



A systematic review and meta-analysis of the potential effect of medicinal plants on innate immunity of selected freshwater fish species: its implications for fish farming in Southern Africa

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Abstract

One of the major bottlenecks affecting the expansion of the freshwater aquaculture sector in developing countries is the outbreak of diseases. Fish farmers have traditionally relied on antibiotics and synthetic chemicals to control diseases. However, antibiotics and synthetic chemicals are associated with negative effects on the environment and consumers and their excessive use has resulted in antibiotic-resistant pathogens. In addition, the majority of freshwater fish farmers, especially small-scale farmers, have limited access to antibiotics and synthetic chemicals due to lack of resources. Medicinal plants have been reported to be suitable replacements in aquaculture, but their usage in aquaculture is still limited. The aim of this paper is to undertake a meta-analysis on the effect of medicinal plants on innate immune response and disease resistance in commonly farmed freshwater aquaculture fish species, namely Mozambique tilapia (*Oreochromis mossambicus*), African catfish (*Clarias gariepinus*), trout (*Oncorhynchus mykiss*), and cyprinids (*Labeo rohita* and *Cyprinus carpio*). The analysis showed that the mean effect size for respiratory burst (-1.90 (95% CI -2.40 ; -1.40), $I^2 = 100\%$, $P = 0$); lysozyme activity (0.05 (95% CI -0.38 ; 0.48), $I^2 = 97\%$, $P < 0.01$); white blood cells (-0.69 (95% CI -1.16 ; -0.23), $I^2 = 89\%$, $P < 0.01$); and phagocytic activity (-1.21 (95% CI -2.08 ; -0.35), $I^2 = 91\%$, $P < 0.01$) was significantly different. Mean effect size for survival rates was not significantly different (2.56 (95% CI 0.10 ; 5.01), $I^2 = 0\%$, $P = 1$). The funnel plots for all parameters were asymmetrical, which indicates possible publication bias or the presence of systematic differences or inconsistencies among studies. Overall, the meta-analysis showed that medicinal plants could enhance immunity and disease resistance in tilapia, African catfish, carp, and trout. These findings reinforce the observation that plant supplements can be used to prevent disease outbreaks in aquaculture. It is therefore recommended that freshwater fish farmers in African regions such as Southern Africa learn to produce their own diets supplemented with plants at their farms in order to prevent diseases in cultured fish.

Keywords Plant supplements · Immunostimulants · Disease prevention · Disease management · Infectious diseases

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Extended author information available on the last page of the article

Introduction

Freshwater aquaculture is one of the most important subsectors of the aquaculture industry. Over the past decade, this industry has recorded impressive growth and it is projected to increase in the future due to the rise in the global demand for affordable animal protein (FAO 2020). In Africa, freshwater aquaculture represents a valuable source of animal protein, especially for poor rural communities. It has been estimated that fish products supply at least 20% of the protein intake in the poorest countries in Africa (FAO 2020). In Southern Africa, the farming of freshwater fish species is considered one of the interventions needed to reduce poverty and create employment opportunities, especially in rural areas where poverty levels are high (Moyo and Rapatsa 2021). The Mozambique tilapia (*Oreochromis mossambicus*) and African catfish (*Clarias gariepinus*) are the most widely cultured freshwater fish species in Southern Africa (Rapatsa and Moyo 2022). Trout (*Oncorhynchus mykiss* and *Salmo trutta*) and carp (*Cyprinus carpio* and *Ctenopharyngodon idella*) are the second most widely cultured freshwater fish species, especially in South Africa (DFFE 2019). In most countries in Southern Africa, the freshwater aquaculture industry is mainly dominated by the small-scale sector, which is expected to play a significant role in the economic development of rural communities. However, the expansion of this sector has often been hampered by high mortality rates caused by infectious diseases, which leads to substantial economic losses each year.

Diseases are prevalent in many aquaculture production systems in the small-scale sector due to poor environmental conditions (e.g., low dissolved oxygen, fluctuations in temperature) (Plumb and Hanson 2011; Pridgeon and Klesius 2012). For instance, the majority of freshwater fish farmers in Southern Africa use earthen ponds as their main culture method. Fish farmed in earthen ponds are exposed to harsh environmental conditions such as low water temperatures, pH fluctuations, and low dissolved oxygen. Poor environmental conditions in earthen ponds lead to stress in fish, which is known to suppress the fish's immune system and increase its susceptibility to opportunistic pathogens (Reverter et al. 2014). The most common causes of diseases in freshwater aquaculture are bacteria, fungi, viruses, and parasites (e.g., Hecht and Endemann 1998; Plumb and Hanson 2011; Pridgeon and Klesius 2012). In earthen ponds, infectious agents such as the bacterium *Aeromonas hydrophila* and the fungus *Saprolegnia parasitica* are common and frequently take advantage of fish under stress. These pathogens have proven difficult to control in production systems because they are part of the fish's environment and mainly affect the fish when it is stressed. Even in recirculating production systems where environmental conditions are usually maintained at optimal levels for the fish, some infectious agents can be accidentally introduced into the systems and cause heavy mortalities. Furthermore, there is a general poor adherence to biosecurity measures on many fish farms in the small-scale sector, which exacerbates the prevalence of pathogenic agents.

Generally, fish farmers have been using antibiotics and synthetic chemicals to treat infectious diseases and reduce farm mortalities (Verschuere et al. 2000). Some of the most commonly used chemical agents to treat infections in aquaculture include formalin, hydrogen peroxide, potassium permanganate, malachite green, and copper sulfate (Nasser et al. 2017). However, the major challenge of using antibiotics and synthetic chemicals to treat disease in aquaculture is that they have become less effective in killing pathogens since their extensive use has resulted in the development of drug-resistant pathogens (Verschuere et al. 2000; Batista et al. 2015). This is usually attributed to the inappropriate use of chemical dosages, which are sometimes too low to kill pathogens, but sufficient to allow them

to develop defense mechanisms to resist treatment. The use of antibiotics and synthetic chemicals is also associated with harmful effects on the environment and consumer health (due to fish accumulating antibiotic residues) (Caruana et al. 2012; Zhang et al. 2016). Moreover, antibiotics and synthetic chemicals are generally expensive and most farmers in the small-scale sector cannot afford them as they often lack adequate resources, which implies that most infections in this sector are left untreated. There is therefore a need to continue to search for effective but environmentally friendly alternatives that can be used to control infectious diseases in freshwater aquaculture, especially in the small-scale sector.

The most promising strategy that can be used to control diseases in fish, especially where the use of antibiotics and synthetic chemicals is restricted, is to strengthen the immunological defense mechanism of the fish by using immunostimulants. An immunostimulant is described as a chemical substance, drug, or action that improves the capacity of the immune response or defense mechanisms such that the animal is more resistant to pathogens (Anderson 1992). A number of immunostimulants have been investigated for their role in modulating the immune defense mechanisms in fish (Anderson 1992; Sakai 1999; Bricknell and Dalmo 2005; Vallejos-Vidal et al. 2016; Vijayaram et al. 2022). Among the immunostimulants that have been tested in fish thus far, natural plant products have been identified as the most promising immunostimulants that can be used to control diseases in aquaculture. Medicinal plants contain a range of bioactive compounds such as phenolics, polyphenolics, alkaloids, quinones, terpenoids, lectins, flavonoids, and polypeptides (Harikrishnan et al. 2011). These compounds have been demonstrated to possess various pharmacological functions such as anti-stress, antioxidant, tonic, immune-stimulatory, anti-inflammatory, antimicrobial, antifungal, anti-parasitic, antiviral, antimicrobial, antifungal, anti-parasitic, and antiviral properties (Bulfon et al. 2015; Van Hai 2015). These functions show that plants can be good candidates to be used to prevent infectious diseases. A number of studies have reported that medicinal plants can enhance innate immune responses (non-specific immune response) in fish (Bulfon et al. 2015; Van Hai 2015; Awad and Awaad 2017; Reverter et al. 2021). The innate defense system is the first line of defense against infectious agents in fish and it plays a major role in preventing infections as well as triggering an adaptive immune response (Whyte 2007). Innate immunity in fish includes both humoral and cellular defense mechanisms and consists of a broad range of cells such as neutrophils, monocytes/macrophages, non-specific cytotoxic cell, natural killer cells, and mast cells and soluble molecules such as complement, transferrins, interferon, total protein (globulin and albumin), anti-proteases, lysozyme, and C-reactive protein (Whyte 2007). Some medicinal plants have been reported to enhance the activities of some of these cells and molecules in a number freshwater fish species (Bulfon et al. 2015; Awad and Awaad 2017). Plant products are also cheaper (depending on factors such as geographic location, the type of plants) and safer because they are biodegradable (Van Hai 2015).

The overall potential health benefits of medicinal plants in aquaculture have been widely reported in various systematic reviews (Bulfon et al. 2015; Van Hai 2015; Awad and Awaad 2017; Kuebutornye and Abarike 2020; Li et al. 2022). Recently, Reverter et al. (2021) carried out a meta-analysis study to investigate the potential effect of plant-supplemented diets on marine and freshwater aquaculture species. These studies have provided comprehensive information indicating that medicinal plants have the potential to improve immunity in both marine and freshwater fish species. However, due to the large number of studies covering numerous plants and fish species in previous reviews, there is a need to conduct more meta-analysis studies and narrow the scope to specific fish species or specific aquaculture sectors in order to generate more information for specific aquaculture sectors. To the best of our knowledge, there is no meta-analysis study that has been performed on

the effect of medicinal plants on immunity and disease resistance of freshwater fish species, namely Mozambique tilapia (*Oreochromis mossambicus*), Nile tilapia (*Oreochromis niloticus*), African sharptooth catfish (*Clarias gariepinus*), trout (*Oncorhynchus mykiss*), rohu (*Labeo rohita*), and carp (*Cyprinus carpio*). Therefore, the aim of this paper was to carry out a meta-analysis of studies focusing on the effect of medicinal plants on enhancing immunity and disease resistance in these fish species. The analysis will generate information that will add to the body of existing literature regarding the understanding of the use of medicinal plants in fish. In addition, this study also focused on the challenges and potential application of medicinal plants in the freshwater aquaculture industry in Southern Africa.

Materials and methods

Literature search

This meta-analysis was carried out according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Moher et al. 2009). An extensive and systematic literature search was undertaken on all published peer-reviewed articles on the effect of diets supplemented with plants on immunity, disease resistance, and survival in tilapia, trout, carp, and catfish. The search included published books, articles in journals, relevant government and agency documents, and gray literature. Literature was accessed through Google Scholar, Google Search engine, ScienceDirect, and Web of Science. The literature search was aimed at scientific articles from all over the world and prioritized articles published within the last 10 years, with older, but relevant articles also included. The search for articles was undertaken from March 2021 to December 2022. Emphasis was placed on using literature that included immunity and disease resistance or survival of tilapia, catfish, trout, and carp. The following keyword combination was used: dietary herbal/medicinal plant supplementation on serum total protein/immunoglobulin/phagocytic activity/white blood cells/lysozyme activity/respiratory burst, phagocytic activity/complement activity/disease resistance/survival in tilapia, African catfish, trout, carp, and trout.

Screening of articles

The selection and screening flow chart is presented in Fig. 1. Titles and abstracts of retrieved articles were transferred into EndNote and duplicates were removed. Articles were screened to assess their eligibility and articles that were deemed irrelevant were removed. The complete texts of articles deemed relevant were then assessed for eligibility based on the criteria described below. Two authors did the search and screening of the literature and disagreements were resolved through discussion and consensus.

Eligibility criteria

Retrieved articles were included in the analyses if they met the following criteria:

1. The study investigated the inclusion of plant material as a supplement and not a replacement of a major ingredient such as fishmeal or soybean meal.

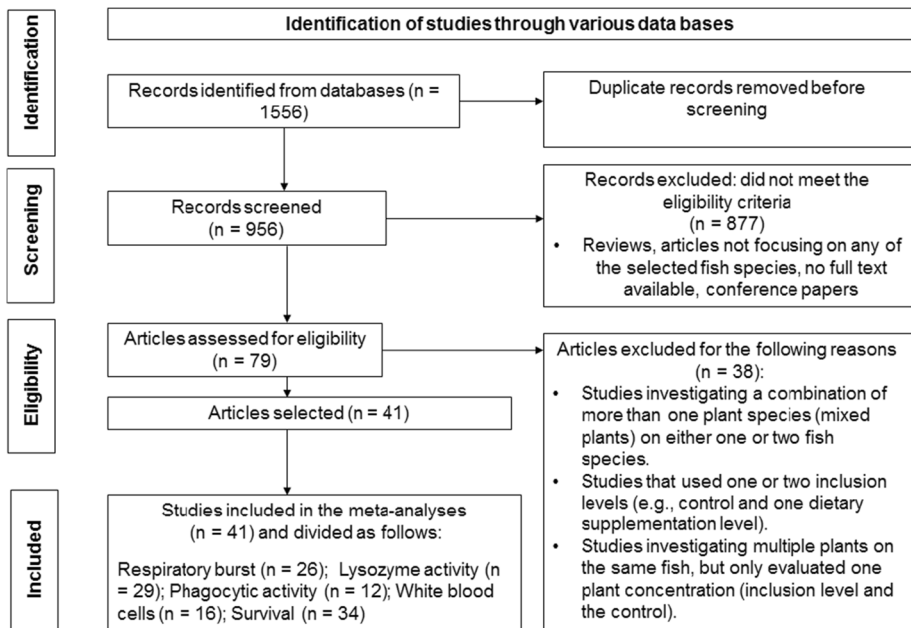


Fig. 1 Flow chart on the selection and screening of articles used in the meta-analysis

2. Studies that reported data for a control (not supplemented with plant material) and at least two experimental diets supplemented with plant material.
3. Studies reporting for at least one of the following parameters for both fish fed with a control diet and fish fed with diets supplemented with a plant material—serum total protein (g dL^{-1}), immunoglobulin (mg mL^{-1}), phagocytic activity, white blood cells (WBCs), lysozyme activity (U mL^{-1}), respiratory burst, phagocytic activity (%), complement activity (ACH50, U mL^{-1}), and survival rate after a challenge with a pathogen (%).
4. Studies with a number of replicates and results reported to include means and standard deviation or standard error either numerically or graphically for each of the parameters.
5. Studies indicating part of the plant (roots, stem, and leaves), plant material (powder, extracts, and essential oils), type of extract used (aqueous, ethanol, methanol), and administration duration (weeks) and inclusion rate (g plant kg^{-1} feed) used.

For studies that investigated the effect of a plant on any of the immune parameters mentioned above and collected results at different time points, only the final time point was considered. Studies that investigated the effect of a combination of more than one plant species (mixed plants) on either one or two fish species were disregarded. Studies that used one inclusion level (e.g., control and one dietary supplementation level) were also disregarded. Studies that investigated multiple plants on the same fish but only evaluated one plant concentration (inclusion level and the control) were also removed from the analysis. A study that investigated more than two plants simultaneously on the same fish, but reported results for each plant separately and had more than two inclusion levels for each plant, was included in the analysis. In the analysis, the results for each plant from such study were recorded as plant one (s1), plant two (s2), plant three (s3), etc., to represent each plant, but the author and year remained the same. There were few articles that reported on

superoxide dismutase (SOD), serum total protein (g dL^{-1}), immunoglobulin (mg mL^{-1}), and complement activity (ACH50, U mL^{-1}) in freshwater fish species fed with diets supplemented with plants. Consequently, these parameters were excluded from the analyses. In all publications that mentioned survival, the name of the pathogen used was recorded.

Data extraction and synthesis

Quantitative and qualitative data extracted from the included studies was compiled into an Excel spreadsheet and included the following information: author name, publication date, name of the plant, study location, fish species, plant material used, and pathogen used. The spreadsheet also contained important information such as means, standard deviations, and standard errors of the immune parameters included in the analysis.

Statistical analysis

All statistical analyses were conducted in R Studio (R version 4.1.1 (2021–08–10)—“Kick Things” Copyright (C) 2021 The R Foundation for Statistical Computing Platform: x86_64-w64-mingw32/x64 (64-bit)). The standardized difference in means (Hedge’s g (g)) was used as the effect size to assess the effect of medicinal plant on lysozyme activity, respiratory burst, WBCs, phagocytic activity, and survival. Mean effect size is a measure of the strength of the relationship between the control group and the experimental groups. The standardized difference in means was calculated for each individual observation or study for each parameter as the difference between the mean of the experimental treatment and the control divided by their pooled standard deviation and multiplied by a correction term to reduce bias from small sample sizes (package esc in R version 3.6). The I^2 statistic was used to estimate heterogeneity across studies according to Harrer et al. (2021). The calculated I^2 value is used to measure the percentage of variability due to heterogeneity rather than chance or sampling error. If the I^2 value is greater than 50%, heterogeneity is considered statistically significant. Forest plots were produced as a means of visualization. Funnel plots were plotted to estimate publication bias. The presence of asymmetry shows possible publication bias while symmetry indicates no publication bias. A sensitivity test was also performed to remove strong outliers from the datasets in order to decrease heterogeneity. The test removes each study from the analysis, one by one, so that it is easier to indicate the combined effect sizes and the associated heterogeneity.

Results

Studies included in the analysis and the parameters they reported on are presented in Table 1. After screening the literature and extracting the data, we obtained 34 studies that investigated the effect of medicinal plants on survival, 26 on respiratory burst (NBT), 29 on lysozyme, 12 on phagocytic activity, and 16 on WBCs. It should be noted that the studies included in the analysis have mostly focused on tilapia (Table 1). Accordingly, there is strong possibility that the observations of the results were largely influenced by the data from tilapia because it accounted for a significant portion of all data, whereas the trout, carp, and African catfish accounted for about a quarter of the data (Table 1). Therefore, the interpretation of the results from the meta-analysis should be treated with circumspection.

Table 1 Sources of data for respiratory burst, lysozyme activity, phagocytic activity, and white blood cells used for meta-analysis

Author	Fish species	Plant species	Parameter reported				
			Survival	Respiratory burst	Lysozyme	Phagocytic activity	WBCs
Divyagnaneswari et al. (2007)	<i>Oreochromis mossambicus</i>	<i>Solanum trilobatum</i> (leaf)	✓	✓	N/A	N/A	N/A
Christyapita et al. (2007)	<i>Oreochromis mossambicus</i>	<i>Eclipta alba</i> (leaf)	✓	✓	N/A	N/A	N/A
Wu et al. (2010)	<i>Oreochromis mossambicus</i>	<i>Toona sinensis</i> (leaf)	✓	✓	✓	N/A	N/A
Baba et al. (2016)	<i>Oreochromis mossambicus</i>	<i>Citrus limon</i> (peel)	✓	✓	✓	N/A	✓
Alexander et al. (2010)	<i>Oreochromis mossambicus</i>	<i>Tinospora cordifolia</i> (leaf)	✓	✓	✓	N/A	N/A
Yilmaz et al. (2013)	<i>Oreochromis mossambicus</i>	<i>Cuminum cyminum</i> (leaf)	✓	N/A	N/A	N/A	N/A
Kirubakaran et al. (2016)	<i>Oreochromis mossambicus</i>	<i>Nyctanthes arbor-tristis</i> (seed)	✓	✓	✓	N/A	N/A
Acar et al. (2015)	<i>Oreochromis mossambicus</i>	<i>Citrus sinensis</i> (orange peel)	✓	N/A	✓	N/A	N/A
Gobi et al. (2016)	<i>Oreochromis mossambicus</i>	<i>Psidium guajava</i> (leaf)	✓	✓	N/A	N/A	N/A
Mbokane and Moyo (2018a)	<i>Oreochromis mossambicus</i>	<i>Artemisia afra</i> (leaf)	✓	✓	✓	N/A	✓
Mbokane and Moyo (2018b)	<i>Oreochromis mossambicus</i>	<i>Moringa oleifera</i> (leaf)	✓	✓	✓	N/A	✓
Wu et al. (2013)	<i>Oreochromis niloticus</i>	<i>Sophora flavescens</i> (root)	✓	✓	✓	✓	N/A
Shalaby et al. (2006)	<i>Oreochromis niloticus</i>	<i>Allium sativum</i> (powder)	✓	N/A	N/A	N/A	N/A
Park and Choi (2012)	<i>Oreochromis niloticus</i>	<i>Viscum album coloratum</i>	✓	✓	✓	✓	N/A
Abdel et al. (2009)	<i>Oreochromis niloticus</i>	<i>Trigonella foenum-graecum</i> (L.) (seeds)	✓	N/A	N/A	N/A	N/A
Yilmaz (2019)	<i>Oreochromis niloticus</i>	Blackberry syrup	✓	N/A	✓	N/A	N/A
Ndong and Fall (2011)	Hybrid tilapia hybrid tilapia (<i>Oreochromis niloticus</i> x <i>Oreochromis aureus</i>)	<i>Allium sativum</i> (bulbs)	N/A	✓	✓	✓	✓
Gabriel et al. (2015)	GIFT	<i>Aloe vera</i> (leaf)	N/A	N/A	✓	N/A	✓
Asadi et al. (2012)	<i>Oncorhynchus mykiss</i>	<i>Nasturtium nasturtium</i> (leaf)	N/A	N/A	✓	N/A	N/A
Baba et al. (2015)	<i>Oncorhynchus mykiss</i>	<i>Lentinula edodes</i> (mushroom)	✓	N/A	✓	✓	✓
Bilen et al. (2011)	<i>Oncorhynchus mykiss</i>	<i>Cotinus coggygia</i> (leaf)	N/A	✓	✓	✓	N/A

Table 1 (continued)

Author	Fish species	Plant species	Parameter reported			
			Survival	Respiratory burst	Lysozyme	Phago-cytic activity
Bilen et al. (2016)	<i>Oncorhynchus mykiss</i>	<i>Capparis spinosa</i> (leaf)	✓	N/A	✓	✓
Adel et al. (2016a)	<i>Oncorhynchus mykiss</i>	<i>Mentha piperita</i> (leaf)	✓	✓	✓	N/A
Baba et al. (2018)	<i>Oncorhynchus mykiss</i>	<i>Olea europaea</i> (L.) (leaf)	✓	N/A	N/A	N/A
Farsani et al. (2019)	<i>Oncorhynchus mykiss</i>	<i>Coriandrum sativum</i> (seed)	✓	N/A	✓	N/A
Doruca et al. (2009)	<i>Oncorhynchus mykiss</i>	<i>Nigella sativa</i> (seeds)	N/A	✓	N/A	✓
Düğenci et al. (2003)	<i>Oncorhynchus mykiss</i>	<i>Viscum album</i> (s1), <i>Urtica dioica</i> (s2), and <i>Zingiber officinale</i> (s3) (leaf)	N/A	✓	N/A	✓
Pratheepa and Sukumaran (2014)	<i>Cyprinus carpio</i>	<i>Euphorbia hirta</i> (leaf)	✓	N/A	N/A	N/A
Soltanian and Fereidouni (2016)	<i>Cyprinus carpio</i>	<i>Lawsonia inermis</i> (leaf)	✓	✓	✓	✓
Adel et al. (2016b)	<i>Cyprinus carpio</i>	<i>Achillea wilhelmsii</i> (leaf)	N/A	✓	✓	N/A
Jafarnejad et al. (2018)	<i>Cyprinus carpio</i>	<i>Zingiber officinale</i> (leaf)	N/A	N/A	N/A	✓
Giri et al. (2015)	<i>Labeo rohita</i>	<i>Psidium guajava</i> (leaf)	✓	N/A	✓	N/A
Sharma et al. (2010)	<i>Labeo rohita</i>	<i>Withania somnifera</i>	✓	✓	✓	N/A
Sahu et al. (2007)	<i>Labeo rohita</i>	<i>Mangifera indica</i> ()	✓	N/A	✓	✓
Sukumaran et al. (2016)	<i>Labeo rohita</i>	<i>Zingiber officinale</i> ()	✓	N/A	✓	N/A
Gupta and Mishra (2014)	<i>Clarias gariepinus</i>	<i>Eclipta alba</i> (leaf)	N/A	N/A	N/A	✓
Veerasingh et al. (2014)	<i>Clarias gariepinus</i>	<i>Polygonum minus</i> (leaf)	✓	N/A	✓	✓
Sheikhlal et al. (2017)	<i>Clarias gariepinus</i>	<i>Euphorbia hirta</i>	✓	N/A	N/A	✓
Mbokane and Moyo (2020a)	<i>Clarias gariepinus</i>	<i>Artemisia afra</i> (leaf)	✓	✓	✓	✓
Mbokane and Moyo (2020b)	<i>Clarias gariepinus</i>	<i>Moringa oleifera</i> (leaf)	✓	✓	✓	✓
Kaleswaram et al. (2011)	<i>Calta calta</i>	<i>Cynodon dactylon</i> (L.)	✓	✓	✓	N/A

N/A, not applicable

The analysis showed that the mean effect size for respiratory burst was significantly different (Fig. 2). The overall effect size of the comparisons between the control and the supplementation levels was -1.90 (95% CI $-2.40; -1.40$) (Fig. 2). The effect sizes of some individual studies were negative. The level of heterogeneity for respiratory burst was significantly high ($P=0$) at $I^2=100\%$ (Fig. 2). The analysis also showed that the plant-supplemented diets had significant effect on lysozyme activity (Fig. 3). The overall effect size of the comparisons between the control and the supplementation levels was 0.05 (95% CI $-0.38; 0.48$) (Fig. 3). The level of heterogeneity for lysozyme activity was also significantly high ($P<0.01$) at $I^2=97\%$ (Fig. 3).

It was also observed that WBCs and phagocytic activity were significantly enhanced ($P<0.01$) in fish fed with medicinal plants compared with fish fed with the control (Figs. 4 and 5). The overall effect size of the comparisons between the control and the supplementation levels for WBCs and phagocytic activity was -0.69 (95% CI $-1.16; -0.23$) and -1.21 (95% CI $-2.08; -0.35$), respectively (Figs. 4 and 5). The level of heterogeneity was significantly high for both parameters, $I^2=89\%$ ($P<0.01$) for WBCs and $I^2=91\%$ ($P<0.01$) for phagocytic activity (Figs. 4 and 5). The effect sizes of some individual studies for both WBCs and phagocytic activity were also negative. Mean effect size for survival rates for fish fed with the diets containing medicinal plants was not significantly different from the control (significantly positive Hedge's g) (Fig. 6). The overall effect size of the comparisons between the control and the supplementation levels was 2.56 (95% CI $0.10; 5.01$) (Fig. 6). The level of heterogeneity for survival rate was significantly low ($I^2=0\%$, $P=1$) (Fig. 6). The funnel plots for respiratory burst, lysozyme activity, WBCs, and phagocytic activity were asymmetrical (Fig. 7), which indicates possible publication bias or the presence of systematic differences among studies.

Discussion

The summary statistics was calculated using the common effect and random effects models, but only the summary statistics from the random effects models is reported in the “Results” section. Random effects model assumes that the study effect is from a distribution of study effects. Heterogeneity is incorporated and the confidence interval is thus wider. The common effect model on the other hand assumes that one true effect underlies all studies and any differences among studies are due to random error. Heterogeneity is thus ignored and the confidence interval is narrower. The random effects model is therefore more appropriate and commonly used in meta-analysis. In this study, the heterogeneity for respiratory burst, lysozyme activity, WBCs, and phagocytic activity was significantly high among studies and the funnel plots were asymmetrical. Asymmetry of funnel plots is used to indicate possible publication bias. I^2 values above 50% are indicative of little homogeneity among studies or the presence of inconsistencies among studies. However, it is noteworthy that the observed asymmetry of the funnel plots may not necessarily indicate the presence of publication bias. Generally, the interpretation of funnel plots is associated with several limitations (Higgins et al. 2003; Page et al. 2021). Inconsistencies among studies might also lead to an asymmetrical funnel plot and a high heterogeneity. Since studies investigating the use of medicinal plants in fish bring together several papers that are highly variable methodologically, a higher heterogeneity and inconsistencies among studies can be expected. In this study, asymmetrical plots may have been caused by factors such as methodological differences (diversity in dosages, length of administration, choice of plant

material (e.g., powder, extract), and inclusion criteria), study quality, and dissemination bias (reporting strategy). According to Higgins et al. (2003), these factors can contribute to a higher heterogeneity in studies. These factors are known to influence the efficacy of medicinal plants in fish, thus increasing the risks of inconsistencies in the results among studies. For example, a specific dosage of a particular medicinal plant can improve immunity in fish, while a similar dose of another plant may fail to elicit the same response in the same fish (Awad and Awaad 2017). Therefore, it is speculated that the observed publication bias or inconsistencies among the studies used in the analysis could be attributable to some of these factors.

Despite the asymmetrical funnel plots and high heterogeneity, the meta-analysis showed that supplementing fish diets with plant products has a significant effect on respiratory burst, WBCs, phagocytic activity, and lysozyme activity of tilapia, African catfish, carp, and trout. The increase in these parameters in fish fed with diets supplemented with plants is an indication that medicinal plants can improve innate immune response in fish. The mode of action of medicinal plants in boosting the immune system in fish is a topic that has not been fully explored. However, medicinal plants have been reported to contain various components that improve the overall health condition of the fish, which enables it to cope with infections. They have been shown to possess a wide range of biological activities such as antioxidant, anti-inflammatory, and anti-stress characteristics, which can improve the general well-being of fish, thus enhancing its immune response against pathogens. In

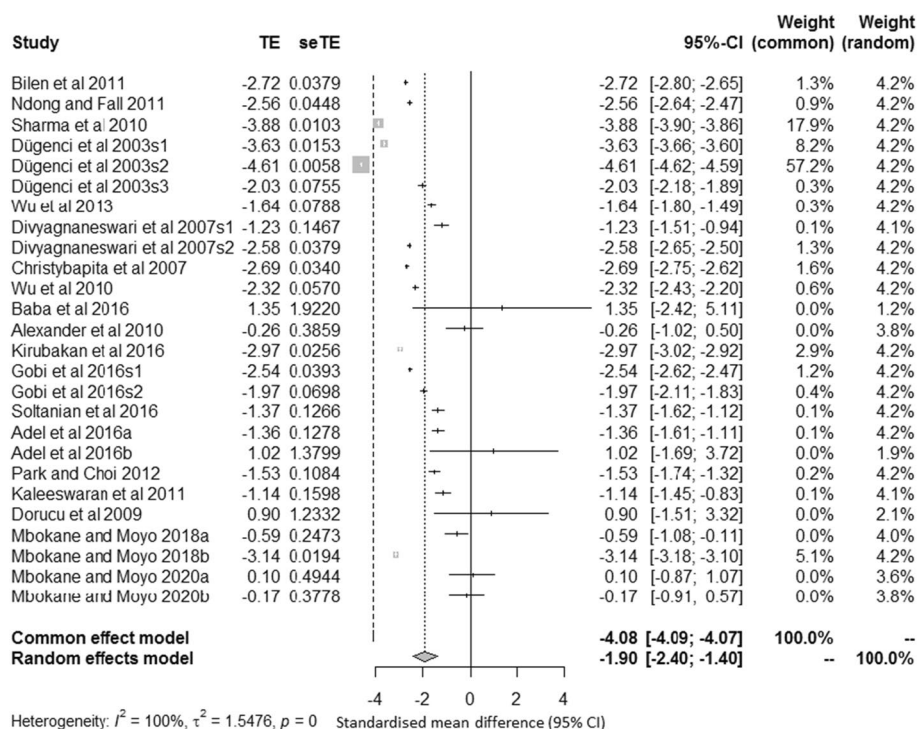


Fig. 2 Forest plot reporting the effect sizes of plants in tilapia, carp, trout, and catfish for respiratory burst (nitro-blue tetrazolium). Key: TE, estimate of treatment effect; seTE, standard error of treatment estimate; CI, confidence interval

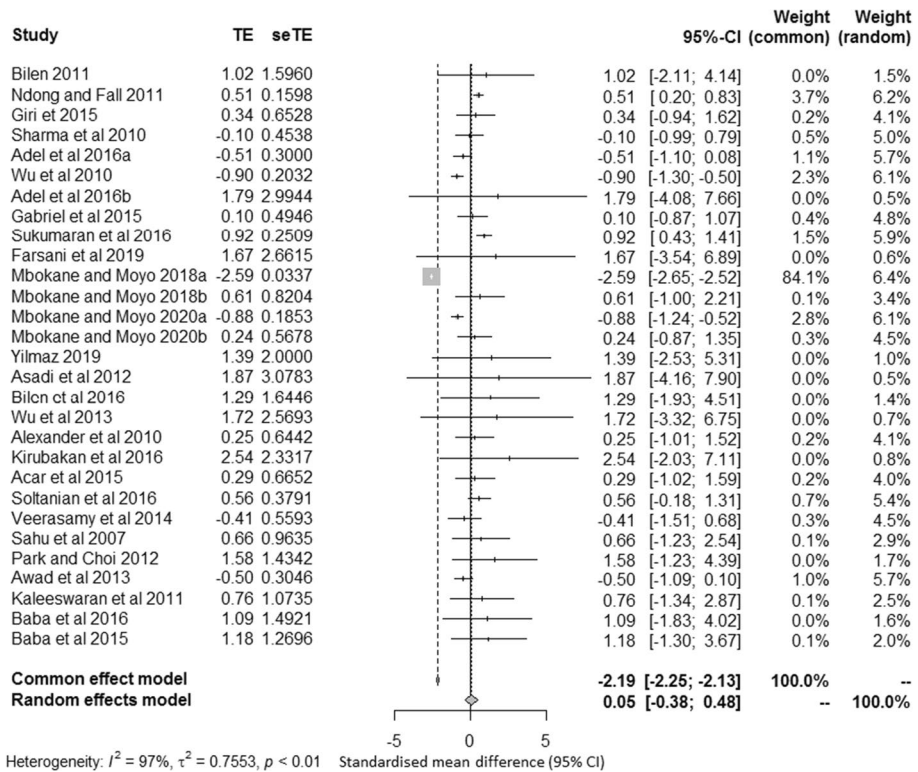


Fig. 3 Forest plot reporting the effect sizes of plants in tilapia, carp, trout, and catfish for lysozyme. Key: TE, estimate of treatment effect; seTE, standard error of treatment estimate; CI, confidence interval

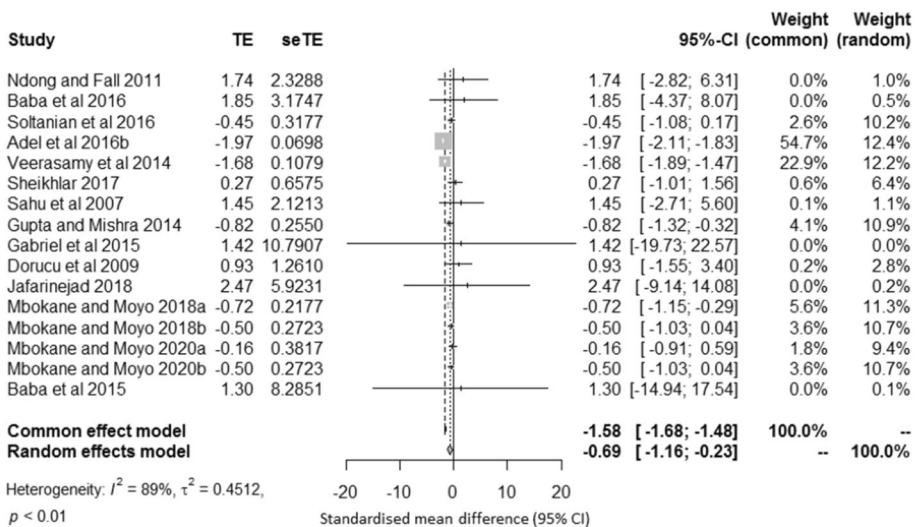


Fig. 4 Forest plot reporting the effect sizes of plants in tilapia, carp, trout, and catfish for white blood cells count. Key: TE, estimate of treatment effect; seTE, standard error of treatment estimate; CI, confidence interval

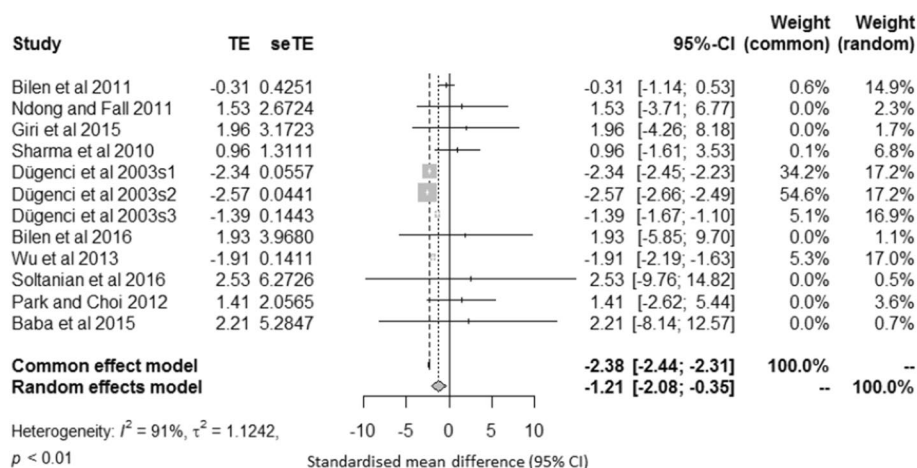


Fig. 5 Forest plot reporting the effect sizes of plants in tilapia, carp, trout, and catfish for phagocytic activity. Key: TE, estimate of treatment effect; seTE, standard error of treatment estimate; CI, confidence interval

vertebrates, the antioxidant system is considered the most important immune defense system against diseases (Meng et al. 2019). The presence of high levels of free radicals (toxic molecules affecting health cells) in fish can lead to chronic oxidative stress-related diseases, leading to poor health and a weaker immune response. On the other hand, medicinal plants have been shown to act as antioxidants. The main function of an antioxidant is to protect the body against the destructive effects of free radical damage. Medicinal plants are known to possess bioactive compounds with redox active molecules that can inhibit the generation of oxygen anions and scavenge free radicals (Chakraborty and Hancz 2011). This action is known to improve the general physiological condition of animals, including fish (Citarasu 2010). The antioxidant activities associated with medicinal plants have been demonstrated to have similar effects to superoxide dismutase, metal ion chelators, and xanthine oxidase inhibitors (Citarasu 2010). Superoxide dismutase (SOD) is an important enzyme that provides an antioxidant defense against oxidative stress in the body. It acts as a therapeutic agent by scavenging free radicals (Younus 2018). Therefore, enhancement of the antioxidant systems in fish is regarded as one of the mechanisms needed to strengthen immunity. The major classes of bioactive compounds known to possess strong antioxidant properties include terpenoids, polyphenols, organosulfides, alkaloids, coumarins, triterpenoids, b-sitosterol, steroidal lactones, volatile oils, and flavonoids (Citarasu 2010; Chakraborty and Hancz 2011). The majority of the plants included in the analysis are known to contain a combination of these compounds, which gives an indication on what other types of plants could be of interest for further research. Medicinal plants have also been reported to contain phytochemicals that may directly stimulate the innate defense mechanisms by acting on receptors of immune cells such as T-cells, natural killer, B-cells, and macrophages as well as triggering the expression of immune-related genes and the production of cytokines (Chakraborty et al. 2013; Bricknell and Dalmo 2005). This may lead to the synthesis of anti-pathogenic molecules, thus increasing disease resistance and overall fish health (Chakraborty et al. 2013). This shows that medicinal plants can also be used as immunostimulants in fish, thus providing greater protection against pathogens.

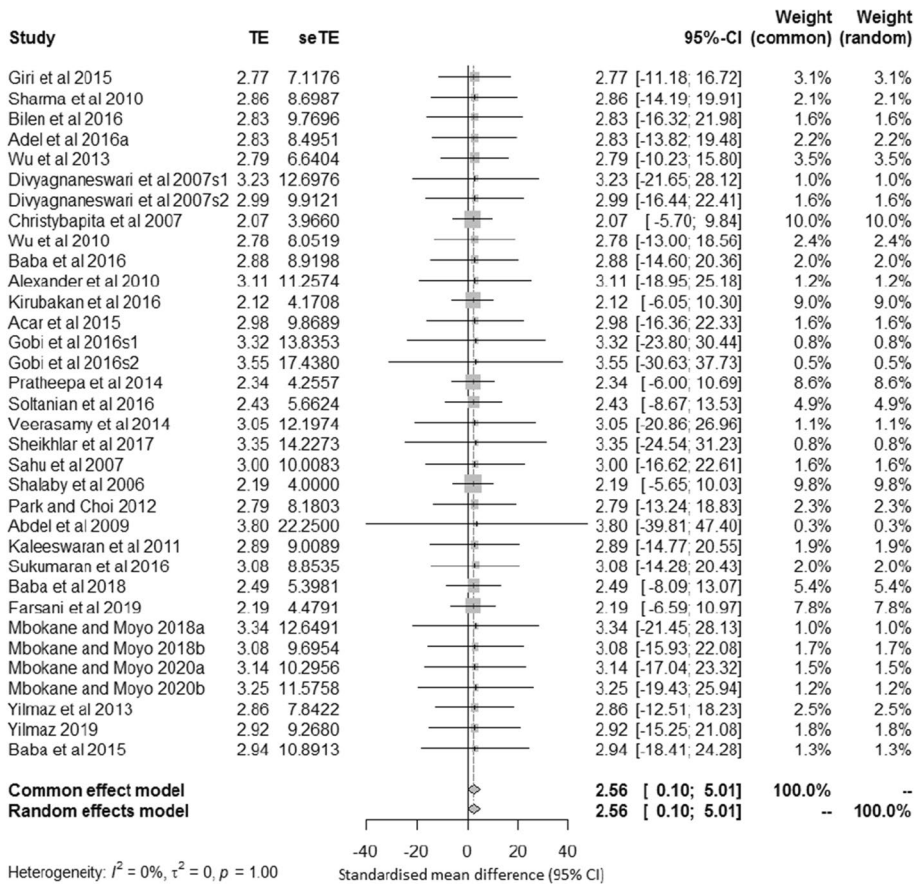


Fig. 6 Forest plot reporting the effect sizes of plants in tilapia, carp, trout, and catfish for survival rate. Key: TE, estimate of treatment effect; seTE, standard error of treatment estimate; CI, confidence interval

Most of the studies included in the analysis reported that survival rate increased in fish fed with diets supplemented with plants following a challenge with a known pathogen. This is usually observed in fish that have displayed an increase in immunological parameters such as WBCs, respiratory burst, phagocytic, and lysozyme activities (and other immune indicators) after feeding with dietary plants. The meta-analysis showed that the addition of plants in fish diets did not have a significant effect on survival rate. It must also be highlighted that heterogeneity for the meta-analysis for survival rates was significantly low (0%), indicating that statistical heterogeneity was minimal among studies. The funnel plot also showed less variation among studies. This is perhaps attributed to the consistency in the reporting style of the results among studies. The majority of studies included in this analysis used pure cultures of *Aeromonas hydrophila* (80%) followed by *Streptococcus* (*Streptococcus agalactiae* and *S. iniae*) (10%) and *Edwardsiella tarda* (10%) as test pathogens. These studies showed that fish fed with the diets supplemented with plants displayed increased resistance against these pathogens compared to fish fed with the control, which displayed low survival. The pathogens used in the studies included in the analysis are among the most virulent pathogenic bacteria in freshwater fish culture and have proven

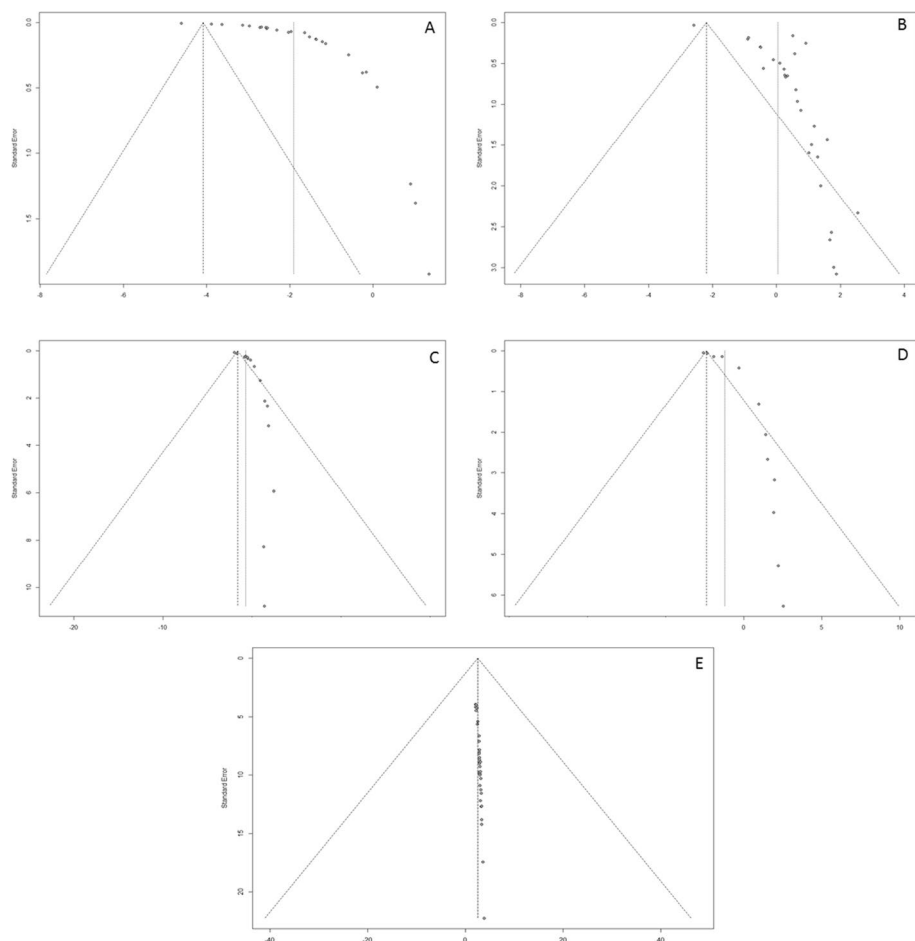


Fig. 7 Asymmetric funnel plots reporting the effect sizes for **A** respiratory burst, **B** lysozyme activity, **C** white blood cells, **D** phagocytosis, and **E** survival rate

difficult to contain. It is therefore of interest to note that infections caused by these pathogens can be managed using medicinal plants as immunostimulants. However, it is noteworthy that all the studies analyzed used pathogenic bacteria and none used fungal or protozoan pathogens in their challenge trials. Fungal pathogens such as *Saprolegnia parasitica* are among the most common opportunistic disease-causing agents affecting a number of freshwater fish species (van West 2006). The protozoan *Ichthyophthirius multifiliis* (Ich) is also a troublesome pathogen in freshwater fish culture. It is thus advisable for future studies to include other pathogenic groups such as fungi and protozoans in the challenge trials in order to determine if the response from fish fed with diets enriched with plant products will be similar to responses reported for bacterial pathogens. Nevertheless, a fish with an improved immunity can be expected to resist a wide spectrum of pathogens including bacteria, fungi, protozoans, parasites, and viruses.

Overall, the current analysis shows that the supplementation of aquafeeds with medicinal plants can prevent diseases in the freshwater aquaculture industry. This observation

is consistent with Reverter et al. (2021)'s analysis that showed that the use of plant supplementation can improve immunity in various fish species. However, the application of medicinal plants in aquaculture is still limited in scope. Generally, the use of plants in aquaculture is still at an exploratory phase, despite several studies over the past two decades having shown their efficacy in strengthening immunity in fish. In Southern Africa, for example, the application of medicinal plants in aquaculture is still limited, save for few farmers who use baths (extracts added to water) to treat external infections. One of the challenges for the lack of wide-scale application of plants in aquaculture could be that there is no consensus on the use of plant supplements in fish diets. Researchers use different methodologies (dosage, material type, treatment duration) when evaluating the inclusion of different plants in fish diets, leading to lack of standardization in application. Furthermore, the efficacy of plants also varies based on its phytochemical composition, which is known to differ among plants due to various factors such as climate, season, soil type, and processing techniques (Zidorn et al. 2005). This suggests that the efficacy of a plant from a particular region can improve immunity while the same plant in another region may fail to produce the same response in the same fish. It also implies that a specific dosage from a particular plant can improve immunity in fish, whereas a similar dosage from another plant cannot elicit the same response in the same fish (Awad and Awaad 2017). It is therefore important for regions to prioritize locally available plants in order to standardize processing techniques. For example, the literature reveals that most of the studies investigating the use of plants in aquaculture are mainly found in Asian (China, India, Thailand, and Korea) and some few countries in the Middle East (Bulfinch et al. 2015). In China, most of the studies have focused on the application of Chinese medicinal plants in aquaculture. Pu et al. (2017) noted that Chinese medicinal plants have long been applied as antibacterial and immunomodulatory agents in aquaculture in China, where they are considered effective replacements for antibiotics, chemicals, vaccines, and other synthetic compounds.

The other challenge limiting the utilization of plants in fish diets is that information is not always well promoted among the targeted end-users. This seems to be the main problem facing farmers in the small-scale freshwater aquaculture sector. In Southern Africa, for instance, very few freshwater fish farmers are aware of the potential use of medicinal plant products in aquaculture. There is thus a need to increase awareness on the potential use of plants as immunostimulants in fish. Efforts should be made to educate and train farmers to process plant material and formulate their own fish diets. Medicinal plants can be easily prepared on the farm without the need for expensive machinery. Although majority of the plants that have been tested in fish are not widely available, farmers should be encouraged to use some of the most popular medicinal plants that have been shown to enhance immunological responses in fish. Medicinal plants such as ginger (*Zingiber officinale*), aloe (*Aloe vera*), garlic (*Allium sativum*), peppermint (*Mentha piperita*), green tea (*C. sinensis*), lemon (*Citrus limon*), cinnamon (*Cinnamomum verum* or *zeylanicum*), garlic chives (*A. tuberosum*), blueberry (*Vaccinium ashei*), rosemary (*Rosmarinus officinalis*), tomato (*Solanum lycopersicum*), mango (*Mangifera indica*), onion (*Allium cepa*), guava (*Psidium guajava*), turmeric (*Curcuma longa*), Sundial lupine (*Lupinus perennis*), nutmeg (*Myristica fragrans*), basil (*Ocimum basilicum* and *sanctum*), and oregano (*Origanum vulgare*) have been reported to enhance immunity in some freshwater fish species (Awad and Awaad 2017). The majority of these plants are widely cultivated in many African regions where they are used as vegetables, spices, and fruit production and most farmers have access to them. There is therefore a need to increase awareness on the use of these plants in aquaculture within the fish farming community.

There are also many medicinal plants in Southern Africa that are widely used in traditional medicine and have the potential to be used in aquaculture. For example, *Moringa oleifera* (moringa) and *Artemisia afra* (wormwood) grow readily across different climates in Southern Africa, and results from previous studies have demonstrated that they can improve immunity in both tilapia and African catfish (Mbokane and Moyo 2018a, 2018b, 2020a, 2020b). *Artemisia afra* has been shown to possess compounds such as flavonoid, phenolic, monoterpenic, aromatic, terpenic, and sesquiterpenes. These compounds are known for various pharmacological activities such as anti-inflammatory, immunostimulatory, antioxidant, antimicrobial, and antifungal properties (Van Vuuren and Viljoen 2006; More et al. 2012). *Moringa oleifera* is also a popular medicinal plant in Southern Africa. The leaves of this plant are rich in bioactive compounds such as flavonoids, phenols, carotenoids, and vitamins (Moyo et al. 2012). Therefore, the use of these plants in freshwater aquaculture can provide a more economically sustainable alternative to prevent diseases and should be strongly recommended for the farmers who have access to them. The long-term benefit to fish farmers is that they will be managing diseases in a cost-effective manner because they will be using cheaper and locally available resources, which is likely to enhance production on farms since fewer resources will be channeled to treating diseases.

Conclusion and recommendations

The meta-analysis performed in this study showed that there is a high potential for the use of medicinal plants in preventing diseases in tilapia, African catfish, trout, and carp culture. The use of plant supplements in fish can benefit the freshwater aquaculture industry the most, especially the small-scale sector where disease management is often poor due to lack of resources. Thus, there is a need to create appropriate platforms to facilitate the transfer of information to fish farmers. It is therefore recommended that local farmers be trained to prepare their own diets supplemented with medicinal plants. Furthermore, it is recommended that detailed studies be undertaken on locally available plants in order to identify more plants that are suitable for the local conditions.

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Author contribution Esau M. Mbokane and Ngonidzashe A.G. Moyo conceived the study and wrote the manuscript. Esau M. Mbokane and Ngonidzashe A.G. Moyo reviewed the literature, collected data, and analyzed and interpreted it.

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Data availability The data that supports the findings of this study is available on request from the corresponding author.

Code availability Not applicable.

Declarations

Competing interests The authors declare no competing interests.

Ethics approval No ethical clearance was required for this study.

Conflict of interest The authors declare no competing interests.

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