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In vitro and *in vivo* action of turmeric oil (*Curcuma longa* L.) against *Argulus* spp. in goldfish (*Carassius auratus*)

Banthita Saengsitthisak[1], Wasana Chaisri[2,3], Raktham Mektrirat[4], Terdsak Yano[2] and Surachai Pikulkaew[2,3*]

[1]*Faculty of Pharmacy, Payap University, Chiang Mai, Thailand*
[2]*Department of Food Animal Clinic, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai, Thailand*
[3]*Research Center of Producing and Development of Products and Innovations for Animal Health and Production, Chiang Mai University, Chiang Mai, Thailand*
[4]*Department of Veterinary Biosciences and Public Health, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai, Thailand*

Abstract

Background: *Argulus* is a common and widespread ectoparasite that causes major parasitic diseases and is a virus and bacteria carrier in the ornamental fish trade.

Aim: The purpose of this study is to determine what types of phytochemicals are present in the essential oil extracted from turmeric rhizome (*Curcuma longa* L.) and to assess the efficacy of turmeric oil in eliminating *Argulus* spp. infestations in goldfish (*Carassius auratus*).

Methods: The chemical composition and quantity of the major substances in essential oils from fresh turmeric rhizome were detected by gas chromatography/mass spectrometry (GC-MS). The antiparasitic effect of turmeric oils on *Argulus* spp. was tested at 12.5, 25, 50, 100, and 200 ppm and compared to 0.25 ppm Neguvon® (the positive control). The percentage of *Argulus* spp. killed, the percentage of the mean mortality rate, and the effectiveness of each test were evaluated.

Results: By using GC-MS analysis, it was possible to identify the primary phytochemical component of turmeric essential oil as b-turmerone. The results obtained from the *in vitro* test indicated that there was a correlation between the concentration of turmeric essential oil and the average mortality rate of fish lice. The mean mortality of fish louse exposed to 200 ppm turmeric essential oil was higher than the mean mortality of fish louse exposed to Neguvon® ($p < 0.05$). In an *in vivo* study, the effectiveness of 12.5 ppm turmeric essential oil against parasites was 44.44%, 55.46%, and 62.83% at 24, 48, and 72 hours, respectively.

Conclusion: In summary, the efficacy of turmeric essential oil against fish louse has been shown both *in vitro* and *in vivo* studies.

Keywords: Turmeric, *Curcuma longa* L., antiparasitic, *Argulus* spp. goldfish, *Carassius auratus*.

Introduction

The aquaculture industry encompasses significant global economic value and can generate substantial revenues for fish growers. Nevertheless, aquaculture operators will encounter a significant challenge in the form of diminished fish production and substantial economic losses resulting from a heightened mortality rate associated with parasites and disease outbreaks in fish farming (Tavares-Dias and Martin, 2017; Ananda Raja *et al.*, 2020, 2022, 2023). Ectoparasites are the predominant parasites affecting fish, as they are known to infest nearly the whole culture system. *Argulus* spp., also referred to as fish lice, are ectoparasitic crustaceans with a broad distribution in aquatic environments. These organisms have the ability to parasitize and develop on a variety of host species (Steckler and Yanong, 2012; Aalberg *et al.*, 2016; Alom *et al.*, 2019; Ananda Raja *et al.*, 2020, 2022). The *Argulus* spp. species engage in feeding and predatory behavior by damaging the epidermis of their host, introducing a toxic substance, and sucking blood, resulting in the development of argulosis disease (Steckler and Yanong, 2012; Wafer *et al.*, 2015; Aalberg *et al.*, 2016; Alom *et al.*, 2019; Ananda Raja *et al.*, 2020, 2022).

The presence of fish lice has emerged as a significant threat to the health of hosts, resulting in various

***Corresponding Author:** Surachai Pikulkaew. Department of Food Animal Clinic, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai, Thailand.
Email: *surachai.pikul@cmu.ac.th; surapikulkaew@gmail.com*

adverse effects such as reduced growth, underweight conditions, hindered mating capabilities, heightened susceptibility to secondary bacterial or fungal infections, and severe skin damage in cases of heavy infestation. The resulting stress experienced by fish can lead to substantial morbidity and mortality rates among the affected animals (Steckler and Yanong, 2012; Wafer *et al.*, 2015; Aalberg *et al.*, 2016; Alom *et al.*, 2019). Various methods have been employed for the control and treatment of *Argulus* spp. (Ananda Raja *et al.*, 2020, 2022; Thakur *et al.*, 2022).

Malachite-green/formalin mixture, trichlorfon, emamectin benzoate, and organophosphate are the main chemicals used to reduce the number of parasites and/or treat diseases in fish; however, these chemicals could lead to negative situations for animals and their surroundings, which prompted drug resistance and negatively impacted the environment (Hakalahti-Sirén *et al.*, 2008; Das *et al.*, 2018; Ananda Raja *et al.*, 2020, 2022; Thakur *et al.*, 2022; Ananda Raja *et al.*, 2023). Therefore, alternative antiparasitic treatments derived from medicinal plants, such as plant extracts and essential oils, may be preferable to conventional chemical treatments due to their efficacy, biodegradability, environmental friendliness, and cost-effectiveness (Kumar *et al.*, 2012). In recent years, a greater emphasis has been placed on the use of medicinal plants in fish aquaculture for the prevention and treatment of a variety of health disorders (Kumar *et al.*, 2012; Thakur *et al.*, 2022). *Curcuma longa* L. (*C. longa*), also referred to as turmeric, is a botanical species belonging to the Zingiberaceae family (Kim *et al.*, 2013; Mitsuwan *et al.*, 2020). The rhizome of turmeric is known to contain a variety of phytochemical components, making it a frequently utilized component for medicinal applications (Kim *et al.*, 2013; Mitsuwan *et al.*, 2020; Kumar *et al.*, 2022). Extensive *in vitro* and *in vivo* tests have been performed with essential oils and extracts, which have been found to exhibit a wide range of pharmacological effects. These effects include antibacterial properties (Méndez *et al.*, 2016; Singh *et al.*, 2017), antioxidant activity (Hefnawy *et al.*, 2016), antimalarial activity (Martinez-Correa *et al.*, 2017), antiviral activity (Sornpet *et al.*, 2017), as well as anti-inflammatory and anti-tumor effects (Araújo and Leon, 2001). In addition, the insecticidal and repellent properties of turmeric rhizome extract were well-known and utilized against insect invaders (Pitasawat *et al.*, 2003). The purpose of this study was to evaluate the phytochemical compounds in the essential oil from turmeric rhizome using gas chromatography/mass spectrometry (GC-MS) and to determine the antiparasitic activity of turmeric oil against *Argulus* spp. parasites that infested goldfish (*Carassius auratus*).

Materials and Methods

Essential oils used in the experiment were obtained from *C. longa* L. cultivated in Chiang Mai province, Thailand. The fresh rhizomes of turmeric were cut and chopped into small pieces. Then, turmeric essential oil extraction was carried out by the hydro-distillation method in Clevenger's apparatus (Hashimoto *et al.*, 2016; Wesołowska *et al.*, 2019; Jaiswal and Naik, 2021). The extracted essential oils were kept in a light-resistant, tightly sealed container and maintained at 4°C before use.

The chemical compositions in the extracted turmeric essential oil were analyzed by GC-MS using an HP 6890 gas chromatograph coupled with an HP 5973 Mass Selective Detector (Agilent Technologies, Foster City, CA). Samples of 1 µl were injected in the electron impact mode. The chemical compounds in the sample were separated on a 30 m long capillary column (HP-5MS), 0.25 mm in diameter, with a 0.25 µm thick stationary phase film [(5% phenyl)-methylpolysiloxane]. The flow rate of helium through the column was kept at 1.0 $ml \cdot min^{-1}$. The total running time for a sample was about 50 minutes. The relative percentage of the essential oil constituents was evaluated from the total peak area of total ion chromatogram (TIC) by apparatus software (Hashimoto *et al.*, 2016; Wesołowska *et al.*, 2019; Jaiswal and Naik, 2021).

For the preparation of stock solution and working test solution, the extracted essential oils were diluted in the organic solvent dimethyl sulfoxide (DMSO) for the preparation of stock solutions following Hashimoto *et al.* (2016). The stock solution had a concentration of 33,000 mg l^{-1} and was composed of 1 g of turmeric essential oils prepared in 29 ml of DMSO in a proportion of 1:29. The prepared essential oil stock solution was diluted in DMSO to obtain 12.5, 25, 50, 100, and 200 mg l^{-1}, which were the different working test solutions in the experiments. The control solutions were water (negative control), Neguvon® (positive control), and 2% DMSO (vehicle control).

Fish used in this experiment were goldfish (*C. auratus*) with an average weight of 12.56 $\pm$ 2.40 g, which were obtained from ornamental fish shops located in Chiang Mai, Thailand, for *in vitro* and *in vivo* studies. These fish were stocked and acclimatized under optimum physicochemical conditions for a period of 14 days, and commercial goldfish pellets were fed daily at 2% of body weight.

For experimental design, *Argulus*-infested goldfish were picked up from ornamental fish shops located in Chiang Mai, Thailand. The fish management followed the standard operating procedure of the Aquatic Animal Medicine Laboratory of the Veterinary Faculty at Chiang Mai University. The eggs of *Argulus* were collected when they were inspected. The *Argulus* eggs were kept in an incubator at 28°C (Sahoo *et al.*, 2013). Ten days after hatching, the *Argulus* larvae were chosen and used to create an artificial *Argulus* infection in healthy goldfish. This was done to test how well turmeric essential oil kills *Argulus* spp. parasites (Kumar *et al.*, 2012; Mitsuwan *et al.*, 2020).

For *in vitro* test, the lice (*Argulus* spp.) were mildly picked up from the infested fish with a plastic tip, and actively moving parasites were collected into a petri dish with the help of a small hairbrush. During this period, fish were anesthetized with 50 mg l^{-1} of MS-222, and their behaviors and responses were also closely observed (Sneddon, 2012). The actively moving parasites were measured by vernier calipers and included the same size organism for further testing. The chosen lice were split into 8 groups of 10 live lice each and put in a Petri dish with 20 ml of different concentrations of working solution in triplicate. The working solution concentrations were 12.5, 25, 50, 100, and 200 mg l^{-1}, which were higher than the concentrations in the control solution groups. The number of organisms killed at 30, 60, 90, 120, and every 1 hour until 24 hours determined the antiparasitic action of various turmeric essential oil concentrations. Parasitic death was considered when the organism did not move after 5 minutes of observation and with a gentle touch with a dressing forceps on some parasites with an incomplete physical appearance (Fig. 1). The monitoring was processed under a stereoscopic microscope (Kumar *et al.*, 2012; Mitsuwan *et al.*, 2020). For *in vivo* test, the toxicity concentration of turmeric essential oil was analyzed to determine the safety of the experimental solution for the animal. To determine the safety dose of the working solution for *in vivo* testing, find tests with 12.5, 25, 50, 100, and 200 mg l^{-1}. The healthy goldfish was subjected to bath treatment with a diluted turmeric essential oil solution at each concentration as described above. During this period, the behaviors and responses of the animals were closely monitored and recorded. The stressed behaviors and responses of fish (such as dyspnea, anxiety, and slow breathing) were observed, and the immediate discontinuation of the experiment was performed. A safe dose of the working solution was selected for further study.

The moderately infested parasite goldfish were sampled for bath treatment with a working solution and control solution, which were divided into four groups (nine infested fish per group). One *Argulus*-infested fish was put into the tank, which contained 1,000 ml of solution as given below:

Group I	*Argulus*-infested fish exposed to purified water;
Group II	*Argulus*-infested fish treated with 0.25 mg l^{-1} of Neguvon®;
Group III	*Argulus*-infested fish exposed to 2% DMSO;
Group IV	*Argulus*-infested fish treated with 12.5 mg l^{-1} of turmeric essential oil.

Parasite mortality was observed and recorded during the study. All experimental animals were observed. The effectiveness of each treatment was determined by comparing the average number of surviving parasites in each treatment group to the control group after a 72-hour period. The antiparasitic efficacy of all groups was calculated using the calculation method described by Wang *et al.* (2009).

$$AE = \frac{[B\text{-}T]}{B} \times 100\%$$

AE = antiparasitic efficacy;
B = the mean number of surviving *Argulus* in negative control; and
T = Mean number of surviving *Argulus* in the treatment.

The linear mixed model was performed to analyze the variance of the mortality rate of parasites and antiparasitic efficacy for *in vitro* and *in vivo* tests, respectively. Either *in vitro* and *in vivo*, the treatment

Fig. 1. Feature of a fish parasite in goldfish under stereoscopic microscopy. (A) Lived organism with mobility. (B) No movement of dead organisms with incomplete physical appearance (arrows).

and time were assigned as the fixed effects in the model. Tukey's *post hoc* test was selected to examine differences among treatments and time. A statistical significance level of 0.05 was considered. Analysis of the data was done using the lme4 package (Bates *et al.*, 2015) in the R statistical program R Core Team (2020).

Ethical approval

The Animal Care and Committee of the Faculty of Veterinary Medicine, Chiang Mai University (FVM-ACUC) (Process number: R17/2557), approved the investigation for use in animal studies.

Results

Plant material and chemical analysis of the essential oil

The extraction yield was 0.7% of the weight of the raw turmeric rhizome. Observed against a white background, turmeric essential oil possessed a fluid consistency and a pale yellow color. In addition, the extracted oil was liquid at ambient temperature. The GC-MS study found that the extracted essential oil of turmeric had a wide range of chemical components. A comprehensive number of components were identified in the extracted oil. The GC-MS chromatogram presented in Figure 2 exhibits a substantial number of peaks, each characterized by distinct chemical constituents. The extracted essential oil contained b-turmerone (39.64%) and alpha-turmerone (13.87%) as the primary chemical components, as indicated in Table 1. In addition, the compounds ar-turmerone, alpha-zingiberene, and beta-sesquiphellandrene were identified, with respective concentrations of 5.87%, 4.13%, and 4.03%.

In vitro test

The results of effectiveness testing of different concentrations of turmeric oil, which were 12.5, 25, 50, 100, and 200 ppm, and control vehicles against the *Argulus* spp. parasite are presented in Figure 3. In the *in vitro* test, 200 ppm of turmeric oil performed the highest antiparasitic activity of up to 100% and killed *Argulus* spp. in 180 minutes. Furthermore, 50 and 100 ppm of essential oil revealed an antiparasitic efficacy of up to 100% and killed in 1,080 and 360 minutes, respectively. However, during observation time, the mean mortality rate (%) in the 12.5 and 25 ppm turmeric oil groups was 36.66 ± 25.16 and 93.33 ± 5.77, respectively. In addition, the antiparasitic efficacy of Neguvon® (the positive control) group showed 86.66 ± 23.09 in 1,440 minutes. Finally, after 24 hours, the results of the clean water group and the DMSO group were 3.33 ± 5.57.

In vivo test

The toxicity of turmeric essential oil was determined at 12.5, 25, 50, 100, and 200 ppm. We found the safe dose of the working solution to be 12.5 ppm. At this concentration, there was a significantly higher antiparasitic effect than in the negative control and the vehicle control ($p < 0.05$). Thus, this concentration was selected to apply in the next experiment.

In vivo antiparasitic efficacy testing demonstrated on infected fish that soaking with 12.5 ppm of turmeric essential oil, dimethyl sulfoxide, and Neguvon® resulted in the results shown in Figure 4. During the observation period, the Neguvon® group achieved the highest percentage decrease in *Argulus* spp. infection in treatment, 94.69 + 0.003. However, 12.5 ppm of essential oil presented 62.83 ± 0.004 of antiparasitic efficacy (%).

Discussion

Curcuma longa L., commonly known as turmeric, was native to tropical South Asia but is now generally harvested in tropical regions of the world. It was botanically related to the Zingiberaceae family (Kim *et al.*, 2013; Setyaningsih *et al.*, 2019; Mitsuwan

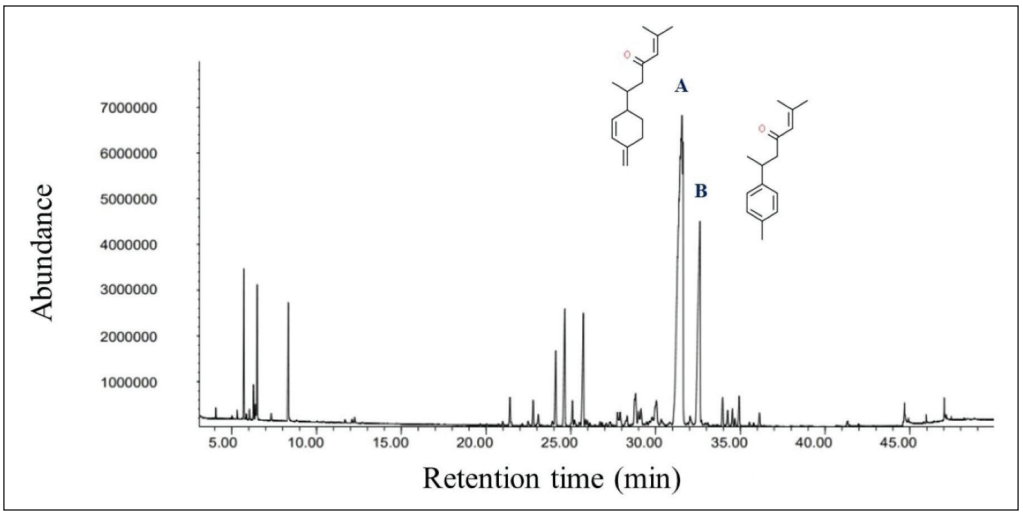

Fig. 2. GC-MS chromatograph for essential oil isolated from *Curcuma longa* L. Chemical structures of the two major constituents identified from the turmeric essential oil, including (A) b-turmerone and (B) ar-turmerone.

et al., 2020; Kumar *et al.*, 2022). The role of essential oil from the rhizome of turmeric performed various pharmacological properties in terms of antibacterial, antiviral, and antiparasitic activities for treating disease in humans and animals (Méndez *et al.*, 2016; Martinez-Correa *et al.*, 2017; Singh *et al.*, 2017; Sornpet *et al.*, 2017). The percentage yield in essential oil extraction in this study was 0.7% of the weight of fresh turmeric rhizome. Similarly, some studies in India have reported that *C. longa* L. essential oil in fresh rhizomes yields between 0.37% and 0.8% in different agroclimatic zones (Sandeep *et al.*, 2016), and between 0.61% and 1.45% in the northern part of the country (Garg *et al.*, 1999). Furthermore, Pino *et al.* (2018), who studied in Amazonian Ecuador, revealed the percentage yield of essential oil in fresh rhizomes was 0.8%. On the other hand, the percentage yield of essential oil that was extracted from dry turmeric rhizome in the USA varied from 1.5% to 5.0% (Li *et al.*, 2011). Moreover, the researchers in Brazil reported a percentage yield of essential oil from dry rhizomes between 3.0% and 5.16% (Guimarães *et al.*, 2020). To date, the chemical compositions in turmeric oil have been investigated and generally contain primarily phenolic compounds and terpenoids, including diarylheptanoids (including those commonly known as curcuminoids), monoterpenes, sesquiterpenes, diterpenes, triterpenoids, alkaloids, and sterols. This present research detected turmerone (59.38%) as the major compound in the extracted oil from fresh rhizomes. Our results were in agreement with previous studies by Pino *et al.* (2018), who reported that turmerone (58.9%) was the most represented class of volatiles from rhizomes grown in Amazonian Ecuador, including ar-turmerone (45.5%) and alpha-turmerone (13.4%). Similar results were performed by Jaiswal and Naik (2021), Guimarães *et al.* (2020), Intirach *et al.* (2012), and Devkota and Rajbhandari (2016), whose data indicated that 58%, 50.05%, 49.21%, and 25.93% of turmerone were revealed as the major constituents, respectively. Nevertheless, the previous research presented the other compounds as the major components in the essential oil, including from fresh rhizomes in Brazil (11% zingiberene) (Gonçalves *et al.*, 2019), from plants cultivated in Korea (27.70%–36.75% alpha-zingiberene) (Hwang *et al.*, 2016), from raw materials grown in Sri Lanka

Table 1. Chemical composition of essential oil from *Curcuma longa* L. obtained by GC-MS analysis. The turmeric oil was extracted from the rhizomes of *Curcuma longa* L. by simultaneous hydro-distillation and analyzed by GC-MS. RT: Retention time.

Peak	Retention time (minute)	Component	Amount (%)
1	24.65	alpha-zingiberene	4.13
2	25.74	beta-sesquiphellandrene	4.03
3	31.51	b-turmerone	39.64
4	31.62	ar-turmerone	5.87
5	32.62	alpha-turmerone	13.87

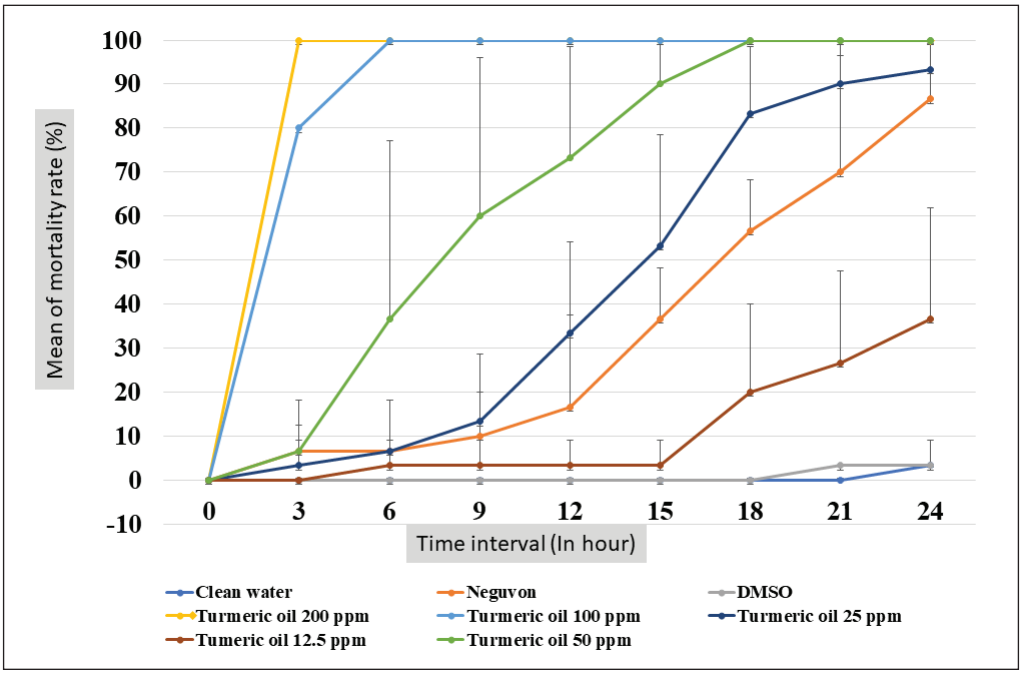

Fig. 3. *In vitro* mortality of *Argulus* spp. treated with different concentrations of turmeric oil compared to controls (negative, positive, and vehicle control).

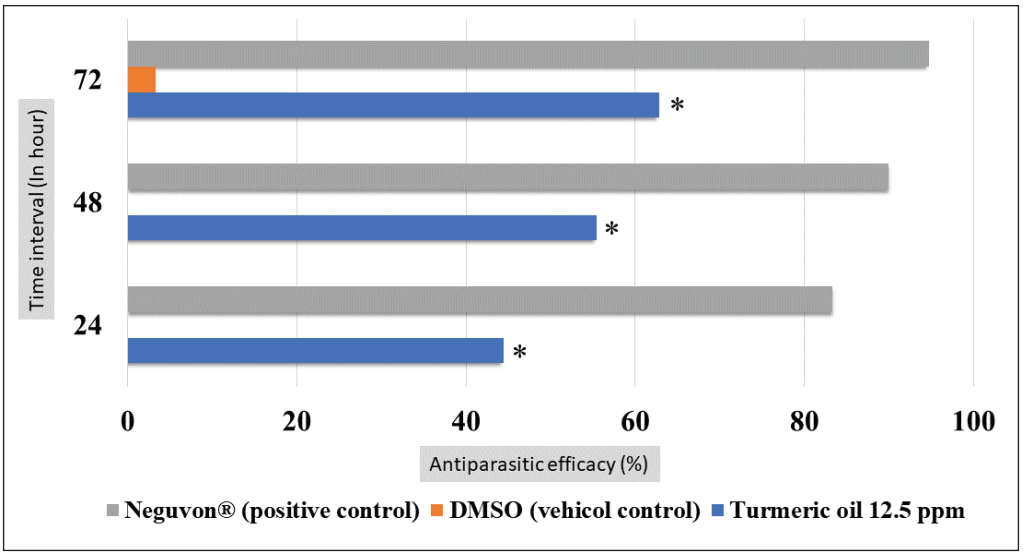

Fig. 4. *In vivo* mortality of *Argulus* spp. treated with 12.5 ppm of turmeric oil compared to positive and negative controls (* indicate significance at $p < 0.05$).

(18.2% alpha-phellandrene) (Herath *et al.*, 2017), and from herbs in Nigeria (13.9% beta-bisabolene) (Usman *et al.*, 2009). In numerous previous studies presented, several factors could interfere with the percentage yield of essential oils and the chemical variation of essential oils in plants, such as temperature, humidity, altitude, ultraviolet radiation, soil and nutrient conditions, seasonality, plant age, methods of collection, drying, and part of the plant (Bowes and Zheljazkov, 2004; Yavari *et al.*, 2010; Tavares *et al.*, 2013; Guimarães *et al.*, 2020).

This research also determined the antiparasitic efficacy of the extracted oil against fish lice in goldfish. We discovered turmeric oil had the ability to fight organisms. *In vitro* studies found a relationship between the concentration of turmeric oil and the mortality rate of *Argulus* spp. The increasing concentration has shown a rising mortality rate for parasites. At 200 mg l^{-1}, essential oil performed the highest anti-parasitic activity. However, an *in vivo* test revealed that 12.5 mg l^{-1} of the testing solution was a suitable dose to treat infested animals without toxic effects. These results were in agreement with the study of the insecticidal and repellent efficacy of extracted oil from turmeric rhizome used against insect pests, as well as Tavares *et al.* (2013), who detected high percentages of ar-turmerone in nonpolar extracts and essential oils that presented anti-repellent activity to *Sitophilus zeamais* and *Spodoptera frugiperda* (Tavares *et al.*, 2013), 50% and 100% of *S. zeamais* were killed by 0.1% of the active substance in 15 days and by 1% of ar-turmerone in 7 days, respectively. Moreover, in the case of *S. frugiperda*, they reported that 1% of the active compound eliminated 58.3% of *S. frugiperda* and reduced the development of this insect (Tavares *et al.*, 2013). However, in the present study, to ascertain the acute toxicity in the fish model, which was evaluated by only monitoring the behaviors and responses of the animals, further experimentation and analysis of the biochemical and histopathological data should be done to understand the mechanism of the acute toxicity of turmeric oil (Ramesha et al., 2016; Bhartia and Rasool, 2021). During our investigation, we observed that fish infested with *Argulus* and exposed to a 2% DMSO solution, which served as the vehicle control for the *in vivo* test, did not exhibit parasite mortality within the first 72 hours. However, we did observe a mortality rate among the fish parasites after the 72-hour point. This finding was caused by the fact that *Argulus* spp. could not complete its lifecycle in the absence of a host (Steckler and Yanong, 2012; Thakur *et al.*, 2022); the use of 2% DMSO had no effect on this result.

Furthermore, when Intirach *et al.* (2012) investigated the larvicidal effects of turmeric oil against *Anopheles cracens*, they noticed that 100 mg l^{-1} of the extracted oil demonstrated promising efficacy with 100% larval mortality (Intirach *et al.*, 2012). In addition, the results of the investigation indicated that the duration to eliminate fish lice in the *in vitro* test was less than in the *in vivo* test. The difference between *in vitro* and *in vivo* tests could be described by the treatment in the *in vitro* experiment: the *Argulus* were separated from the host with no protection, so the organisms were directly contacted with a chemical solution. However, under *in vivo* conditions, the essential oil was obstructed from approaching parasites by the scales and fins of the host, and the covered mucous on the fish body and lice, produced by the immune response of the fish, might also interfere with the contact between *Argulus* spp. and chemical reagent (Mitsuwan *et al.*, 2020).

On the other hand, the mechanism of antiparasitic activity of turmerone oil was not clarified, but it could be explained by Blenau *et al.* (2012), who reported antiparasitic mechanism action of volatile substances from herbal plants presented through inhibited and stimulated various substances and receptors such as octamine, tyramine, acetylcholine, and Gamma-aminobutyric acid (Blenau *et al.*, 2012). In addition, the toxicity effect of essential oil in our study might be justified by the research of Kostyukovsky *et al.* (2002). High concentrations of essential oil from medical plants performed insecticidal and repellent activity by inhibiting the acetylcholinesterase enzyme but also could eliminate insects and vertebrate hosts, likely through organophosphates such as Neguvon® (Kostyukovsky *et al.*, 2002). Due to this, it was important to acquire an appropriate dose of turmeric essential oil to fight against fish lice. *Argulus* spp. infesting fish caused an increase in diseased animals and a rise in the mortality rate of hosts, which was a serious problem and led to economic loss. The efficacy of antiparasitic treatment and acute toxicity using turmeric essential oil to cure diseased fish was confirmed by this present study. The primary active compounds in the extracted oil were also discovered. These might encourage the use of natural substances as an alternative way for the control and treatment of disease in aquaculture that have safety, low adverse effects, are decomposable, and have eco-friendly properties. Drug resistance from the overuse of hazardous chemicals, which is a serious global problem, might be solved.

Conclusion

Antiparasitic activity in numerous traditional medicinal plants has been investigated. However, the efficacy of turmeric essential oil against *Argulus* spp. infected fish is infrequently studied. Our research demonstrates that turmeric oil has a high potential for eradicating *Argulus* spp. Thus, the essential oil extracted from this plant can be used as an alternative treatment to the traditional chemicals that were responsible for a variety of ecological and health issues, such as persistent hazards in the environment, adverse effects on animal and human health, and a particular resistance to medications and chemicals. Further research is required to determine the mechanisms of action underlying the antiparasitic and toxic effects of turmeric oil's active ingredients. In addition, a pharmaceutical preparation of turmeric oil could be created that is simple to use in fish aquaculture and has a precise dosage.

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Conflict of interest

The authors declare that they have no competing interests.

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Authors contributions

BS and SP are project administration and conceptualization methodology. BS, WC, RM, and SP performed investigation, and validation, BS, TY, and SP performed data curation, formal analysis, and visualization. BS wrote the original draft preparation. BS and SP are reviewing, editing, and approval of the final draft. All authors have read and agreed to the published version of the manuscript.

Data availability

The data that supports the findings of this study are available from the corresponding author, upon reasonable request.

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