

Qualitative Phytochemical Screening of Medicinal Plants and Their Prospectus as Natural Therapeutics in Aquaculture

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Abstract— In the present study, three medicinally significant plant species, viz., *Artemisia absinthium*, *Matricaria chamomilla*, and *Thymus vulgaris*, were selected for qualitative screening of phytochemicals, and crude extracts of the plants were used to evaluate their antimicrobial activity against *Staphylococcus aureus*. The aerial parts of the species were sequentially extracted with ethyl acetate and reconstituted with 70% ethanol; qualitative analysis was planned for detection of the presence of major phytochemicals. Disk diffusion method was used to evaluate the antibacterial activity of all the three extracts at different concentrations. Phytochemical screening results indicated the presence of different secondary metabolites, viz., alkaloids, flavonoids, phenols, glycosides, and terpenoids. Disk diffusion assay results indicated that *Thymus vulgaris* showed high-level antibacterial activity even at low concentrations, with increasing inhibition zones of 8.95 mm at 0.1 mg/ml to 22.65 mm at 100 mg/ml; *Matricaria chamomilla* showed the highest inhibition zone of 35.80 mm at 100 mg/ml, showing high efficacy at high concentration, while *Artemisia absinthium* showed moderate activity, with inhibition zones of 0.90 mm at 0.1 mg/ml to 8.90 mm at 100 mg/ml. The results indicate that all three plant extracts contain potential secondary metabolites that could be used as preventive agents in the diet of fish to inhibit bacterial infection.

Keywords— Medicinal plants, phytochemical screening, *Staphylococcus aureus*, aquaculture.

I. INTRODUCTION

India's aquaculture production in 2022 was 10.23 million tonnes (FAO, 2024), while total fish production in Jammu and Kashmir for 2023–2024 was 28,000 tonnes (FAO, 2024). India's aquaculture, including Jammu and Kashmir, is still facing repeated problems due to disease outbreaks caused by bacteria, fungi, and parasites, leading to high fish mortality and heavy economic losses (Kalaria et al., 2024). The indiscriminate use of conventional chemical and antibiotic control methods, though initially successful, has led to grave issues like the development of antibiotic-resistant disease-causing agents, contamination of the environment, and accumulation of toxic residues in fish, toxic to the aquatic ecosystem as well as human consumption (Kalaria et al., 2024). Additionally, the use of chemicals and antimicrobial agents has been associated with a number of other issues, including bioaccumulation, contamination of water, and contamination of the surrounding natural ecosystems.

Compared to these synthetic chemicals, the use of phytochemical-rich plant extracts is promising alternatives for their use in aquaculture (Manzoor et al., 2025). These toxic effects have led to banning or restricting some chemical treatments in some areas and have triggered greater interest in safer and more sustainable alternatives, like plant therapeutics (Panigrahi & Azad, 2007). Studies are in progress on medicinal plants and plant extracts because of their high antibacterial, antiviral, and antiparasitic activities as well as due to their ability to stimulate increasing the immunity of aquatic animals, thus becoming a potential alternative to manage disease in a sustainable way (Jeyavani et al., 2022; Semwal et al., 2023).

Medicinal plants such as garlic and ginger are being used in aquaculture to stimulate fish immunity and increase growth, thus serving as a natural replacement for antibiotics. In humans, these plants have therapeutic activities that include anti-inflammatory, antioxidant, antimicrobial, and cardioprotective activities. They are used in the treatment of gastrointestinal disorders, infections, and chronic conditions, with consequences generally being lower side effects compared to man-made drugs (El-Seedi et al., 2019; Marwat et al., 2009). Ecologically, cultivation and use of these plants improve biodiversity, enhance sustainable agriculture, and reduce the utilization of chemical drugs, which aligns with the principles of green chemistry and works towards reducing environmental degradation (El-Seedi et al., 2019). Plant extracts of plants like garlic and ginger have in aquaculture been found to have the potential to boost the immune system, growth rate, and resistance to disease of fish, thereby providing a natural alternative to antibiotics while working towards the creation of healthier aquatic ecosystems (El-Seedi et al., 2019).

Use of these plants in traditional and conventional medicine gives an integrated philosophy of health that focuses on prevention, natural healing, and harmony with the environment, principles that align with Islamic values (El-Seedi et al., 2019; Marwat et al., 2009). Moreover, these natural products are considered generally inexpensive, easily accessible, and environmentally friendly, which aligns with their use in organic and sustainable aquaculture practices. In addition, they have the ability to boost growth, reduce stress, and generally improve aquatic cultured animals' well-being (Semwal et al., 2023).

Aquaculture is increasingly looking for natural, plant-based therapeutics to enhance disease management and minimize the use of synthetic chemicals. In this regard, medicinal plants such as *Artemisia absinthium*, *Thymus vulgaris*, and *Matricaria chamomilla* have gained prominence based on their rich phytochemical content and varied biological activities, including antioxidant, antimicrobial, and anti-inflammatory activities (Hbika et al., 2022). *Artemisia absinthium* is characterized by the presence of phenolic acids, flavonoids, sesquiterpene lactones, and tannins, all of which are accountable for its antioxidant, immunomodulatory, hepatoprotective, and insecticidal activities (Trifan et al., 2022). Likewise, *Thymus vulgaris* is rich in phenolic compounds such as caffeic and rosmarinic acids, which are accountable for its strong antioxidant, antimicrobial, and antiproliferative features. These compounds are present in sizeable amounts in *Thymus vulgaris* extracts, and research has established that the antioxidant activity of the plant is squarely related to its phenolic content, particularly caffeic and rosmarinic acids. Moreover, *Thymus vulgaris* extracts have been proven to contain the potential to inhibit the proliferation of varied bacterial and fungal species, and its total phenolic content has been proven to strongly correlate with antiproliferative activity against cancerous cell lines (Rafael Mascoloti Spréa et al., 2022; Taghouti et al., 2020). *Matricaria chamomilla* is gifted with a range of properties, including antioxidant, anti-inflammatory, antimicrobial, antiparasitic, hepatoprotective, neuroprotective, wound healing, and bioremediation, which are attributed to its flavonoid content (particularly luteolin, apigenin, and quercetin), sesquiterpenes (such as chamazulene and α -bisabolol), and potential to accumulate and resist heavy metals (Joumaa & Jamilah, 2022).

The ethyl acetate extraction has been identified as a superior technique for the isolation of bioactive medium-polarity compounds, including phenolics and flavonoids, that are often responsible for antioxidant, antimicrobial, and anti-inflammatory activity (Hbika et al., 2022). For example, ethyl acetate-derived *Artemisia absinthium* extracts are rich in polyphenols and flavonoids and possess good antioxidant and enzyme inhibitory activity (Hbika et al., 2022). *Thymus vulgaris* ethyl acetate fractions possess high thymol and carvacrol content with high antioxidant and anti-inflammatory activity (Saleem et al., 2022). Ethyl acetate extraction makes it easier to isolate major bioactive compounds from those plant sources, which are essential to their therapeutic effect (Trifan et al., 2022; Szopa et al., 2020). Routine qualitative phytochemical screening of these plants reveals the presence of major bioactive classes, including phenolics, flavonoids, tannins, and terpenoids, responsible for their biological activity and therapeutic potential (Hbika et al., 2022). Utilization of such plant extracts as growth promoters in aquaculture can potentially improve the health of fish, enhance disease resistance, and encourage more sustainable and environmentally friendly aquaculture practices (Trifan et al., 2022).

To isolate ethyl acetate extracts of *Thymus vulgaris*, *Matricaria chamomilla*, and *Artemisia absinthium*, the conventional method is to dry and grind the aerial parts of each respective plant. The plant material is then macerated in ethyl acetate for some time, typically 24 to 72 hours, at room temperature under stirring at intervals (Saleem et al., 2022; Alkufeidy et al., 2021). After the maceration step, the mixture is filtered such that the liquid extract is separated from the remaining plant material. The filtrate is then concentrated in volume using reduced pressure by a rotary evaporator to yield a semi-solid or solid crude extract rich in bioactive constituents (Saleem et al., 2022). The extraction method is effective in isolating phenolic compounds, flavonoids, and essential oils, which are responsible for the antioxidant, antimicrobial, and anti-inflammatory activity

elucidated in the above-botanical species (Saleem et al., 2022). For *Thymus vulgaris*, the ethyl acetate extracts have been shown to be potent antioxidants and antibacterial agents, and this activity can be attributed to constituents like thymol and carvacrol (Alkufeidy et al., 2021). The same extraction steps are followed for *Matricaria chamomilla* and *Artemisia absinthium*, as elucidated in extensive phytochemical and antimicrobial assessment studies (Stanojević et al., 2004).

II. MATERIALS AND METHODS

2.1 Plant Specimen Collection and Identification:

Artemisia absinthium was harvested from Hatbora Ganderbal, *Thymus vulgaris* from Benehama Ganderbal, and *Matricaria chamomilla* from Rangil Ganderbal.

Artemisia absinthium was recognized from its diagnostic micromorphological characters, particularly the T-shaped non-glandular trichomes on the upper and lower leaf surfaces and more dense occurrence of glandular trichomes than in related species, which are the key diagnostic characters of the species. *Thymus vulgaris* was recognized from its diagnostic glandular trichomes, particularly the peltate and capitate ones, which are responsible for the aromatization of the herbaceous plant and are manifested on the leaf surface. *Matricaria chamomilla* is recognized from finely divided leaves and glandular trichomes, along with its diagnostic flower morphology. The identification of the three species was also supported by their diagnostic anatomical and micromorphological characters, which are well documented in botany literature and are suitable criteria for the identification of species (European Pharmacopoeia, 11th ed., 2023).

2.2 Drying of Plant Material:

For each of the plant species, 1 kg of fresh aerial parts were collected. The plant material was cleaned well to eliminate soil and dirt, and shade dried for 14 days to maintain its phytochemical integrity.

2.3 Preparation of Crude Extract by Maceration Using Ethyl Acetate as Solvent:

2.3.1 Preparation of Plant Material:

Plant specimens of chamomile, thyme, and artemisia were first collected, each weighing 1 kg. The specimens were shade-dried to prevent the degradation of heat-labile phytochemicals, resulting in lower weights of 165.2 g for chamomile, 112.6 g for thyme, and 97.5 g for artemisia. The dried materials were next pulverized with a mortar and pestle in order to reduce particle size, thus enhancing the surface area for solvent contact and extraction efficiency.

2.3.2 Weighing of Plant Material:

The plant materials were precisely weighed and later transferred into individual 1000 ml borosilicate glass containers.

2.3.3 Cold Maceration Process:

Cold maceration is a delicate extractive procedure that maintains the thermolabile constituents and is well-suited to enhance the yield of bioactive compounds. The vessels holding the plant material were loaded with an addition of 830 ml of ethyl acetate, which was employed as the extracting solvent. The vessels were maintained at an angle and at ambient temperature for 72 hours. The shaking was done intermittently throughout the maceration period to promote the release of bioactive compounds into the solvent.

2.3.4 Filtration and Sequential Extraction:

After a 72-hour lapse, the solution was filtered to yield liquid extract and marc (plant residue). The marc was then re-extracted with more ethyl acetate, thus maximizing the retrieval of phytochemicals. The second filtrate was collected, and the combined volume of the filtrates for each of the plants was collected: 450 ml for *Artemisia*, 620 ml for *Thymus*, and 600 ml for *Matricaria*.

2.3.5 Concentration of Extracts:

The filtrates were concentrated under rotary evaporation at 70°C for 45 minutes to approximately 30 ml. The procedure removed most of the solvent without undermining the integrity of the extracted compounds. The concentrated extracts were then transferred to china dishes and dried further at a water bath at 50°C for 10 minutes to obtain the final crude extracts.

2.4 Qualitative Phytochemical Screening of Crude Extracts:

2.4.1 Drying and Reconstitution of Extracts:

A small quantity of each plant extract was oven-dried at 60°C for 2 minutes, then air-dried for 10 minutes. Dried extracts (300 mg each) were dissolved in 30 mL of 70% ethanol to prepare a stock solution (10 mg/ml).

2.4.2 Phytochemical Tests:

2.4.2.1 Test for Phenols: Folin-Ciocalteu Test:

In Folin-Ciocalteu test, 1 mL of extract was taken in a test tube and 0.5 mL of Folin-Ciocalteu reagent was added with 1 mL of 10% sodium hydroxide. The contents were mixed and incubated for 5 minutes at room temperature. Formation of a blue-gray color indicates the presence of phenols (Atiya et al., 2024).

2.4.2.2 Test for Flavonoids: Alkaline Reagent Test

In alkaline reagent test, few drops of 10% sodium hydroxide solution were added to 1 mL of extract in a test tube and then 2N HCl was added to neutralize the solution. Formation of an intense yellow color, which becomes colorless upon addition of dilute acid, confirms flavonoids (Shaikh & Patil, 2020).

2.4.2.3 Test for Alkaloids: Wagner's Test

In Wagner's test, a few drops of Wagner's reagent (iodine in potassium iodide) were added to 1 mL of extract solution prepared by mixing 500 mg of dried extract in 8 ml of 1% HCl, warmed and filtered in a test tube. Formation of a reddish-brown precipitate indicates the presence of alkaloids (Shaikh & Patil, 2020).

2.4.2.4 Test for Glycosides: Keller-Kiliani Test

In Keller-Kiliani test, 1.5 mL of glacial acetic acid was added to a test tube containing one drop of 5% ferric chloride and 1 mL of extract. One mL of concentrated sulfuric acid was also added along the sides of the test tube at last. Formation of a reddish-brown color indicates the presence of glycosides (Çilesizoglu et al., 2022).

2.4.2.5 Test for Terpenoids: Salkowski's Test

In Salkowski's test, 1 mL of extract was added with 2 mL of chloroform in a test tube, then 2 mL of concentrated sulfuric acid was carefully added down the sides of the test tube. A reddish-brown coloration at the interface confirms terpenoids (Sharma et al., 2020).

2.5 Antibacterial Activity of Plant Extracts Against *Staphylococcus aureus*:

2.5.1 Preparation of Plant Extract Solutions:

Different concentrations (0.1 mg/ml, 0.25 mg/ml, 1 mg/ml, 10 mg/ml, 50 mg/ml, and 100 mg/ml) of each plant extract (*Artemisia absinthium*, *Matricaria chamomilla*, and *Thymus vulgaris*) were prepared using the serial dilution method with 70% ethanol as the solvent. A 70% ethanol solution was also prepared to serve as a negative control.

2.5.2 Preparation of Agar Plates and Broth:

Nutrient agar plates and nutrient broth were prepared according to standard microbiological protocols. A 24-hour sterility test was performed to confirm the sterility of the agar plates and broth.

2.5.3 Preparation of Bacterial Suspension:

A glycerol stock of *Staphylococcus aureus* was obtained and inoculated into nutrient broth, followed by incubation at 37°C for 24 hours to achieve optimal bacterial growth.

2.5.4 Inoculation of Agar Plates:

An L-shaped spreader was used to evenly distribute the bacterial suspension over the entire surface of the agar plates to ensure uniform growth.

2.5.5 Drying of Plates:

The inoculated plates were allowed to dry at room temperature for a few minutes to ensure the surface was not excessively moist before applying the discs.

2.5.6 Application of Extract-Infused Discs:

Forceps were sterilized using a Bunsen burner and used to place sterile filter paper discs, each infused with a specific concentration of plant extract, onto the surface of the inoculated agar plates. Discs containing only 70% ethanol were included as negative controls.

2.5.7 Incubation:

The plates were incubated at 37°C for 24 hours.

2.5.8 Measurement of Inhibition Zones:

After incubation, the diameter of the clear zones (zones of inhibition) around each disc was measured in millimeters. Larger zones indicated greater antibacterial activity.

2.5.9 Calculation of Minimum Inhibitory Concentration and Dose-Response Relationship:

The MIC is determined by identifying the lowest concentration of each extract that produced a visible zone of inhibition against *S. aureus*. The relationship between extract concentration and the diameter of inhibition zones was analyzed to assess the dose-dependent antibacterial effect of each plant extract.

III. RESULTS

3.1 Plant Extraction Yield and Characteristics of Final Crude Extract:

The dried plant materials (97.5 g, 112.6 g, and 165.2 g of *Artemisia absinthium*, *Thymus vulgaris*, and *Matricaria chamomilla* respectively) with ethyl acetate yielded plant extract residues that ranged from 2.83 to 9.85 g. The highest yield of plant extract was obtained from *Matricaria chamomilla* (9.85 g) followed by *Thymus vulgaris* (6.63 g), while *Artemisia absinthium* gave the lowest extract yield (2.83 g).

The final crude extracts displayed different consistencies: *Thymus vulgaris* yielded a powdery extract, while *Matricaria chamomilla* and *Artemisia absinthium* produced scrape-like residues. These differences are attributed to the unique phytochemical compositions and solubility profiles of each plant species.

TABLE 1
EXTRACTION YIELD OF PLANT SPECIES

Plant Species	Initial Weight (g)	Final Weight (g)	Yield (%)
<i>Artemisia absinthium</i>	97.5	2.83	2.9
<i>Thymus vulgaris</i>	112.6	6.63	5.88
<i>Matricaria chamomilla</i>	165.2	9.85	5.96

3.2 Qualitative Phytochemical Screening:

The phytochemical screening of ethyl acetate extracts revealed the presence of various secondary metabolites, with variation among the three plant species.

TABLE 2
QUALITATIVE PHYTOCHEMICAL SCREENING OF PLANT EXTRACTS

Plant Species	Alkaloids	Flavonoids	Glycosides	Phenols	Terpenoids
<i>Artemisia absinthium</i>	-	++	++	++	-
<i>Matricaria chamomilla</i>	+	++	++	++	+
<i>Thymus vulgaris</i>	++	++	-	++	++

Note: (+) = Present; (-) = Absent

3.3 Antibacterial Activity:

The antibacterial potential of all three plant extracts was scrutinized against *Staphylococcus aureus*. The antibacterial potential was tested in vitro using the disc diffusion test. Discs were loaded with 0.1, 0.25, 1, 10, 50, and 100 mg/ml concentrations each of the extracts, dissolved in 70% ethanol, and the same solvent was used as a negative control. The use of 70% ethanol as a negative control was successful in showing that the reported antibacterial effects are because of the plant extracts themselves, as indicated by a zone of inhibition of 0.0 ± 0.0 mm.

TABLE 3
ZONE OF INHIBITION (MM) OF PLANT EXTRACTS AGAINST STAPHYLOCOCCUS AUREUS AT DIFFERENT CONCENTRATIONS

Plant Species	Concentration (mg/ml)	Zone of Inhibition (mm) Mean $\pm$ SE
<i>Artemisia absinthium</i>	0.1	0.90 ± 0.099
	0.25	4.75 ± 0.250
	1	4.90 ± 0.099
	10	6.90 ± 0.099
	50	7.80 ± 0.20
	100	8.90 ± 0.099
<i>Thymus vulgaris</i>	0.1	8.95 ± 0.050
	0.25	9.60 ± 0.40
	1	10.70 ± 0.299
	10	11.60 ± 0.40
	50	19.85 ± 0.149
	100	22.65 ± 0.350
<i>Matricaria chamomilla</i>	0.1	1.10 ± 0.099
	0.25	1.75 ± 0.149
	1	6.80 ± 0.20
	10	9.75 ± 0.025
	50	12.70 ± 0.299
	100	35.80 ± 0.20
Negative Control (70% Ethanol)	-	0.0 ± 0.0

3.4 Minimum Inhibitory Concentration (MIC):

The MIC of all plant extracts was 0.1 mg/ml with different inhibition zones, the highest being observed in *Thymus vulgaris*.

TABLE 4
MINIMUM INHIBITORY CONCENTRATION (MIC) OF PLANT EXTRACTS AGAINST STAPHYLOCOCCUS AUREUS

Plant Species	MIC (mg/ml)	Zone of Inhibition at MIC (mm) Mean $\pm$ SE
<i>Artemisia absinthium</i>	0.1	0.90 ± 0.099
<i>Thymus vulgaris</i>	0.1	8.95 ± 0.050
<i>Matricaria chamomilla</i>	0.1	1.10 ± 0.099

Values are mean of two replicates (n=2) $\pm$ standard error

3.5 Dose-Response Relationship:

According to Figure 1, for all three extracts, in general, there is a trend of zone of inhibition rising with increasing concentration, particularly at low concentrations. This confirms that the antibacterial activity is dose-dependent. *Artemisia absinthium* extract possesses the minimum zone of inhibition at most concentrations, *Thymus vulgaris* possesses a moderate zone of antibacterial activity, and *Matricaria chamomilla* extract possesses the maximum antibacterial activity, particularly at higher concentrations. It possesses a sudden increase in zone of inhibition at higher concentrations, which confirms its greater efficacy and potency than the other two extracts.

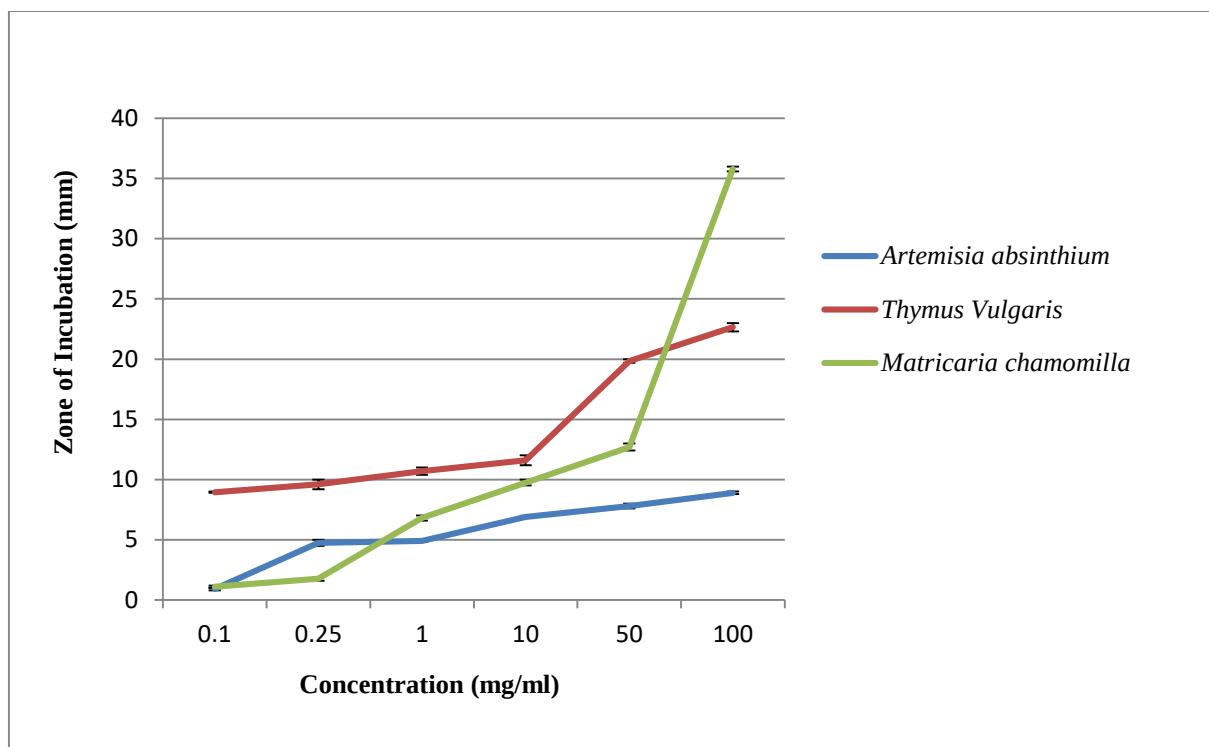
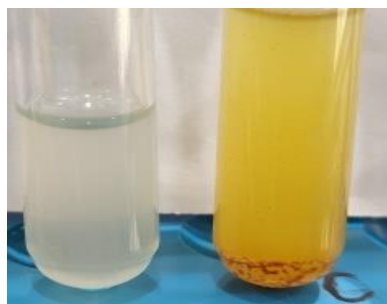
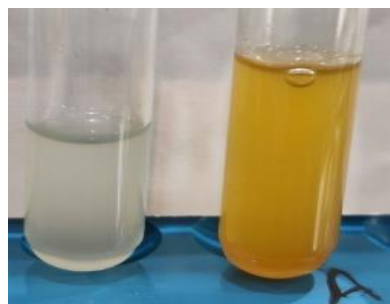


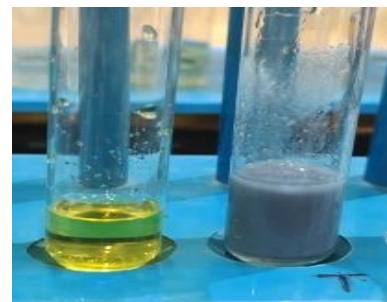
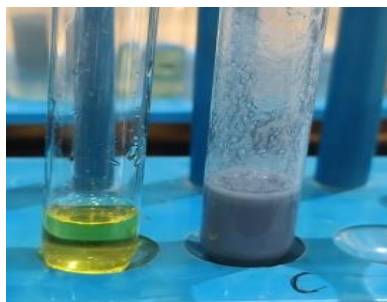
FIGURE 1: Dose-Response Curve - Zone of Inhibition vs. Concentration for All Three Plant Extracts



Test for Flavonoids



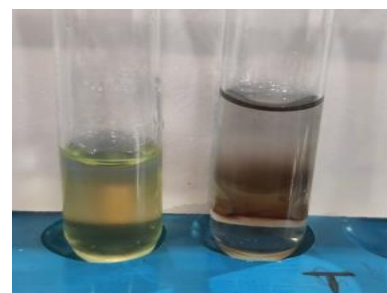
Test for Alkaloids



Test for phenols



Test for Glycosides

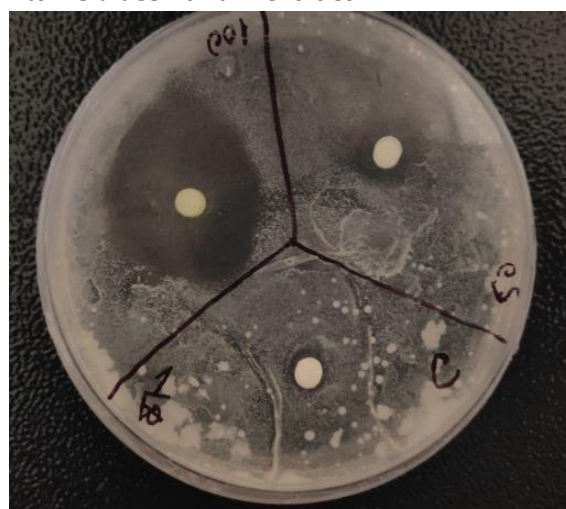


Test for Terpenoids

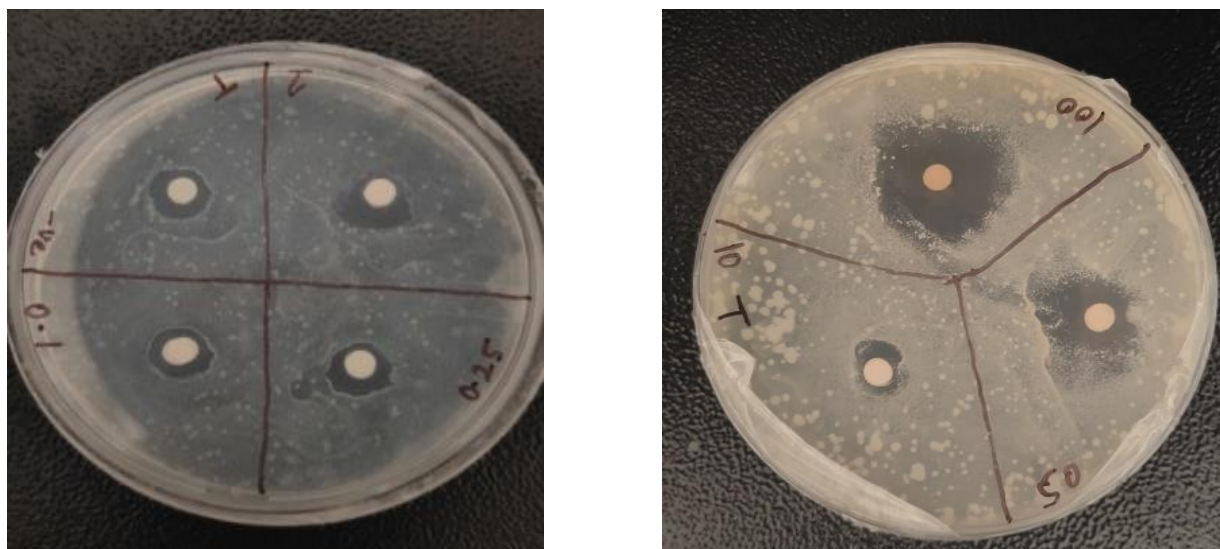
FIGURE 1: Phytochemical screening



ZOI at different concentrations of *Artemisia absinthium* extract



ZOI at different concentrations of *Matricaria chamomilla* extract



ZOI at different concentrations of *Thymus vulgaris* extract

FIGURE 3: Showing ZOI at different concentrations of *Matricaria chamomilla* extract

IV. DISCUSSION

4.1 Extraction Yield:

The plant extracts were observed to fall between 2.83-9.85 g. *Matricaria chamomilla* extract had the highest amount of extract (9.85 g), followed by *Thymus vulgaris* which yielded 6.63 g, and *Artemisia absinthium* which yielded the least extract of 2.83 g. This study complements previous data by Chuo et al. (2022) suggesting that solid-liquid extraction methods with 2-10% yields remain consistent with sprayed plant extract yields while varying depending on the species and solvents applied. This affirms the work of Lefebvre et al. (2021) who emphasize that solvent choice and plant matrix characteristics are key determinants of both yield and selectivity. Overall, extraction yields of this study are consistent with the literature for conventional methods. Chuo et al. (2022), Lefebvre et al. (2021), Rajkovic et al. (2020), and El Maaiden et al. (2022) all underlined the importance of optimizing both solvent and technique to maximize yield and preserve the quality of the extract.

4.2 Phytochemical Composition:

The qualitative phytochemical analysis conducted on the ethyl acetate extract of *Artemisia absinthium* showed the presence of flavonoids, glycosides, and phenols, with the absence of alkaloids and terpenoids. This aligns with the observations made by Trifan et al. (2022) who noted that *Artemisia absinthium* had abundant phenolic acid and flavonoid content, particularly in the polar extracts, and alkaloids and terpenoids were rather infrequent in significant quantities in methanol or ethyl acetate. Their metabolite profiling revealed a number of phenolic acids and flavonoids within the aerial parts, which corroborates the findings of abundant phenolic and flavonoid content (Trifan et al., 2022). Moacă et al. (2019) also observed significant chlorogenic acid and other phenolic constituents in ethanolic extracts, with lesser focus on the terpenoid and alkaloid content. Goud (2018) also supported the abundant phenolic and flavonoid content in methanolic extracts of *A. absinthium*.

In the case of *Matricaria chamomilla*, the results of this study showed the presence of all the tested groups, with the prominence of flavonoids, glycosides, and phenols, as well as moderate amounts of alkaloids and terpenoids. This corresponds with the study done by Spréa et al. (2022) where it was noted that *Matricaria chamomilla* has numerous secondary metabolites which include flavonoids (apigenin and luteolin), phenolic acids, glycosides, terpenoids, and variably dependent alkaloids with the extraction method used. Rahmani and Ouahrani (2022) also noted the presence of the majority of phytochemical groups in the methanolic extracts which had high solubility of the active constituents, therefore supporting findings of this study. Boudieb et al. (2018) proved that flavonoids, tannins, alkaloids, and glycosides were documented in some qualitative screenings, which further supports the findings.

Concerning *Thymus vulgaris*, the screening documented important alkaloids, flavonoids, phenols, and terpenoids, with the exception of glycosides which were not detected. Spréa et al. (2022) reported that *Thymus vulgaris* was richly phenolic, containing also flavonoids and terpenoids, while glycosides were less detected, particularly in the more apolar solvents. This also supports the findings of this study: absence of glycosides and the prominence of other groups.

In general, the qualitative findings for these three species corroborate the available literature, especially concerning the high concentrations of flavonoids and phenols. The varying occurrences of alkaloids, glycosides, and terpenoids may arise from the chemistry of the species and the solubility of the extracting solvent (Trifan et al., 2022; Moacă et al., 2019; Goud, 2018; Spréa et al., 2022; Rahmani and Ouahrani, 2022; Boudieb et al., 2018).

4.3 Antibacterial Activity:

The antibacterial potential shown by these plant extracts is in agreement with several earlier studies that have reported the antimicrobial activity of *Artemisia absinthium*, *Thymus vulgaris*, and *Matricaria chamomilla* against several pathogenic bacteria, including *Staphylococcus aureus*.

Artemisia absinthium has been reported to possess medicinal activities including antiparasitic and antimicrobial activities (Abid et al., 2023). Its extracts were found to possess activity against numerous bacterial strains by studies, which have oftentimes been attributed to the presence of compounds like sesquiterpene lactones (like artemisinin) and essential oils (Abid et al., 2023). For example, studies show that *Artemisia absinthium* essential oil and extracts have been able to suppress the growth of *S. aureus*, which is consistent with this study (Hrytsyk et al., 2022). Antibacterial activity can be varied based on the plant part, extraction method, and geographical location (Abid et al., 2023).

Thymus vulgaris is well known to have strong antimicrobial activity, with this being largely due to its high phenolic content of compounds such as thymol and carvacrol (Pereira et al., 2021). These have been documented well to cause cell death in bacteria through disrupting the bacterial cell membrane (Pereira et al., 2021). The result of the current study that *Thymus vulgaris* exhibited a large inhibition zone is consistent with a very large body of literature reporting its high performance against *S. aureus* and other resistant bacteria (Pereira et al., 2021). Research has even shown that the essential oil of *Thymus vulgaris* is used to treat methicillin-resistant *S. aureus* (Nostro et al., 2007).

Matricaria chamomilla has traditionally been applied in medicine because of its antioxidant, anti-inflammatory, and antimicrobial activities (Singh et al., 2019). The antibacterial property of the plant is mainly attributed to the essential oil compounds, α -bisabolol and chamazulene, and flavonoids and coumarins (Singh et al., 2019). While chamomile extracts are less active compared to thyme extracts at low concentrations, they have been shown to be inhibitory to *S. aureus* at higher concentrations, thus making it a natural antimicrobial compound (Singh et al., 2019).

4.4 Minimum Inhibitory Concentration:

The MICs of all plant extracts was 0.1 mg/ml with varying inhibition zones, which was highest in *Thymus vulgaris* (8.95 ± 0.05 mm). In this study, the minimum inhibitory concentration (MIC) of the *Artemisia absinthium*, *Thymus vulgaris*, and *Matricaria chamomilla* extracts against *Staphylococcus aureus* was 0.1 mg/ml. Comparing findings of this study with similar research, it is clear that ethyl acetate extracts are much stronger than most of those reported in the literature. For example, *Artemisia absinthium* and similar *Artemisia* species research commonly reports MICs ranging from 0.165 to 2.64 mg/ml against *S. aureus*, meaning the ethyl acetate extract is active at much lower concentration (Hrytsyk et al., 2022). For *Thymus vulgaris*, essential oil MICs against *S. aureus* are commonly 0.6–10 mg/ml, thus the finding of this study at 0.1 mg/ml is at the lower, more active end of this range (Aouadhi et al., 2020). For *Matricaria chamomilla*, reported MICs for chamomile essential oil are commonly 20–40 mg/ml, which is much higher than reported in this study (Aouadhi et al., 2020). This suggests that the extraction process (cold maceration) with ethyl acetate and reconstituting with 70% ethanol may have concentrated active compounds more than traditional essential oil or aqueous extractions. Overall, findings of this study show that these plant extracts, which were made with ethyl acetate as solvent using cold maceration, have much greater antibacterial activity against *Staphylococcus aureus* than most previously reported extracts or oils of the same species (Hrytsyk et al., 2022; Aouadhi et al., 2020).

V. CONCLUSION

The phytochemical analysis of *Artemisia absinthium*, *Thymus vulgaris*, and *Matricaria chamomilla* revealed the presence of flavonoids, terpenoids, alkaloids, glycosides, and phenols in their extracts. Different concentrations of all three plant extracts exhibited potent inhibitory activity, with varying inhibition zone diameters, against *Staphylococcus aureus*. The reconstituted ethanolic extracts of these plants showed a broad spectrum of activity. This study demonstrated that ethyl acetate extracts of *Artemisia absinthium*, *Matricaria chamomilla*, and *Thymus vulgaris* are rich in diverse phytochemicals, including phenolic acids, flavonoids, and other bioactive compounds, as confirmed by qualitative phytochemical screening. The integration

of *Artemisia absinthium*, *Matricaria chamomilla*, and *Thymus vulgaris* as natural therapeutics in aquaculture presents a promising strategy to address the challenges of antibiotic resistance and disease management.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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