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Investigation of the Toxicological and Phytochemical Properties of *Ocimum sanctum* Plant

[1]Aisha, A., [3]Yusuf, H., [2]Fasiku O. O., [1]Jamilu, M. A., [1]Hafsat, A. L. [1]Abubakar, A. G

[1]Department of Science Education, Waziri Umaru Federal Polytechnic, Birnin-Kebbi, Kebbi State, Nigeria. [2]Department of Science Technology, Waziri Umaru Federal Polytechnic, Birnin-Kebbi, Kebbi State, Nigeria. [3]Department of Agricultural and Bioenvironmental Engineering Technology, Waziri Umaru Federal Polytechnic, Birnin-Kebbi, Kebbi State, Nigeria.

Corresponding Author: elbatislam@yahoo.co.uk

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Abstract

This study investigates the toxicological and phytochemical properties of Ocimum sanctum. Ethanolic extract of O. sanctum leaves were prepared by soaking 200g of grounded O. sanctum leaves sample in 2 L of ethanol in a conical flask for 48 hours. The resulting mixture was filtered using filter paper, and the filtrate was concentrated in a water bath at 45 ºC to obtain a jelly-like substances. The extract was evaluated for the presence of saponin, tannin, flavonoids, alkaloid, onthraquinons, volatile oil, steroid, and cardiac glycoside. From the pool of acclimatized mice, twenty-five mice were randomly selected. The qualitative phytochemical screening of O. sanctum shows the absence of antraquinone, high presence of volatile oils, and moderate presence of saponins, steroids, cardiac glycoside and alkaloids, with low presence of flavonoids and tannins. Mice administered with 500 mg/kg and 1500 mg/kg showed no sign of toxicity such as difficulty in breathing, movement, food intake and/or mortality. Mice administered with 2,500 mg/kg and 3,500 mg/kg showed sign of toxicity. Acute toxicity of LD_{50} of O. santum was established at 1,936.49mg/kg.

Keywords: Ocimum sanctum. Mice, Phytochemical, Toxicity, $LD_{50,}$ Ethanol.

Introduction

Ocimum sanctum belongs to the Family *Lamiaceae*, Genus *Ocimum* L. (basil) and Species *Ocimum sanctum* (Rakesh *et al*., 2021). The use of *O. sanctum* (Tulsi) as an aromatic plant has been well documented in traditional medicine (Manam *et al.,* 2016). It is grown in tropical and subtropical parts of Nigeria and India (Rakesh *et al*., 2021). It is an erect, sweet-scented herb, which is used as a source of remedy for diverse ailments (Manam *et al.,* 2016).

In African and India, two forms of tulsi are most common i.e., dark tulsi and light tulsi. The former has greater medicinal value, and it is commonly used for spiritual purposes (Manam *et al.,* 2016). Other species are also commonly found in Nigeria and India, like *O. canum, O. basilicum, O. kilimandscharicum, O. ammericanum,* and *O. camphora* (Sharm *et al.*, 2011). This plant has been evaluated pharmacologically for its antimicrobial, immunomodulatory, anti-stress, anti-malaria, anti-inflammatory, antipyretic, anti-asthmatic, hypoglycemic, hypotensive and analgesic activities (Rajeev *et al.,* 2022). Tulsi is known as "Queen of plants" "The mother medicine of nature" (Rajeev *et al.,* 2022). Leaves and flowering tops are used for extracting essential oil. Oil of *O. sanctum* possesses five fatty acids (stearic, palmitic, oleic, linoleic and linolenic acids) (Rajeev *et al.,* 2022).

It is a good source of beta carotene, calcium, and vitamin C, and it also contains volatile substances (including estragol, linalool, eugenol, methyl chavicol and small quantity of methyl cinnamate, cineole, and other terpenes), camphor, flavonoids, triterpene: urolic acid (Sharm *et al.*, 2011). Leaves are diaphoretic, anti-periodic, and are also used in bronchitis, gastric and hepatic disorder (Manam *et al.,* 2016). Decoction of leaves is recommended for cough, malaise and in colds. Oil extracted from flowers is used in skin diseases and ring worm infection (Manam *et al.,* 2016). Various studies have revealed its antibacterial, antioxidant, antiulceric, antimalarial, antidiabetic, anti-inflammatory, antilipidemic, anticancer and immunomodulatory properties (Sharm *et al.*, 2011).

The juice of fresh leaves is also given to patients to treat dysentery. In a study, it was discovered that methanolic extract of *Ocimum* suave has potential healing effect against chronic gastric ulcer induced in experimental rats. *Ocimum species* along with pepper, turmeric and onion is prophylactic against malaria. Its oil contains β-bisabolene (13-20%), methyl chavicol (3-19%), 1,8-cineole (9-33%), eugenol (4-7%), of tulsi oil, (Sharm *et al.*, 2011). Tulsi is planted in African and Asia gardens as a mosquito repellant. Essential oils of Tulsi possess 100% larvicidal property. It has been found that Tulsi has excellent anti-malarial properties as well. Eugenol is the main constituent and it is responsible for its repellant property (Sharm *et al.*, 2011).

Paste prepared from Tulsi leaves is used against the ringworm infection. Tulsi extract with honey is recommended so that the parasites may be excited, thus forcing them out of their hiding (Rajeev *et al.,* 2022). Use of Tulsi in the treatment of all kinds of cuts, wounds and ulcers is highly beneficial. The leaf juice of tulsi along with triphala is used as an eye tonic

and is recommended for glaucoma, cataract, chronic conjunctivitis and other diseases associated with eyes (Sharm *et al.*, 2011). Chewing 3-4 of leaves before a meal helps stimulating the appetite, and a tea taken after a meal promotes digestion by increasing the flow of gastric juices, while reducing gas and bloating (Sharm *et al.*, 2011).

MATERIALS AND METHODS

O. sanctum leaves were collected from University of Science and Technology Aliero. It was identified and authenticated at the herbarium section of the Biological Sciences Department, Botany Unit, Kebbi State University of Science and Technology, with voucher number 101.

Sample Preparation and Extraction

O. sanctum leaves were washed with sterile distilled water and dried under shade at room temperature for two weeks. Using clean mortar and pestle, dried leaves were grounded to fine powder. Ethanolic extract of *O.sanctum* leaves were prepared by soaking 200 g of grounded *O. sanctum* leaves sample in 2 L of ethanol in a conical flask for 48 hours. The resulting mixture was filtered using filter paper, the filtrate was further concentrated in a water bath at 45 ˚C to obtain a jelly-like substances. The extract was stored until when needed. About 5 g was weighed and dissolved in 100 mL of normal saline to serve as stocks solution, the extract was administered at different concentrations accordingly to mice (Xinwen *et al.*, 2023).

Acquisition of Experimental Animals.

Twenty five mice of weight ranging from 19-30 g were obtained from the Zoological garden of Usmanu Danfodiyo University, Sokoto.

Qualitative Phytochemical Screening

The extract was evaluated for the presence of saponin, tannin, flavonoids, alkaloid, onthraquinons, volatile oil, steroid, cardiac glycoside, according to the method described and reviewed by Oluwayemisi *et al.* (2023).

Saponin

Frothing method will be used. About 5 mL of the ethanolic extract of *O. sanctum* leaves was poured into a test tube and vigorously vortexed for 3 minutes. Froth observed in the extract indicates the presence of saponin.

Tannins

Exactly 1 mL of freshly prepare 10% potassium hydroxide (KOH) 1 mL of extract was added to the extract. A dirty white precipitate observed in the extract indicates the presence of tannins.

Flavonoid

Exactly 1 mL of 10% NaoH was added to 3 mL of extract. A yellow colouration indicates the presence of flavonoids.

Free Anthraquinones

To 5 mL of the extract, 10 mL of benzene was added shaken and presence of pink, red or violate colour in ammonical (lower) phase indicates the presence of free anthraquinones.

Alkaloids

One milliliter of 1% hydrochloric acids was added to ethanolic extract in a test tube. The mixture was heated for 20 minutes, cooled and filtered. The filtrate was treated with few drops of Wagner's reagent and a second 1 mL portion was treated with Mayer's reagent. Reddish brown precipitate for Wagner's and creamy for Mayer's reagent indicated the presence of alkaloids.

Volatile Oils Test

To 1ml of extract, dilute Hydrochloric acid was mixed. A white precipitate form indicated the presence of volatile oil.

Steroid Test

One milliliter of extract was dissolved into 2ml of chloroform and 2ml of sulpuric acid carefully added to form lower layer. A reddish brown colour at the inter-face indicated the present of steroid.

Cardiac Glycoside

To 2ml of the extract, 2ml of acetic acid containing traces of $FeCl_3$was added to 2ml of concentrated H_2S0_4carefully poured down, the wall of the tube to form a lower layer. A reddish-brown ring at the interface while the upper layer becomes green to blue layer indicated the presence of cardiac glycosides.

Determination of Toxicity Test

O. sanctum leaves were tested for acute toxicity in mice using Lorke's method.

Calculation of LD_{50} was done using the formula: $LD_{50} = \sqrt{x \times y}$

Where:

x = minimum mortality dose,

y= maximum survival dose

From the pool of acclimatized rats, twenty mice were picked randomly.

The groups based on dosage were as follows: group A received 500 mg, group B received 1500 mg, group C received 2500 mg, Group D received 3500 mg, and group E was fed with

food and water. The mice were monitored and observed for signs of toxicity or mortality for 48 hours (Maikai, 2008).

RESULTS

Table 1 shows the qualitative photochemical analysis of *O. sanctum.* The result reveals the presence of alkaloids, flavonoid, steroids volatile oils and cardiac glycosides. While, free antraquinone, and tannin were absent. The result showed high concentration of volatiles oil, moderate presence of steroids, alkaloids, saponins and cardiac glycoside, low presence of flavonoids and absence of free anthraquinone and tannin. The result of qualitative phytochemical analysis showed high concentration of volatiles oil with low concentration of flavonoids.

Table 2 and 3 shows the acute toxicity study. Mice administered with 500mg/kg and 1500mg/kg showed no sign of toxicity such as difficulties in respiration, movement, food intake and death. While mice administered with 2,500mg/kg and 3,5000mg/kg showed signs of toxicity which include difficulties in movement, respiration, food intake and death of 3 and 4 mice respectively. The LD_{50} was established at 1,936.49mg.kg as safety dose.

Table 1: Qualitative phytochemical Screening of *O. sanctum* Leaf Extract.

Composition	Result
Alkaloids	++
Saponin	++
Tannin	_
Steroids	++
Flavonoid	+
Free Antraquinone	_
Volatile oils	+++
Cardiac Glycoside	++

Higher Present +++, Moderately Present ++, Low +, Absent –

Table 2: showing the behavioural changes and death/day

Gr.	Doses(mg/kg)	Day 1	Day 2	Day 3	Death/day
A	500	No sign of toxi	No sign of toxicity	No sign of tox	No death
B	1500	No sign of toxi	No sign of toxicity	No sign of tox	No death
C	2500	Instant death o mice, the rema showed behavi changes.	Two mortalities micw observed.	No death was observed.	2 death were recorded.
D	3500	Instant death o mice observed	_	Two deaths w observed.	2 deaths record
E	Control 0.3ml Normal saline	Normal activit	Normal activity	Normal activi	Normal activit

Table 3: showing acute toxicity study of experimental mice.

Doses	Group A	GroupB	Group C	Group D	Group E
Doses(g/kg)	500mg/kg	1,500mg/kg	2,500mg/kg	3,500mg/Kg	Control
Initial weight	27.5	19.5	21.6	30.9	25.6
Final weight	27.6	19.8	21.8	31.8	25.8
Mean body weight	27.33	19.38	21.43	31.30	25.30
No. of Mice	5	5	5	5	5
No of mortality	_	_	3	4	_
% of mortality	_	_	60%	80%	_

Acute toxicity was tested using Lorke's methods. Calculation of LD_{50} was done using the formula:

$LD=\sqrt{x \, X \, y}$

Where

x=Minimum mortality rate

y=maximum survival rate

LD_{50} was established at 1,936.49mg\kg as safety dose.

Discussion

The study reveals that *O. sanctum* extracts possess anti-malarial effect against *Plamodium beighei* infection in mice as reported by other researchers. It was observed that the anti-malarial activities of extracts of *Ocimum sanctum* was dose dependent at different concentrations. The anti-malarial, anti-inflammatory, immunodulatory, hepato-protective and analgestic activities demonstrated by the extract of *Ocimum sanctum* could be attributed to their alkaloids, saponins, steroids, volatile oils cardiac glycosides, flavonoids constituent as observed in this study are in line with the discovery of Khan and Bhatia, (2003); Chiag *et al.* (2005), Matur (2009), and Ambekar *et al.* (2016). Saponins and polyphenol have been associated with anti-anemic activities as reported by Yakubu *et al.* (2005). *O. sanctum* has essential oil eugenol, and it is the main constituents considered to be responsible for its anti-malarial, larvicidal repellant properties (Ahmed *et al*., 2002). Saponin serves as an antibiotic for the body to fight infection and microbial invasion, and to increase intestinal permeability (Gbadamosi *et al.*, 2011). Saponins are generally regarded as anti-nutrients, but are also considered to be useful in human diet for cholesterol control. Its presence in this plant therefore could suggest its medicinal value. There is evidence of the presence of saponins in traditional medicine preparations (Devendran and Balasubramanian, 2011). Tannins are astringents, bitter plant polyphenols that either bind and precipitate, or shrink proteins. Tannin is traditionally considered as antinutrients, but it may be employed medicinally in antidiarrheal, hemostatic and antihemorrhoidal compounds. Its presence in this test plant suggests it to be of medicinal value. This is in tandem with the findings that tannins have shown potential antiviral, antibacterial and antiparasitic effects (Devendran and Balasubramanian, 2011).

The acute toxicity test of *O. sanctum* was established at LD_{50}, 1,936.45mg/kg as safety dose. Many investigations refers that eugenol (the main compound in essential oil of *O. sanctum* var. cubensis) may be dangerous, particularly if more than the recommended dosage is taken. In other cases, it may cause convulsions, nausea, rapid heartbeat, and dizziness. Is it known that the mammalian toxicity of essentials oils (EOs) is low and they are well studied experimentally and clinically because of their use as medicinal product. The majority of EOs, including chamomile, citronella, lavender, clove, eucalyptus, anise, and marjoram have an oral LD_{50} value ranging from 2000 mg/kg to 5000 mg/kg in rats (Chil *et al*., 2017).

Conclusion

Conclusively qualitative phytochemical screening shows high presence of volatile oils, moderate presence of alkaloids, saponins, steroid, cardiac glycoside, low presence of flavonoids and absence of tannin and free antraquinone. The $LD_{50}$was established at 1,936.49mg/kg as safety dose as observed in acute toxicity at LD_{50}. The modulation may be attributed principally to the active presence of volatile oils which were found in high concentration in this plant and others like saponins, alkaloids, steroids, and cardiac glycosides which were found in moderate concentration, and low Flavonoids.

Further research is recommended to understand the histology effects of *Ocimum santum on mice and the* main constituents in *O. sanctum* extract responsible for anti-malaria, anti-inflammatory, anti-oxidants, anti-microbial, immunodulatory, eye diseases anti-fungal antibacterial activities.

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