

Investigation Of In-Vitro Antihelminthic Activity Using Ethanolic & Aqueous Extract Of Origanum Majorana Plant Leaves

N. Manjusri¹, Y. Sridhar², Dr.G.Vijay Kumar³, S.Murali⁴

^{1,2,3,4} KGR Institute of Technology and Management, Rampally Vill, Kesara Mdl, Medchal Dist-501301, Telangana, India

Corresponding Author :

N. Manjusri Department Of

Pharmacology, KGR Institute Of

Management And Technology Email

ID: nmanjusri99@gmail.com

DOI: 10.47750/pnr.2022.13.S09.962

Abstract

Research on novel medications is important since the majority of commercially available anti-helminthic products have problems with the emergence of helminth resistance. Most of the nearby plants have medical properties, making them a natural alternative source for gastrointestinal nematode anti-helminth drugs. Examining the chemical makeup of Origanum majorana plant leaves and determining their anti-helminthic effectiveness are the study & main goals. In this work, chemical components from the leaves of the Origanum majorana plant are extracted using both aqueous and alcoholic methods. An earthworm called *Pheritima posthuma* is employed to carry out in-vitro anthelmintic activities since it shares the same anatomical characteristics. Furthermore, it shares a physiological resembling traits with the human gastrointestinal roundworm. The earthworms, which were almost the same size, were dispersed into the 1 ml of the chemical formulation made from plant extracts in groups of around six apiece. The time it took for paralysis and death to occur was measured if there was no movement or very little movement, excluding when the worms were violently shaken. The earthworms were said to be paralysed when there was no movement seen other than when they were shook ferociously. The findings were recorded for each concentration based on the paralysis time and death time. The major evidence for this was the lack of movement in the worms in hot water or when shaken. Their motility may be observed to completely reduce or stop at zero when they die, and this is followed by a change in body colour. The anthelmintic activity of the Majorana leaves & ethanolic and aqueous extracts was dose- or concentration-dependent, requiring the lowest amount of time to cause paralysis (P) and death (D) at a concentration of 150 mg/ml. Therefore, Origanum aqueous and ethanolic extracts, at various extraction concentrations, demonstrate their anthelmintic activity.

Key words: Anti-helminth drugs, Nematode

Introduction

Origanum majorana, is a tender perennial herb of 'origanum' genus. It is commonly known as for its sweet marjoram and native to cyprus, antolia {turkey} and naturalised in parts of Mediterranean region especially Egypt. It is cultivated all over the world in different parts of india, hungary and USA because of the usage due to the fragrance and flavours. Majorana was initially used by Hippocrates as an antiseptic agent it is a well-liked home remedy for chest infection, cough, sore throat rheumatic pain, nervous disorders, cardiovascular disease epilepsy insomnia;

skincare flatulence and stomach disorders.

Materials And Methods

1. Aqueous extraction of ORIGANUM MAJORANA:

The Origanum Majorana leaves were cleaned, dried, and powdered using 100g of dried powder and 500ml of water for 45 minutes in a beaker with a heating mantle. It was permitted to continue until all of the sample material used for extraction had been utilized. The extract that is so produced is then treated to solvent evaporation to produce the extract in crystalline/slurry form, which was employed for preliminary analysis and anti-helminthic research.

2. Soxhlet Apparatus Ethanolic Extraction Of Origanum Majorana

- After being cleaned, dried, and crushed into powder, origanum leafs are placed 150 g on filter paper and into the Soxhlet apparatus sleeve.
- A solid substance containing the target component is put into the Soxhlet extractors main chamber within a bushing constructed of thick filter paper. On top of a flask carrying the extraction solvent, this soxhlet extractor is situated. Soxhlet then installed a condenser.
- The solvent is then refluxed. After rising to the distillation arm, these solvent vapors stream into the solid cartridge cavity. All solvent vapours are cooled and drip-fed into the solids chamber housing due to the condenser.
- Solid material is progressively infused with a heated solvent in this chamber. Hot solvent would dissolve the desired chemical. An auxiliary branch of the syphon automatically empties the Soxhlet chamber when it is almost full, and the solvent then flows back down the distillation flask. For a few hours or days, this cycle was repeated again. A fraction of the non-volatile component will dissolve in the solvent once each cycle. These chosen chemicals are concentrated in the distillation flask after a number of cycles. The benefit of this method is that only one batch of hot solvent is recycled, as opposed to many parts being heated and then passed through the sample.
- The chemicals to be extracted are obtained after the solvent is evaporated, frequently using a rotary evaporator. The extracted solids insoluble component is kept in the tube and is often discarded.

Methodology

Study of the Anti-helminthic activity

The *Pheritima posthuma* common Indian earthworm, which has physiological and anatomical characteristics with human intestinal roundworm parasites, was used to evaluate the anthelmintic activity in accordance with the approach.

Five groups of about equal-sized Indian earthworms, each with six worms, were released into a chosen formulation in a volume of one milliliter. Group one serves as a control initially, receiving only saline solution, The second group is treated conventionally, with Albendazole 10 mg/ml. Test third group with Origanum majorana 50 mg/ml concentrations are given to dose-1. The fourth group is the dose -2 with origanum majorana doses of (100 mg/ml), Fifth group serves as the dose 3 receiving origanum majorana at about (150 mg/ml) concentrations.

The length of time needed for each individual worm to become paralysed or die was observed. The same kind of of each container has six worms inside of it. The earthworms; inability to move other than when they were violently disturbed was measured as the paralysis time. After

determining that earthworms were not moved by forceful shaking or by being submerged in hot water up to 50°C, the time of death of the worms was recorded. Death was only declared after the worms lost their ability to move and began to lose colour.

Group I (Normal Control): Normal saline

Group II (Standard treated): Albendazole(10mg/ml)

Group III (Dose-1): (50 mg/ml) concentrations of *origanum majorana*

Group IV (Dose-2): (100 mg/ml) concentrations of *origanum majorana*

Group V (Dose-3): (150 mg/ml) concentrations of *origanum majorana*

Results And Discussion

The phytochemical screening carried out on aqueous and ethanolic extract of *origanum majorana* indicates the presence of phytoconstituents such as Alkaloids, flavonoids, tannins, saponins, phenolic compounds, Carbohydrates, glycosides, resins, terpenoids, cardiac glycosides and steroids.

Table 1: Preliminary phytochemical test of *origanum majorana* aqueous and ethanolic extract

Phytochemical test	Results
Alkaloids	+
Flavonoids	+++
Saponins	+
Tannins	++
Phlobatannins	-
Glycosides	+++
Sterols	+
Resins	++
Phenols	+
Anthraquinones	-
Terpenoids	++
Cardiac glycosides	++

(+) indicates presence, (-) indicates absence

Table 2 Effect of Ethanolic extract of ORIGANUM MAJORANA on Indian Earth Worms (*Pheritima posthuma*)

GROUP	TREATMENT (DOSE)	TIME FOR PARALYSIS OF WORMS (mins)	TIME FOR DEATH OF WORMS (mins)
(NORMAL CONTROL)	Saline	-	-
(STANDARD CONTROL)	10mg/ml of Albendazole	23 ± 5	60 ± 5
(EEOM DOSE-1)	50mg/ml of EEOM	46 ± 5	70 ± 5
(EEOM DOSE-2)	100mg/ml of EEOM	38 ± 5	63 ± 5
(EEOM DOSE-3)	150mg/ml of EEOM	32 ± 5	58 ± 5

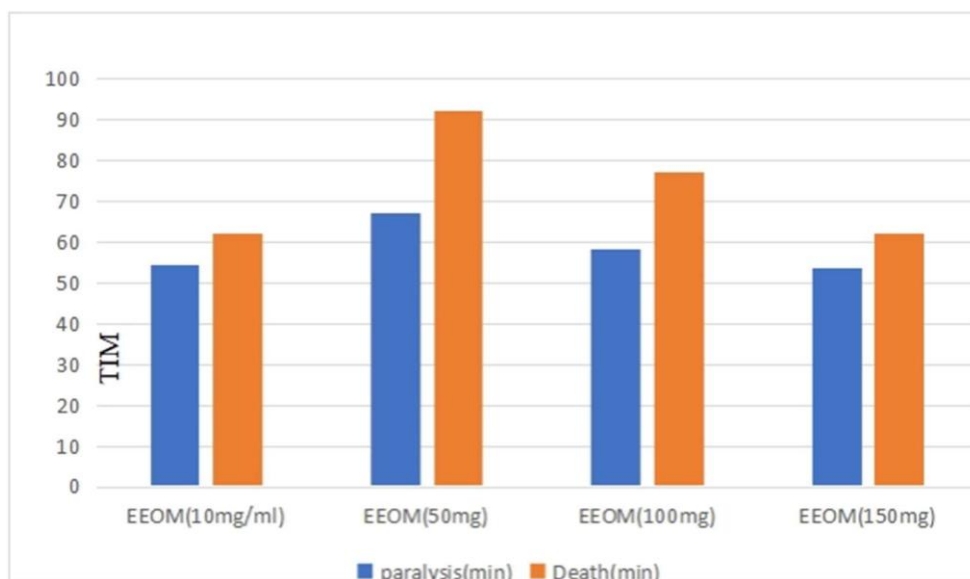
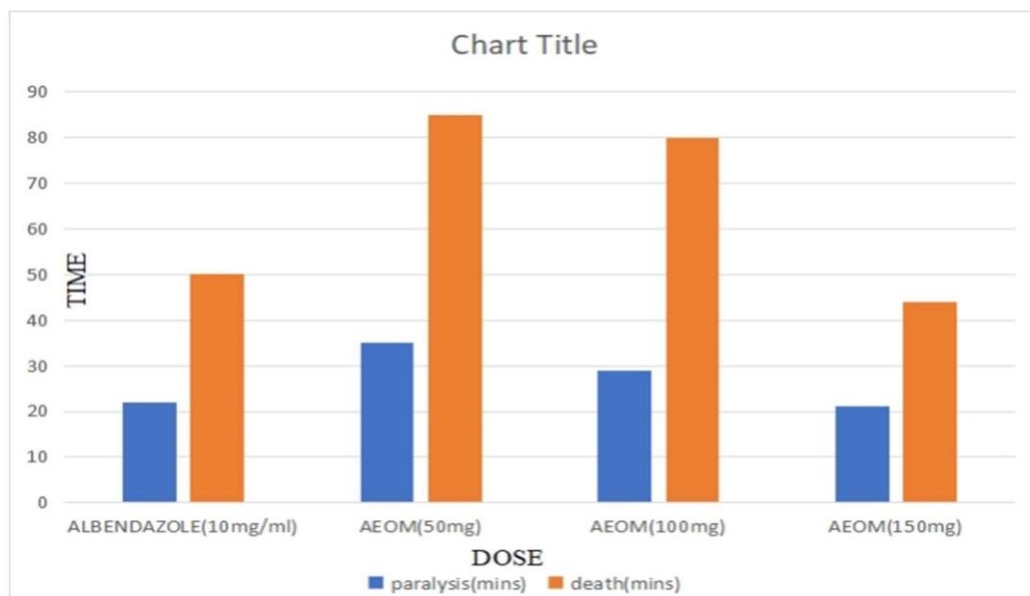


Figure 1 Bar Graph Representing Anti-Helminthic Activity Of Ethanolic Extract Of Origanum Majorana

Table 3: Effect Of Aqueous Extract Of Origanum Majorana On Indian Earth Worms(Pheritima Posthuma)

GROUP	TREATMENT (DOSE)	TIME FOR PARALYSIS OF WORMS (mins)	TIME FOR DEATH OF WORMS (mins)
(NORMAL CONTROL)	Saline	-	-
(STANDARD CONTROL)	10mg/ml of Albendazole	22 ± 5	50 ± 5
(EEOM DOSE-1)	50mg/ml of AEOM	35 ± 5	85± 5
(EEOM DOSE-2)	100mg/ml of AEOM	29:39± 5	80± 5
(EEOM DOSE-3)	150mg/ml of AEOM	21± 5	44± 5

Figure 2 Bar Graph Representing Anti-Helmenthic Activity Of Aqueous Extraction Of Plant Origanum Majorana



Discussion

Preliminary phytochemical study of the ethanolic extract of *ORIGANUM MAJAORANA* leaves revealed the presence of a number of phytochemical components, including phenols, alkaloids, flavonoids, saponins, glycosides, Phytosterols, resins, cardiac glycosides and terpenoids.

This in-vitro study proved that the times required for *Pheretima posthuman*'s paralysis (P) and death (D), which were reported in Tables 1 and 2, showed anthelmintic action by utilizing an ethanol and aqueous extracts of the plant's leaves.

The experiment was carried out on earthworms because of their physical resemblance to human gastro-intestinal roundworm parasites and also because they are widely available. The investigation's standard medicine, ALBENDAZOLE, demonstrated its effectiveness by enhancing chloride ions; transport across the earthworm muscle membrane, producing hyperpolarization, and by reducing excitement, which ultimately led to muscular relaxation and flaccid paralysis.

In comparison to the aqueous extraction, the ethanolic extract of *Origanum Majaorana* (EEOM) leaves showed anthelmintic activity in a dose-dependent manner, taking the least time for paralysis (P) and death (D). Thus, *Origanum Majaorana*'s ethanolic and aqueous extract shown anthelmintic efficacy at various dosage concentration.

Conclusion

The results of the current study showed that an ethanolic and water-based extract of *Origanum Majaorana* leaves contains phytochemicals like alkaloids, carbohydrates, flavonoids, glycosides, saponins, phytosterols, resins, terpenes, a few cardiac glycosides, and phenols, exhibited strong anti-helminthic activity and was nearly as effective as the standard drug albendazole. The inclusion of sterols, glycosides, and flavonoids may contribute to the effectiveness of the anthelmintic action. As a result of the aforementioned studies, it can be said with certainty that the aqueous and ethanolic extract of *Origanum Majorana* has anti-helminthic action.

Bibliography

1. Prema and Neeru Vasudev, *Origanum majorana* L. – Phyto-pharmacological review
2. Dipali saxena , shiv kumar Jayant , Kamakshi soni , kratika Neekhra
3. *ORIGANUM MAJORANA* :A POTENTIAL HERB FOR FUNCTIONAL FOOD
4. *MAJORANA HORTENSIS* (M.): A REVIEW UPDATE Boddu V N Satya Kumar*, Rupesh Kumar.M, Tamizhmani.T, Fsalu Rahiman OM, Mohamed Niyas K
5. Aspects of mycorrhizal colonization in adaptation of sweet marjoram (*Origanum majorana* L.) grown on industrially polluted soil Marieta HRISTOZKOVA1,*, Maria GENEVA1, Ira STANCHEVA1, Madlen BOYCHINOVA1, Efrosina DJONOVA2
1Department of Plant Mineral Nutrition and Water Relation, Institute of Plant Physiology and Genetics,
6. *Oregano: the mountain of joy on taste buds* , Dr. Dhruvo Jyoti Sen D.Pharm., B.Sc. (Hons), B.Pharm. (Hons), M.Pharm., Ph.D., FICS, CChem FIC (India), CChem FRSC (UK), CSci (UK), AOM (USA) Professor, Department of Pharmaceutical Chemistry, Shri Sarvajani Pharmacy College
7. Helminths Introduction WWW.ENCYCLOPEDIA.COM
8. www.ncbi.nlm.nih.gov/books/NBK8282
9. <http://www.slideshare.net/mobile/AbarnaAbi1/soxlet-apparatus>
10. An Updated Review on Traditional Uses, Taxonomy, Phytochemistry, Pharmacology and Toxicology of *Origanum majorana* Bimala Tripathy 1, *, S Satyanarayana 2, K Abedulla Khan 3, K Raja 4 1St. Mary's Pharmacy College, Deshmukhi (V), Near Ramoji Film city, Greater Hyderabad - 508284, Telengana, India. 2Professor, Avanthi Institute of Pharmaceutical Sciences, Vizianagaram, Andhra Pradesh, India. 3 Department of

clinical Pharmacy and Pharmacology, IBN SINA NATIONAL COLLEGE FOR MEDICAL STUDIES, Al Mahjar, Jeddah-22421, K.S.A. 4 Executive, Microbiology-QC, M/s Jodas Expoim Private Ltd, Hyderabad, India

11. <http://www.slideshare.net/mobile/jsugusugu/earthworms-10130462>

12. <http://Wikipedia.org/wiki/Earthworms>

13. Origanum majorana Essential Oil—A Review of Its Chemical Profile and Pesticide Activity

14. by Eleni Kakouri ¹ORCID,Dimitra Daferera ¹,Charalabos Kanakis ¹ORCID,PanagiotaKyriaki Revelou ¹ORCID,Eleftheria H. Kaparakou ¹ORCID,Sofia Dervisoglou

2,Dionysios Perdikis ² andPetros A. Tarantilis ^{1,*}ORCID

15. The Preservative Effects of Marjoram (*Origanum majorana* L.) And Zataria (*Zataria multiflora*) Essential Oils on Common Carp (*Cyprinus carpio*) Surimi Quality during Frozen Storage

16. Fatemeh Pourmollaei,Sahand Sarvari Nouri¹ORCID Icon,Ali Jafarpour¹ORCID Icon &Amir Mokhtarpour

17. Pages 763-773 | Published online: 16 Jun 2021

18. Download citation <https://doi.org/10.1080/10498850.2021.1937423>

19. 14. Insecticidal activity of Origanum majorana L. essential oil as anti-cholinergic agent

20. Sivaprasath Prabu, Dapeng Jing, Viswanathan Chandran, Preethy Mathew

21. First published: 17 June 2020 <https://doi.org/10.1111/1748-5967.12459>Citations: 3

22. Anti-Nematodal Essential Oils with Activity against Anisakis † Jorge M. S. Faria

1,2,* and Inês V. da Silva 3,4 dichloromethanol extract of *Schinus molle* Eur Rev Med Pharmacol Sci . 2016;20(2):372-80.

1. K. Belhamel , A. Abderrahim , R. Ludwig Chemical composition and antibacterial activity of the essential oil of *Schinus molle* L. grown in Algeria International Journal of Essential Oil Therapeutics (2008) 2, 175-177

2. M. D. Barrachina , R. Bello, M. A. Mart'inez-Cuesta, E. Primo-Y'ufera and J. Espluges Analgesic and Central Depressor Effects of the Dichloromethanol Extract from *Schinus molle* L. Phytotherapy Research, Vol. 11 , 317–319 (1997)

3. Ram Kumar Kumawat, Suresh Kumar And Sunil Sharma Evaluation Of Analgesic Activity Of Various Extracts Of *Sida Tiagii* Bhandari Acta Poloniae Pharmaceutica nDrug Research, Vol. 69 No. 6 pp. 1103ñ1109, 2012

4. Rumpa Bhowmick , Md Shahid Sarwar , Syed Masudur Rahman Dewan , Abhijit Das1 , Binayok Das , Mir Muhammad Nasir Uddin , Md Siddiqui Islam and Mohammad Safiqul Islam In vivo analgesic, antipyretic, and anti-inflammatory potential in Swiss albino mice and in vitro thrombolytic activity of hydroalcoholic extract from *Litsea glutinosa* leaves Bhowmick et al. Biological Research 2014, 47:56

5. Manjunath NOokala Krishna murthy , Srinivas Gundagani , Chandrashekhar Nutalapati , Usharani Pingali Evaluation of Analgesic Activity of Standardised Aqueous Extract of *Withania somnifera* in Healthy Human Volunteers using Mechanical Pain Model Journal of Clinical and Diagnostic Research. 2019 Jan, Vol-13(1): FC01-FC04

6. Mohamad Ali Hijazi Evaluation of Analgesic Activity of *Papaver libanoticum* Extract in Mice: Involvement of Opioids Receptors Evidence-Based Complementary and Alternative Medicine / Volume 2017

7. Sook-Ha Fan Evaluation of Analgesic Activity of the Methanol Extract from the Galls of *Quercus infectoria* (Olivier) in Rats vidence-Based Complementary and Alternative Medicine / Volume 2014

8. Bhavesh K. Lalan, Evaluation of Analgesic and Anti-Inflammatory Activity of Extract of *Holoptelea Integrifolia* and *Argyrea Speciosa* in Animal Models 2015 Jul

9. Swapnil R Experimental evaluation of analgesic and anti-inflammatory activity of simvastatin and atorvastatin Indian J Pharmacol. 2012 Jul-Aug

10. Bhavesh M. Vyas Evaluation Of Analgesic Activity Of Methanolic Extract Of *Crotalaria Burhia* Buch.-Ham. Roots In Rats And Mice International Journal Of Pharmaceutical Sciences And Research ISSN 0975-8232

11. Asha D. Jadhav An Experimental Evaluation Of Analgesic Activity Of Telmisartan International Journal Of Pharmaceutical Sciences And Research

12. Esam Qnais Evaluation of Analgesic Activity of the Methanol Extract from the Leaves of *Arum Palaestinum* in Mice and Rats Biomed Pharmacol J 2017;