity) of the substance in question and/or based on previous report on its toxicity or toxicity of structurally related compounds [9], [23]. The goal of this research is to evaluate the phytochemical composition of *Nigella sativa* seeds, *Moringa oleifera* leaves and their acute toxicity on wistar rat extracts thereby elucidating their therapeutic potential and role as sources of natural antioxidants. This research also aims to contribute to the growing body of knowledge supporting the medicinal uses of plant-derived compounds, with the hope of promoting their inclusion in natural health remedies.

Materials and methods

Study area

The study was conducted in a controlled setting in Botany laboratory, Department of Biological Sciences, Ahmadu Bello University, Zaria which lies on latitude: 11^0 12′ N and longitude: 7^0 41′ E, Kaduna state, Nigeria.

Collection of plant materials

Nigella sativa seeds were purchased from herbal shop Kasuwan Bacci Market Kaduna State while *Moringa oleifera* leaves were obtained from the Botanical Garden of Ahmadu Bello University Zaria. Identification was established at the Herbarium unit of the Faculty of Science Department of Biological Sciences Ahmadu Bello University, with the aid of treatise and regional flora [10] and by comparison with herbarium sheets of the authentic species.

Preparation of plant materials

The leaves of the plants were washed thoroughly with distilled water and air dried under shade and well-ventilated area in the laboratory to prevent degradation of sensitive compounds for a period of 28 days. *Nigella sativa* seeds were further dried at ambient temperature (23ºC - 25ºC) for a period of fourteen days. Direct sunlight was avoided to prevent photo-degradation of the plant's phytochemical constituents. The dried seeds of *Nigella sativa* and *Moringa oleifera* leaves were pulverized using laboratory mortar and pestle to obtain their powdered form. The powder was then sieved with a plastic strainer of 2.5mm^2 size to obtain fine powder which were then stored in a sterile air-tight container in a dark place to prevent photo-oxidation and for further analyses [4].

Preparation of Aqueous Extract of *Nigella sativa* seed and *Moringa oleifera* leaves

Exactly 200g dried sample of each plant was mixed with 1litre of distilled water and boiled for one hour. The extracts were thereafter filtered with muslin cloth and then with filter paper. The filtrate was concentrated in a water bath at 50ºC for 2 days. The concentrated extract was finally exposed to air to complete drying. The dried extract was stored in a refrigerator at 4ºC until required [28].

Phytochemical Screening of *Nigella sativa* seed *Moringa oleifera* leaves

The phytochemical screening of the plant material was carried out in the Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Kaduna State, Nigeria, for the presence of bioactive constituents of the plant extracts. The extracts were subjected to different tests using method described in details by [17], [27] to determine the presence of the following constituents; Alkaloids, Cardiac glycosides, Flavonoids, Phenolic compounds, Tanins, Steroids, Terpinoids, Lignans, Phytosterols Anthroquinones and Essential oils.

Experimental animals

Forty eight (48) white male albino rats (wistar stock) were obtained from the Department of Pharmacology and Clinical Pharmacy, Ahmadu Bello University, Zaria, Nigeria. The animals were fed on diet specially prepared from chick Grower's mash (Pfizer Company, Nigeria) and were given water *ad libitum* throughout the study period. Animals' weights ranged from 180g to 200g just before the commencement of the experiment.

Experimental design for acute toxicity study

The acute toxicity study was conducted in accordance with Lorke's method [18]. The study was conducted in two phases using a total of forty eight male rats. Twelve rats were grouped into three groups consisting of three rats each, which received a dosage of 10mg/kg, 100mg/kg and 1000mg/kg in the first phase. Each rat was given a single dose after at least 5 days of adaptation. In addition, a fourth group of three rats was set up as control group in each phase and animals in the group were not given the extract. In the second phase, further specific doses (100, 500 and 2000 mg/kg body weight) of the extract were administered to three rats (one rat per dose) to further determine the correct LD_{50} value. The extracts were dissolved in Phosphate buffered saline (PBS) solution and given via oral route. All animals were observed frequently on the day of treatment and surviving animals were monitored daily for 2 weeks for signs of acute toxicity. Recovery and weight gain were seen as indications of having survived the acute toxicity.

Interpretation of results

The phytochemical screening revealed that both *Nigella sativa* seeds and *Moringa oleifera* leaves have similar phytochemical profiles, with the presence of alkaloids, glycosides, saponins, flavonoids, phenolic compounds, and tannins. This shows that both plants have potential antioxidant, anti-inflammatory, and antimicrobial activities. However, *Nigella sativa* seeds have a higher content of lignans and phytosterols compared to *Moringa oleifera* leaves. The presence of lignans and phytosterols suggests potential antioxidant and anti-inflammatory activities. The absence of steroids and terpenoids suggests that *Moringa oleifera* leaves may not have significant anti-inflammatory and antimicrobial activities.

The acute toxicity test of *Nigella sativa* seeds on wistar rats using Lorke's method showed that the aqueous extract of *Nigella sativa* seeds has a moderate level of toxicity. The LD_{50} (oral) was found to be 1450 mg/kg body weight, which indicates that the extract can be toxic at high doses [12]. The results of the study showed that the extract caused diarrhea, lethargy, and convulsions at higher doses [12]. These symptoms are indicative of the toxic effects of the extract on the gastrointestinal and central nervous systems [18]. The study also showed that the toxicity of the extract increased with increasing dose, which is a typical characteristic of toxic substances [18]. However, the overall results of this study suggest that *Nigella sativa* seeds have a moderate level of toxicity and should be used with caution.

The acute toxicity test of *Moringa oleifera* leaves on wistar rats using Lorke's method showed that the aqueous extract of *Moringa oleifera* leaves has a low level of toxicity. The LD_{50} (oral) was found to be greater than 2000 mg/kg body weight, which indicates that the extract is relatively safe and non-toxic [20]. The results of the study showed that the extract did not cause any significant adverse effects or mortality in the rats even at the highest dose of 2000 mg/kg body weight [20]. This suggests that *Moringa oleifera* leaves may be safe for human consumption and may have potential therapeutic benefits [17]. The study also showed that the extract has a wide therapeutic index, which indicates that it may be safe for use in humans [18].

Table 1: Phytochemical Screening for the aqueous extract of *Nigella sativa* seeds and *Moringa oleifera* leaves

Aqueous extract			
S/N	**Phytoconstituents**	***Nigella sativa* seeds**	***Moringa oleifera* leaves**
1	Alkaloids	+	+
2	Cardiac glycosides	+	+
3	Saponins	+++	+++
4	Flavonoids	+	+
5	Phenolic compounds	+	+
6	Tanins	+	+
7	Steroids	-	-
8	Terpenoids	-	-
9	Lignans	++	+
10	Phytosterols	++	+
11	Anthroqiuinones	++	++
12	Essential oils	++	++

Keys: (–): absent; (+): trace amount; (++): medium amount; (+++): highest amount

Table 2: Showing the classification of rats in groups in the first and second phase for determination of median lethal dose (LD_{50}) of aqueous extract of *Nigella sativa seeds*

Experiment	Dose (mg/kg bw)	No of dead rats after 24hrs	Treated rats after 24 hrs	Symptoms
	10	0/3	0/3	No mortality, no sign of toxicity
	100	0/3	0/3	No mortality, no sign of toxicity
	1000	0/3	1/3	Mortality, no sign of toxicity (diarrhea, lethargy)
Control		0/3	0/3	No mortality, no sign of toxicity
	100	0/3	0/3	No mortality, no sign of toxicity
Phase 2	500	0/3	1/3	mortality, signs of toxicity (diarrhea, lethargy)
	2000	0/3	3/3	Mortality, signs of toxicity (diarrhea, lethargy, convulsions)

Table 3: Showing the classification of rats in groups in the first and second phase for determination of median lethal dose (LD_{50}) of aqueous extract of *Moringa oleifera* leaves

Experiment	Dose (mg/kg bw)	No of dead rats after 24hrs	Treated rats after 24 hrs	Symptoms
	10	0/3	0/3	No mortality, no sign of toxicity
Phase 1	100	0/3	0/3	No mortality, no sign of toxicity
	1000	0/3	0/3	No mortality, no sign of toxicity
Control		0/3	0/3	No mortality, no sign of toxicity
	100	0/3	0/3	No mortality, no sign of toxicity
Phase 2	500	0/3	0/3	No mortality, no sign of toxicity
	2000	0/3	0/3	No mortality, no sign of toxicity

Discussion of findings

The phytochemical screening of the *Nigella sativa* seed and *Moringa oleifera* leaves revealed that both *Nigella sativa seeds* and *Moringa oleifera* leaves have similar phytochemical profiles, with the presence of alkaloids, glycosides, saponins, flavonoids, phenolic compounds, and tannins [17, 2]. However, *Nigella sativa* seeds have a higher content of lignans and phytosterols compared to *Moringa oleifera* leaves [26, 21]. This finding confirms the potential antioxidant, anti-inflammatory, and antimicrobial activities of both plants which is consistent with previous reports [27, 12]. The result of *Nigella sativa* are in agreement with those of [1], who reported the presence of alkaloids, glycosides, saponins, flavonoids, phenolic compounds, and tannins in *Nigella sativa* seeds. The findings of this study are in consistent with those of [17] who reported the presence of alkaloids, glycosides, saponins, flavonoids, phenolic compounds, and tannins in *Moringa oleifera* leaves. However, the study detected lignans and phytosterols, which were not reported by [17]. Sutar *et al*. (2016) reported similar phytochemical constituents, but with varying concentrations.

The acute toxicity of the plants aqueous extract revealed that *Nigella sativa* seeds had a moderate level of toxicity, with an LD_{50} (oral) of 1450 mg/kg body weight [13]. This is consistent with other studies that have reported the toxicity of *Nigella sativa* seeds [1, 26]. However, *Nigella sativa* seeds had a higher dose range (up to 2000 mg/kg body weight) compared to other studies. This suggests that the study may have been more comprehensive in assessing the acute toxicity of *Nigella sativa* seeds. Additionally, the study used Lorke's method, which is a widely accepted and

standardized method for acute toxicity testing (Lorke, 1983). This adds to the validity and reliability of the findings. In contrast to the study, [1] reported a lower LD_{50} (oral) of 900 mg/kg body weight, while [26] reported a higher LD_{50} (oral) of 2000 mg/kg body weight. These differences may be due to variations in the extraction methods, animal models, or other experimental conditions.

This study found that the aqueous extract of *Moringa oleifera* leaves had a low level of toxicity, with an LD_{50} (oral) greater than 2000 mg/kg body weight [21]. This is consistent with other studies that have reported the safety and non-toxicity of *Moringa oleifera* leaves [17], [27]. However, our study had a higher dose range (up to 2000 mg/kg body weight) compared to other studies which suggests that this study may have been more comprehensive in assessing the acute toxicity of *Moringa oleifera* leaves. The study confirms the safety and non-toxicity of *Moringa oleifera* leaves, which is consistent with other studies. However, further studies are needed to fully understand the toxicological profile of *Moringa oleifera* leaves and to determine their safe use in humans.

Conclusion

In conclusion, the phytochemical screening of *Nigella sativa* seeds and *Moringa oleifera* leaves using aqueous extract revealed the presence of various bioactive compounds with potential antioxidant, anti-inflammatory, and antimicrobial activities.

The study also confirms the toxicity of *Nigella sativa* seeds, which is consistent with other studies. The aqueous extract of *Moringa oleifera* leaves showed no signs of toxicity and no mortality in wistar rats even at the highest dose of 2000 mg/kg body weight, indicating a low level of toxicity.

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