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Combined *in vitro* and computational study on the anthelmintic activity of the aqueous ethanolic root extract of *Ocimum gratissimum*

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Abstract
This Research investigated the anti-helminthic activity of aqueous ethanolic *Ocimum gratissimum* root extract through *in silico* molecular docking and *In-vitro* Worm paralysis and death assay study. The fresh plant root part was carefully collected, properly identified, and authenticated, was thoroughly washed, shade-dried, and then coarsely ground into a fine powder. The dried powder was subjected to hot extraction method using the Soxhlet extraction technique with aqueous: ethanol (2:8) as the solvent. Subsequently, the presence of phytochemical constituents was determined using standard qualitative chemical tests. Further molecular docking was done using 5JLE protein carried by TLC for better binding score compounds. Later *in-vitro* worm paralysis and death assay study were carried out using 5groups of Indian earthworms (*Pheretima posthuma*) each group with 2 worms of same size and length for plant extract and one group for Standard drug (Albendazole). Collected earthworms were washed with normal saline (9%w/v) to remove all the dirt matter and waste surrounding their body,10ml different concentrations of plant *Ocimum gratissimum* root extract (25, 50, 100, 150, 200mg/ml) were used and compared with 25mg/ml Std drug Albendazole, tested to determine the time of paralysis and death of worms, while the negative control received saline solution. The results revealed a concentration-dependent increase in antihelminthic activity, as indicated by the progressive reduction in both paralysis and death times with increasing extract concentration. The highest concentration (200 mg/mL) produced paralysis and death within 21 min and 24.5 min respectively, which were comparable to that of Albendazole (26.5 min and 34.5 min). Statistical analysis using the unpaired t-test showed that the lower concentrations (25–100 mg/mL) exhibited significant differences ($p < 0.05$) compared to the standard, while higher concentrations (150–200 mg/mL) showed no significant difference ($p > 0.05$), confirming similar efficacy to the standard drug. The study concludes that the plant extract possesses potent antihelminthic activity in a dose-dependent manner and could serve as a promising natural alternative to synthetic anthelmintic agents.

Keywords: *Ocimum gratissimum*, soxhlet extraction, anti-helminthic, molecular docking, tlc, worm paralysis and death assay

Introduction

Helminthic infections are among the most widespread diseases globally, affecting people of all ages—particularly in developing and underprivileged nations. Parasitic infestations, including helminthiasis, represent a major health concern in tropical regions such as Asia and Africa, impacting more than 2.5 billion individuals worldwide. Helminths cause significant health issues in humans and animals across the globe, with a higher prevalence in developing countries [1].

Various species of helminths infect both humans and animals, among which intestinal roundworms like *Pheretima posthuma* (Annelid) are the most common. It is estimated that around 200 million people experience severe health complications related to these parasites, with nearly half of them being school-aged children suffering from heavy infections. Helminthic infections can lead to numerous clinical symptoms such as dysentery, diarrhea, nausea, vomiting, loss of appetite, weight loss, acidity, and anemia. Other symptoms may include respiratory problems, dermatological conditions, and neurological disorders such as epilepsy caused by neurocysticercosis. Furthermore, helminthic infections can weaken the immune system and interfere with the body's response to other diseases like tuberculosis, HIV, and malaria [2].

Although these infections are primarily restricted to tropical regions, they can also affect travelers visiting these areas, and some parasites are capable of surviving even in temperate climates [3]. Helminthiasis is a condition characterized by the infestation of worms such as pinworms, roundworms, or tapeworms within the human body. These worms mainly inhabit the gastrointestinal tract but can also invade the liver and other organs. Infected individuals excrete helminth eggs through their feces, which contaminate the soil in regions with poor sanitation [4]. Other individuals may contract the infection by consuming contaminated food or water containing eggs or larvae, or through skin penetration by infective larvae present in the soil (as in the case of hookworms). Parasitic infections can cause serious illnesses such as filariasis (leading to elephantiasis), onchocerciasis (river blindness), and schistosomiasis [5].

According to WHO reports, synthetic drugs are currently the primary treatment option for helminth infections in humans. However, these drugs are often expensive, inaccessible to millions, and associated with adverse side effects. Consequently, there is growing interest in exploring herbal medicines for their potential anthelmintic properties.

The emergence of resistance to most commercially available anthelmintic drugs has become a serious global issue. In addition, these drugs are frequently unaffordable, scarce, or inaccessible—particularly for low-income populations and farmers in both developing and developed nations [6]. These challenges have led to an increased focus on herbal remedies as alternative anthelmintic agents [7]. Hence, the scientific evaluation of medicinal plants traditionally used for their anthelmintic potential has gained significant importance [8]. Proper screening and validation of such

plants could provide promising, sustainable, and eco-friendly alternatives to existing synthetic drugs [9]. *Ocimum gratissimum* (fig 1), commonly known as clove basil or African basil, is widely distributed across India and renowned for its significant therapeutic potential. The plant contains a rich diversity of bioactive compounds, including essential oils, flavonoids, alkaloids, tannins, phenolic compounds, terpenoids, and glycosides, which collectively contribute to its extensive pharmacological activities. Owing to these bioactive constituents, *O. gratissimum* is extensively used as a nutritional supplement and flavoring agent in both traditional and modern preparations throughout tropical, subtropical, and warm temperate regions.

Fig 1: *Ocimum gratissimum*

Pharmacological studies have demonstrated that *O. gratissimum* exhibits a wide spectrum of biological activities, including antimicrobial, anti inflammatory, antidiabetic, antidiarrheal, antiurolithiatic, antioxidant, antimutagenic, insecticidal, and anticancer effects. Therefore, the present study was conducted to determine the anti-helminthic activity of *Ocimum gratissimum* root using *in vitro* method, such as worm paralysis and death assay.

Materials and Methods

Materials

Chemical and Reagents

- 1% Dimethyl sulfoxide
- Normal saline solution
- Distilled water
- Aqueous ethanol (solvent used for extraction)
- Chloroform, methanol, ethyl acetate (Mobile phase used in TLC).

Equipment

- Soxhlet apparatus
- Desiccator
- Clean apparatus (used in qualitative analysis)
- Volumetric flasks (used in dilutions preparation)
- TLC apparatus

Software

- PubChem (for downloading ligands)
- Auto Docking tools (for molecular docking)
- Biovia (for 2D binding structure).
- Python and Excel

Methodology

1. Plant sample

Fresh roots of *Ocimum gratissimum* were collected from the local area of Bangalore district, Karnataka. The roots were properly identified and authenticated at the Central Ayurveda Research Institute, Bangalore. The collected fresh roots were thoroughly washed with water, shade-dried at room temperature, and then ground into a coarse powder, which was stored in an air-tight container for further use.

2. Preparation of Extract

The course powdered material was subjected to successive extraction using the Soxhlet apparatus with aqueous ethanolic as solvent.

3. Soxhlet Extraction

Approximately 100 g of the dried root coarse powder was packed in a thimble and sealed securely. Successive solvents (Ethanol and distilled water in ratio 8:2) were placed in a round-bottom flask containing 500 ml solvent (400ml ethanol and 100ml water). The flask was heated, allowing the solvent to evaporate, condense, and repeatedly wash over the drug material. This process was continued until a concentrated extract was obtained [10,11]. After completion of extraction, the solvent was removed under reduced pressure using a rotary flash evaporator, and the resulting extract was stored in a desiccator containing anhydrous calcium chloride to absorb residual moisture. Finally, the phytochemical constituents present in the root extract of *Ocimum gratissimum* were qualitatively analyzed using standard chemical tests followed by TLC for better docking score compounds.

4. Molecular docking

Certain phytochemicals which are present in roots of *Ocimum gratissimum* (Kaempferol, Epicatechin, Luteolin, Quercetin, Reservatrol, Sinapic acid, Apigenin, Chlorogenic acid, Gallic acid) [12-18] were downloaded from PubChem and subjected to docking study using Auto docking tools to find the binding score of these compounds with 5JLE protein [19]. 2D binding picture of ligand-protein interaction was obtained using Biovia software.

5JLE Mechanism: In anti-helminthic studies, 5JLE is known to be a glutamate-gated chloride channel (GluCl) protein, this channel is essential for the neural signaling and muscle function of helminths (worms), binding to or blocking the 5JLE protein (GluCl) channel, causing increased chloride ion influx, this leads to hyperpolarization of nerve or muscle cells, resulting in paralysis and death of the worm.

Therefore, blocking or modulating 5JLE impairs normal neuronal transmission in helminths → loss of motility → paralysis → expulsion or death of the parasite.

5. Thin layer paper Chromatography

Out of certain phytochemicals of *Ocimum gratissimum* (Kaempferol, Epicatechin, Luteolin, Quercetin, Reservatrol, Sinapic acid, Apigenin, Chlorogenic acid) showed better docking score than std drug score to these compounds' TLC were performed [20].

6. Anthelmintic Modeling Procedure

Pheretima posthuma (Indian earthworms) were collected from local regions of Bangalore district, Karnataka.

The worms were used to evaluate the anthelmintic activity of Petroleum ether and Ethanol extracts at varying concentrations, in comparison with the standard drug Albendazole. All test and standard solutions were freshly prepared before the commencement of the experiment.

Each solvent extract of *Ocimum gratissimum* was diluted to obtain concentrations of 25, 50, 100, 150, 200 mg/mL, while the standard drug Albendazole was diluted to a concentration of 25 mg/mL. The worms were thoroughly washed with normal saline and placed in Petri dishes maintained at room temperature. The respective extract and standard solutions were then introduced into the Petri dishes accordingly. The mean paralysis time and death time of the worms were recorded in minutes. Paralysis time was noted when the worms ceased all visible movement, whereas death time was confirmed when the worms showed complete loss of motility and discoloration of their body surface [21].

Unpaired (independent) Student's t-test was used to compare the mean paralysis time and mean death time of worms treated with different concentrations of the plant extract against the standard drug (Albendazole 25 mg/mL).

Results and Discussion

1. Percentage yield of an extract:

Weight of dried plant powder used=100g

Weight of extract obtained after solvent evaporation=12.3g

Then,

$$\text{Percentage yield (\%)} = \frac{\text{Weight of extract}}{\text{Weight of plant powder used}} * 100$$

Percentage yield (%) = 12.3 /100 *100 = 12.3% was the % Yield.

2. Qualitative Phytochemical chemical test of *Ocimum gratissimum* results

Table 1: Qualitative chemical test results

Sl No	Phytochemical test	Test name	Results
1.	Flavonoids	Ferric chloride test	Positive
		Sodium hydroxide test	Positive
		Lead acetate	Positive
2.	Alkaloids	Mayer's test	Negative
		Dragendroff's test	Positive
		Hager's test	Positive
3.	Glycosides		
		Sterol glycoside test	Positive
	Cardiac glycoside test	Keller-Killani test (Cardiac)	Positive
		Salkowski test (Cardiac)	Positive
	Anthraquinone glycoside test	Borntrager's test	Negative
4.	Phenols	Ferric chloride test	Positive
5.	Terpenes	Salkowski test	Positive
6.	Tannins	Ferric chloride test	Positive
		Phlobatannin test	Negative
7.	Saponins	Foam test	Positive
8.	Steroids	Salkowski test	Positive

3. Molecular docking results

Compounds Kaempferol, Epicatechin, Luteolin, Quercetin, Reservatrol, Sinapic acid, Apigenin, Chlorogenic acid and Gallic acid which are present in *Ocimum gratissimum* root part was subjected to docking with 5JLE protein using Auto docking tools and compared with Std drug (Albendazole)docking score.

Table 2: Docking scores of compounds with 1CX2 protein

Compounds	Docking score	Docking score of Albendazole (Std drug)
Kaempferol	-8.36	-7.6
Epicatechin	-9.22	-7.6
luteolin	-9.03	-7.6
Quercetin	-8.84	-7.6
Reservatrol	-8.43	-7.6
Sinapic acid	-8.07	-7.6
Apigenin	-9.14	-7.6
Chlorogenic acid	-11.32	-7.6
Gallic acid	-6.05	-7.6

2D binding of Chlorogenic acid (-11.32) with 5JLE protein shown below.

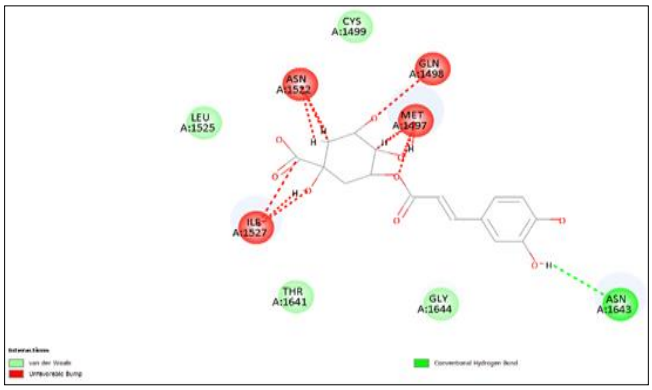

Fig 2: Binding picture

4. Thin layer paper Chromatography

Kaempferol, Epicatechin, Luteolin, Quercetin, Apigenin, Chlorogenic acid (were the compounds which showed better binding score than Std Albendazole drug) TLC was performed for these compounds

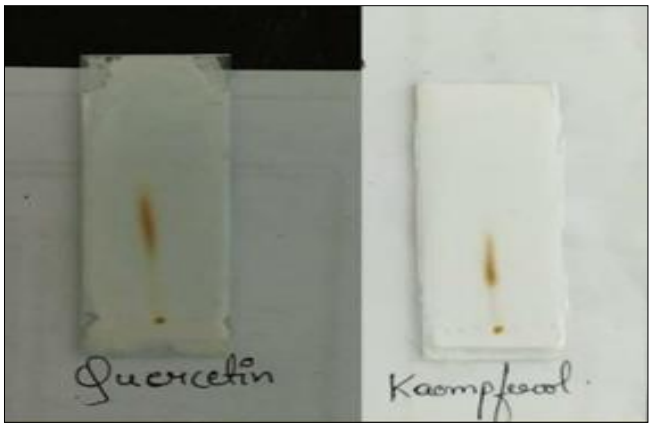

Fig 3: Quercetin, Kaempferol TLC

Quercetin(flavanoid): Ethanol: water (1:9) was used as mobile phase

Rf = Distance by solute/Distance by solvent =2.4/5.6 =0.4

Kaempferol(flavanoid): Methanol: water (1:9) as mobile phase

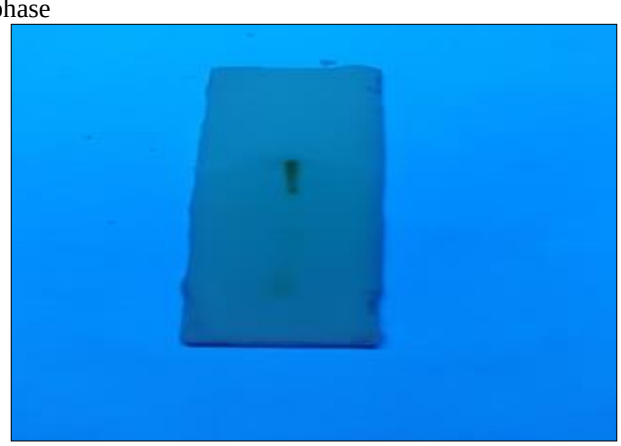

Fig 4: Ephicatechin Chloroform: methanol 9.5:0.5 Rf: -4/5.2=0.76

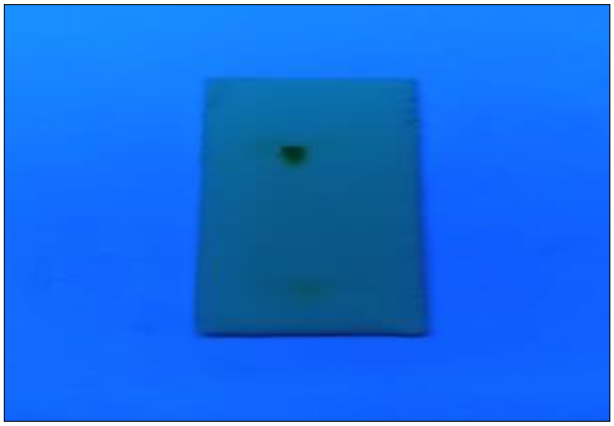

Fig 5: Luteolin Chloroform: methanol 9:1 Rf: -3.8/5.6=0.67

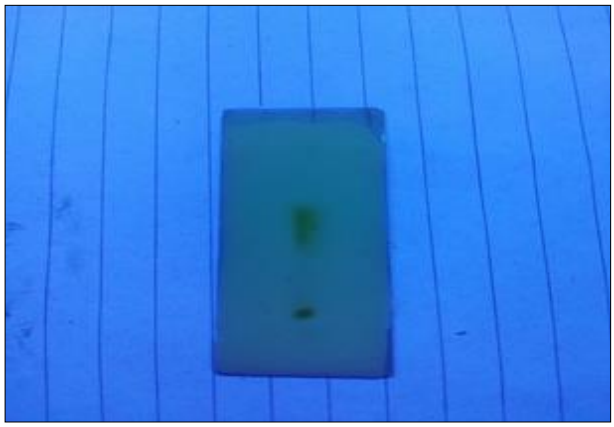

Fig 6: Apigenin Chloroform: Methanol 9:1 Rf: -3.5/5.5= 0.6

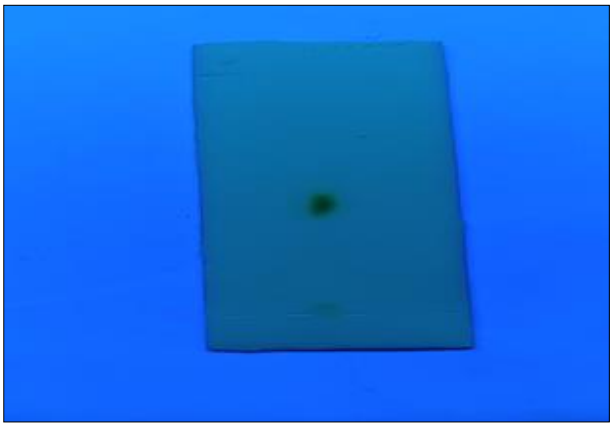

Fig 7: Ellagic acid Methanol: water 8.5:1.5 Rf: -2.8/5.4=0.51

5. Worm paralysis and death assay

Table 3: Worms paralysis and death time

Treatment	Time of Paralysis(min)	Time of Death(min)
Negative Control (Normal saline)	282,295	309,317
Std Albendazole(25mg/ml)	24,29	33,36
Plant root extract		
25mg/ml	95,105	108,112
50mg/ml	68,70	82,85
100mg/ml	42,48	58,63
150mg/ml	30,32	37,40
200mg/ml	20,22	24,25

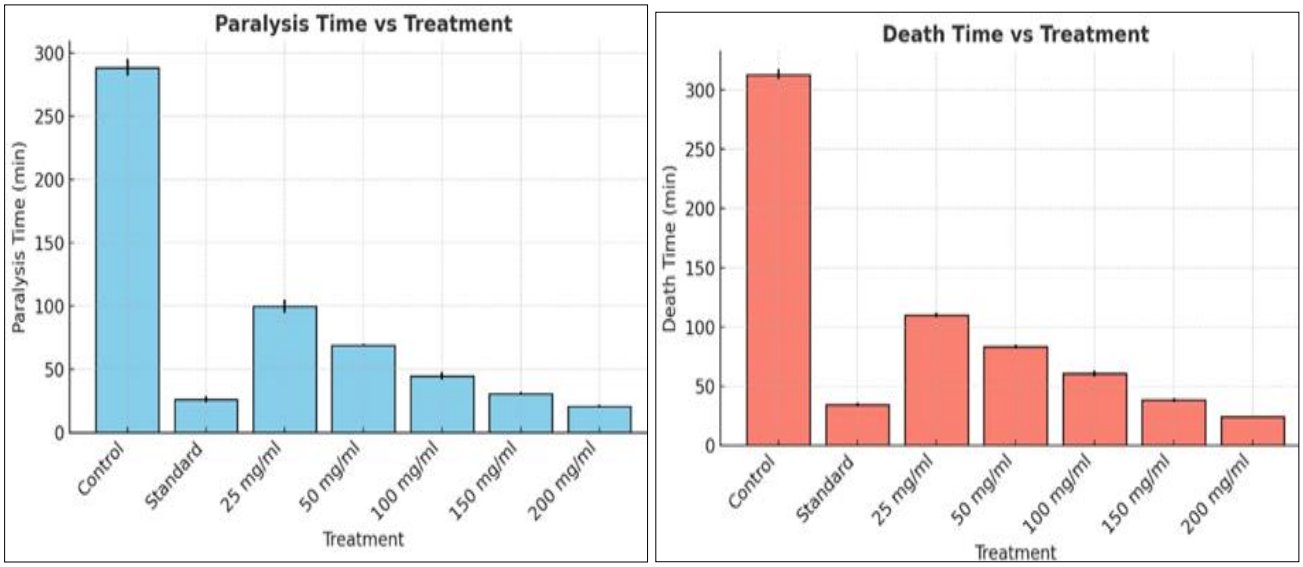

Fig 8: Graphical representation of paralysis and death time vs concentration

Further conducted unpaired t-tests (using Python) between: Each plant extract concentration vs. the standard Albendazole (25 mg/ml) for both paralysis time and death time.

Independent t-test results

Table 4: t-test results

Extract Conc	t-value (Paralysis)	p-value (Paralysis)	t-value (Death)	p-value (Death)	Interpretation
25mg/ml	13.148	0.0168	30.200	0.0016	Highly significant difference
50mg/ml	15.784	0.0186	23.099	0.0019	Highly significant difference
100mg/ml	4.737	0.0444	8.918	0.0222	Significant difference
150mg/ml	1.671	0.2966	1.886	0.2000	Not significant
200mg/ml	-2.043	0.2403	-6.325	0.0702	Not significant (close to Std)

Conclusion

Presence of certain phyto-constituents were confirmed by chemical qualitative test followed by TLC (of those compounds which showed better docking score than Std drug with 5JLE protein), highest binding score was shown by Chlorogenic acid coumpound of about (-11.32) where as Std drug Diclofinac sodium showed (-7.6).

The anti-helminthic potential of *Ocimum gratissimum* plant root extract was assessed through the *in-vitro* worm paralysis and death assay, using Albendazole as the reference/standard drug.

Lower concentrations (25 mg/mL, 50 mg/mL, 100 mg/mL) show significantly higher paralysis and death times ($p < 0.05$) compared to Albendazole → weaker activity.

Higher concentrations (150 mg/mL and 200 mg/mL) show no significant difference ($p > 0.05$), indicating that their effect is comparable to the standard Albendazole drug.

Hence, the p-values obtained from the t-test indicate that the plant extract shows a significant dose-dependent increase in antihelminthic activity. Extract concentrations of 150 mg/mL and 200 mg/mL exhibited no statistically significant difference ($p > 0.05$) compared with the standard Albendazole (25 mg/mL), confirming comparable efficacy at higher doses.

Further proper *in vivo* and advanced molecular studies are warranted to substantiate these findings and to elucidate the specific molecular pathways responsible for its anti-helminthic effect.

Compliance with ethical standards

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Reference

1. Kamrun Nahar, Muhammad Ashikur Rahman, Most Nazma Parvin, Shammy Sarwar. Evaluation of Anthelmintic Activity of Aqueous Leaf Extract of *Clitoria ternatea* Linn. S Journal of Pharmacy and Sciences,2010:3(1):46–48.
2. Kapoor LD. Handbook of Ayurvedic Medicinal Plants. Herbal Reference Library, USA, Florida, CRC Press, 2017, 82.
3. Al-Mofleh IA, Alhaider AA, Mossa JS, Al-Sohaibani MO, Rafatullah S, Qureshi S. Protection of gastric mucosal damage by *Coriandrum sativum* L pretreatment in Wistar albino rats. Environmental Toxicology and Pharmacology,2006:22(1):64–69.
4. Patsnaik AK, Cotati D, Pareta SK, Patra KC, Harwansh RK. Analgesic and anti-inflammatory activities of *Buchanania lanzan* Spreng. Research Journal of Pharmacy, Biology and Chemical Sciences,2011:2(1):419–425.
5. Gupta S, Raghuvanshi M, Jain D. Comparative studies on anti-inflammatory activity of *Coriandrum sativum*, *Datura stramonium* and *Azadirachta indica*. Asian

Journal of Experimental Biology and Science,2010:1:151–154.
6. Ajayeoba EO, Oncha SA, Olarenwaju OT. *In vivo* anthelmintic property of *Buehholria cariaca* and *Gyandropsis gyancha* extract. Pharmaceutical Biology,2001:39(1):127–210.
7. Guha P. Betel leaf, the neglected green gold of India. Journal of Human Ecology,2006:19(2):87–93.
8. Chopra RN, Nayer SC, Chopra IC. Glossary of Indian Medicinal Plants. SIR, New Delhi, 1956, 160.
9. Kelly JD, Hall CA. Resistance of animal helminths to anthelmintic. Advances in Pharmacology,1979:16:9–12.
10. De Castro ML, Garcia-Ayuso LE. Soxhlet extraction of solid materials: an outdated technique with a promising innovative future. Analytica Chimica Acta,1998:369(1):1–10.
11. Keotuonye C, Uronnachi E, Nwakile C, Attama A. *Ocimum gratissimum* essential oil: A review of extraction methods, phytochemical constituents, pharmacological uses and formulation approaches. Journal of Chemical and Biological Research,2023:3:1178–1196.
12. Ugbogu OC, Emmanuel O, Agi GO, Ibe C, Ekweogu CN, Ude VC, *et al*. A review on the traditional uses, phytochemistry, and pharmacological activities of clove basil, *Ocimum gratissimum* L. Heliyon,2021:7(11):3.
13. Offiah VN, Chikwendu UA. Antidiarrhoeal effects of *Ocimum gratissimum* leaf extract in experimental animals. Journal of Ethnopharmacology,1999:68:327–330.
14. Prabhu KS, Lobo R, Shirwaikar AA, Shirwaikar A. *Ocimum gratissimum*, A review of its chemical, pharmacological and ethnomedicinal properties. The Open Complementary Medicine Journal,2009:1:1–15.
15. Pandey AK, Chowdhury AR. Composition of the essential oil of *Ocimum gratissimum* grown in Madhya Pradesh. Journal of Medicinal and Aromatic Plant Sciences,2000:22(23):26–28.
16. Matasyoh LG, Josphat CM, Francis NW, Miriam GK, Anne WTM, Titus KM. Chemical composition and antimicrobial activity of the essential oil of *Ocimum gratissimum* L growing in Eastern Kenya. African Journal of Biotechnology,2007:6:760–765.
17. Keita SK, Vincent C, Jean-Pierre S, Bélanger A. Essential oil composition of *Ocimum basilicum* L, *O. gratissimum* L and *O. suave* L in the Republic of Guinea. Flavour and Fragrance Journal,2000:5:339–341.
18. Jirovetz L, Buchbauer G, Ngassoum MB, Ngamo LT, Adjoudji O. Combined investigation of the chemical composition of essential oils of *Ocimum gratissimum* and *Xylopia aethiopica* from Cameroon and their insecticidal activities against stored maize pest *Sitophilus zeamais*. Ernahrung,2005:29:55–60.
19. Nisha M, Senthilraja P, Thirumurugan K. Molecular docking studies of albendazole and other benzimidazole derivatives with β-tubulin protein from *Ascaris suum*, PDB ID: 5JLE. Journal of Parasitic Diseases,2016:40(4):1374–1380.
20. Bele AA, Khale A. An overview on thin layer chromatography. International Journal of Pharmaceutical Sciences and Research,2011:2(2):256.
21. Goswami S, Pandey A, *et al*. An *in vitro* evaluation of the anthelmintic activity of *Hedychium spicatum* rhizomes and *Zingiber zerumbet* rhizomes on the *Pheretima posthuma* model: A comparative study. Pharmacognosy Research,2011:3(2):140–142.