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Phytoconstituents Profile of *Acorus calamus*, *Cardiospermum halicacabum* and *Solanum nigrum* as Potential Source of Fish Feed Supplementation with Reference to the Bioactive Compounds

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Abstract: The growing demand for high quality food has induced a rapid expansion of the aquaculture sector. On the other hand, this sector has to overcome numerous challenges and problems triggered by the adoption of intensive farming systems, such as stress and high susceptibility to diseases. The improper use of chemicals and antibiotics has led to the development of antibiotic resistance in fish, with consequent health risks for consumers. Natural additives are increasingly used in aquaculture and among these; medicinal plants are constantly under investigation as safe and eco-friendly alternatives to chemicals. Currently, medicinal plants are being tested in aquaculture as a safe and eco-friendly substance to modulate immune status, enhance growth performance and prevent fish disease. It is well known that many active compounds are responsible for potential bioactivities. Hence, the present study aimed to identify bioactive compounds from traditional medicinal plants, *Acorus calamus, Cardiospermum halicacabum* and *Solanum nigrum* in order to establish the phytochemical profile to develop the potential aqua supplementary feed for aqua hatchery production. For this study the different part of medicinal plants, Spadix of *Acorus calamus* (Obtained from Siddha Medicine Shops in Chennai), leaf of *Cardiospermum halicacabum* and leaf of *Solanum nigrum* were collected from Vengodu Village in Cheyyar taluk, Tiruvannamalai district, Tamil Nadu and the crude bioactive compounds were extracted using ethanol. The different phytoconstituents and the volatile compounds were identified by standard methods and Gas Chromatography and Mass Spectroscopy (GC-MS) techniques. The result of the phytochemical analysis shows the presence of different phytoconstituents such as alkaloids, phenol, saponins, flavonoids, steroids, terpenoids and tannins in three crude extract. In GC-MS analysis, the three medicinal plant species recorded totally 39 volatile compounds. The number of compounds recorded in *A. calamus, C. halicacabum* and *S. nigrum* is 14, 13 and 12, respectively. All these bioactive compounds showed common biological activities like antioxidant, antimicrobial, cytotoxic and Antioxidant activity without toxicity. Hence, the ethanolic extracts of *A. calamus, C. halicacabum* and *S. nigrum* can used in aquaculture industry with a view to providing safe and eco-friendly compounds for replacing antibiotics and chemical compounds as well as to enhance immune status and control fish diseases.

Keywords: Medicinal plants, Aquaculture, Bioactive compounds, Fish feed, Phytochemicals, *Acorus calamus*, *Cardiospermum halicacabum*, *Solanum nigrum*

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Introduction

Freshwater aquaculture is one of the most important and fastest growing food producing industries worldwide (Nielsen *et al.*, 2016; Rocha *et al.*, 2022). In developing countries, freshwater aquaculture plays an important role as an alternative source of cheap animal protein (Mohanty *et al.,* 2016; Soliman *et al.,* 2016). It is also considered an important sector that can create job opportunities for poor communities (Germain *et al.,* 2015; Kaleem and Sabi, 2021). Lack of good quality feeds, disease outbreaks, and poor quality of broodstock has been identified as among the key factors hindering production and profitability of the fish culture (Ina-Salwany *et al.,* 2019; Munguti *et al.,* 2022). Therefore, there is a need for freshwater fish farmers to find alternative sources of feed ingredients that can be used to replace expensive ingredients such as fishmeal or feed additives that can be used to promote growth performance, disease resistance, and reproduction.

One of the cheapest and promising alternative sources of feed additives include the use of medicinal plants (Vijayaraj and Kumaran, 2017; Dawood *et al.,* 2018). Plants contain various bioactive compounds such as terpenoids, alkaloids, flavonoids, tannins, saponins, pigments, phenolics, steroids, and essential oils (Vijayaraj and Kumaran, 2017). A number of these compounds found in most medicinal plants possess various pharmacological functions such as antistress, appetite stimulation, growth promotion, immunostimulation, aphrodisiac, antimicrobial, antifungal, and antiparasitic properties (Citarasu, 2010; Vijayaraj and Kumaran, 2017; Reverter *et al.,* 2017; Vijayaraj *et al.,* 2018; Jana *et al.,* 2018). This shows that plants can be used as feed additives or substitutes for conventional feed ingredients in aquafeeds fish. Several studies have shown that plants can boost fish's growth performance and disease resistance. Some plants have been reported to enhance the development of commercially available fish species (Citarasu, 2010; Vijayaraj and Kumaran, 2017; Reverter *et al.,* 2017; Jana *et al.,* 2018). Among them, the *Acorus calamus*, *Cardiospermum halicacabum*, and *Solanum nigrum* are considered suitable feed additives in aquafeeds because they contain adequate amounts of proteins, vitamins, essential amino acids, unsaturated fatty acids, and minerals (Ramya and Jayakumararaj, 2009; Govindan *et al.,* 2020) that can meet the nutritional requirements for some of the widely cultured fish species.

Conversely, the identification of active compounds is of utmost importance to know the pharmacological activity of medicinal plants (Taylor *et al*., 2001; Dutra *et al*., 2016). Several methods, namely, chromatography, spectroscopy etc. are adapted to isolate, identify and quantify bioactive phytochemicals from natural sources (Vijayaraj *et al*., 2019). Although different studies are available on *A. calamus*, *C.halicacabum*, and *S. nigrum* but to the best of our knowledge, very few investigations have delineated its chemical profile based on aqua feed. Therefore, the present study aimed to identify bioactive compounds from *A. calamus*, *C. halicacabum*, and *S. nigrum* in order to establish the phytochemical profile of this plant to identify the potential aqua supplementary feed for fresh waster fishes.

Materials and Methods

Collection and Identification of experimental plants:

The Bulk samples (7±1 kg) of different part of medicinal plants such as Spadix of *Acorus calamus* was obtained from Siddha Medicine Shops in Chennai, the leaf of *Cardiospermum halicacabum* and *Solanum nigrum* were collected from Vengodu Village in Cheyyar taluk, Cheyyar, Tiruvannamalai district, Tamil Nadu (Latitude: 12.6630445;

Longitude: 79.5367251) by hand picking. Morphological characterization and identification of the collected samples were carried out by following the descriptions from Motley (1994), DeFilipps and Krupnick (2018) and Knapp *et al.* (2019). The collected samples were shade dried at room temperature (28 ±2 º C). The shade dried plant materials were ground into a coarse powder using a mortar and pestle for further crude extraction process.

Extraction of Crude Bioactive Compound:

For the extraction of crude compound from different parts of medicinal plants, the coarse powdered plant material was extracted with ethanol by using soxhlet apparatus. The solvent were removed under reduced pressure to get crude extract and stored for further phytochemical studies.

Investigation primary Phytoconstituents:

In the present study, the ethanolic extracts of medicinal plants were subjected to qualitative phytochemical test using standard procedure to detect various phytoconstituents present in them (Harborne, 1998).

Identification of Bioactive Compounds:

Identification of the bioactive compounds was carried out using Gas Chromatography and Mass Spectroscopy (GC-MS) techniques. The GC-MS analysis of the bioactive compounds from different crude extracts of medicinal plant was done using Agilent Technologies GC systems with GC-7890A/MS-5975C model (Agilent Technologies, Santa Clara, CA, USA) equipped with HP-5MS column (30 m in length × 250 μm in diameter × 0.25 μm in thickness of film). The spectroscopic detection of GC – MS involved an electron ionization system which utilized high energy (70 eV). The pure helium gas (99.95%) was used as a carrier gas with flow rate of 1 ml/min. The initial temperature was set at 50 to 150 °C with increasing rate of 3 °C per min and holding time of about 10 min. Finally, the temperature was increased to 300 °C at 10 °C per min. One microliter of the prepared 1% of the extracts diluted with respective solvents was injected in a splitless mode. Relative quantities of the chemical compounds present in each of the extracts were expressed as percentage based on peak area produced in the chromatogram. The identification of components was based on NIST libraries as well as comparison of their retention indices. The constituents were identified after comparison with those available in the computer library (NIST and Willey) attached to the GC-MS instrument and the results obtained have been tabulated.

Results and Discussion

Use of herbal plants as additives in fish feed has recently been the subject of extensive research (Dawood *et al.,* 2018). The materials used for fish supplements are leftovers from companies that create herbal foods, drinks, and supplements for humans. As a result, their price is lower for use as an ingredient in feed supplements (Sogbesan and Ugwumba, 2008). Some of the common medicinal plants such as turmeric, ginger, curcuma, aromatic ginger, noni, catappa leaves, betel leaf, and others are herbal plants that are frequently used as supplements in aqua feed (Dagar *et al.,* 2020). The primary advantages of providing fish herbal supplements are that they have a better digestive system, need less feed, have increased appetites, and develop faster each day (Chris *et al.,* 2018; Lieke *et al.,* 2020). Studies that have been done employing herbal plants as supplements in fish feed have produced positive outcomes. This may serve as the foundation for the use of herbal plants in fish feed in the creation of aquaculture systems to improve the production, endurance, and health of fish. Hence, in this study we have selected three potential medicinal plants, *A. calamus*, *C. halicacabum*, and *S. nigrum* to establish the phytochemical profile of these plants and to prove the potential aqua supplementary feed for fresh waster fishes with reference to the bioactive properties.

Hence, there is a constant need to increase productivity in aquaculture, particularly to improve growth rate, feed utilization as well as stress resistance of fish (Chakraborty *et al.*, 2014;

Aly and Albutti, 2014). Because of consumer concerns and strict regulations in many countries, the use of synthetic chemicals, hormones and antibiotics is becoming unviable and natural compounds are more acceptable to the public (Chakraborty *et al*., 2014; Aly and Albutti, 2014). A wide variety of chemical compounds are found in plants, and many of them have beneficial effects on appetite, growth and the immune status of fish acting through different mechanisms (Awad and Awaad, 2017). The phytochemicals contained in herbs may enhance the innate immune system, possess antimicrobial capabilities, and are redox active molecules with antioxidant characteristics that may help to improve the general physiological condition of fish (Chakraborty *et al*., 2011, 2014). Many studies have discussed the values of phytochemicals as feed additives (Chakraborty *et al.,* 2011, 2014). Another paramount concern related to phytochemicals is their endocrine modulator effect that can be applied both in aquaculture targeting the production of table fish and the growing sector of commercial fish production.

Plant extracts contain a vast source of different molecules (alkaloids, flavonoids, pigments, phenolics, terpenoids, steroids, and essential oils), which have intrinsic bioactivities on animal physiology and metabolism (Chakraborty *et al.*, 2011, 2014; Vijayaraj and Kumaran, 2017). These substances, widely known as phytochemicals, have been in use since the beginning of recorded history in the diet of humans and terrestrial animals. More recently, different studies have shown that many of them may also have beneficial effects on appetite, growth, stress, and the immune status of fish, acting through different mechanisms (Chakraborty *et al.*, 2011, 2014; Syahidah *et al.,* 2014; Vijayaraj and Kumaran, 2017). These phytochemicals contained in different plant sources may enhance the innate immune system; possess antimicrobial capabilities, and redox active molecules with antioxidant characteristics that may help to improve the general physiological condition of fish (Chakraborty *et al.*, 2011, 2014). The use of phytochemicals as feed additives is currently a popular field in aquaculture, and it has been heavily investigated in recent years. There is an adequate quantity of evidence to conclude that dietary supplementation of phytochemicals improves growth, stimulates the immune response, and improves antioxidant status in finfish (Chakraborty *et al*., 2011, 2014). The result of the phytochemical analysis shows the presence of different phytoconstituents such as alkaloids, phenol, saponins, flavonoids, steroids, terpenoids and tannins in three crude extract. Number of compounds recorded in the three medicinal plant species ranged between five and nine. Maximum of nine compounds extracted from A*. calamus* and eight from *C. halicacabum* and six compounds from *S. nigrum*. Phenol, flavonoids and steroids occurred in the extracts of all the three species of medicinal plants. Number of other phytochemicals showed variation pertaining to occurrence in the three species of medicinal plants (Table 1). Hence, these three plants can be used as potent feed additives/supplementary in the aquaculture sector.

Medicinal plants are the resources of new drugs (Vijayaraj *et al.*, 2018; Avarave *et al*., 2022). Many of the modern medicines are produced indirectly from the medicinal plants (Avarave *et al.*, 2022). They have contributed many ingredients to fight against various diseases and illness. The analysis and extraction of plant material play an important role in the development, modernization and quality control of herbal formulations (Chakraborty *et al.*, 2011, 2014). Studying of medicinal plants also facilitates to comprehend plant toxicity and also helps to protect human and animals from natural poisons (Vijayaraj *et al.*, 2019, 2022). Medicinal plants are at great interest to the researcher in the field of biotechnology, as most of the drug industries depend on medicinal plants for the production of pharmaceutical compounds (Gomathi *et al.*, 2015; Hosseinzadeh *et al.*, 2015; Anand *et al.*, 2019). The chemical features of these constituents differ considerably among different species (Gomathi *et al.*, 2015; Hosseinzadeh *et al.*, 2015; Anand *et al.*,

Table 1: Phytochemical analysis of three medicinal plants

Phytochemical	*A. calamus*	*C. halicacabum*	*S. nigrum*
Alkaloids	+	+	-
Phenol	+	+	+
Saponins	+	-	-
Flavonoids	+	+	+
Steroids	+	+	+
Phytosterol	-	+	+
Glycosidase	+	+	+
Carbohydrates	+	+	+
Protein	+	+	+
Lipid	+	+	+

2019). GC-MS method used for the analysis of the obtained extracts can be an interesting tool for testing the amount of some active compounds in herbs used in cosmetic, drugs, pharmaceutical or food industry. Hence, the GC-MS is used to determine and identify compounds present in a plant sample. GC-MS plays an essential role in the phytochemical analysis and chemotaxonomic studies of medicinal plants containing biologically active components. The GC-MS analysis results of three medicinal plant species recorded totally 39 volatile compounds. The number of compounds recorded in *A. calamus, C. halicacabum* and *S. nigrum* is 14, 13 and 12, respectively (Tables 2-4).

The ethanolic extract of *A. calamus,* contains 14 volatile compounds such as 1-Propenyl-aziridine (3.25), Propylure (4.12), Imidazo[4,5-e][1,4]diazepine-5,8-dione, 1,4,6,7-tetrahydro-1,4, 7-trimethyl-2-nitro (4.252), Thymol (4.649), 2-Propen-1-one (5.642), Caryophyllene (6.077), 2,5-Cyclohexadien-1-one (6.834), 2,5-dimethyl-4-[(2,4,5-trimethylphenyl) imino] (7.439), Chalcone (7.675), N - Methyl - 1 - adamantaneacetamide (10.077), [1,2,4]Triazolo [1,5-a]pyrimidine-6-carboxylic acid, 4,7-dihydro-7-imino, ethyl ester (10.256), 3-Methylindole-2-carboxylic acid, 4,5,6,7 -tetrahydro-, ethyl ester (10.937), N-(2-Acetylcyclopentylidene) cyclohexylamine (11.476) and Silicic acid (12.299). Among these compounds 2-Propen-1-one (5.642), 3 - Methylindole - 2 - carboxylic acid, 4,5,6,7-tetrahydro-, ethyl ester (207.273) and N - (2-Acetylcyclopentylidene) cyclohexylamine (11.476) needs to identify the biological properties. Most of the compounds are responsible for common activities like anti-inflamatory, antimicrobial and cytotoxic and Antioxidant activity (Table 2, Fig.1).

The ethanolic extracts of *C. halicacabum*, contains 13 bioactive compounds such as Propane, Dichloroacetic acid, D-Carvone, 2,5-Cyclohexadien-1-one, 3, 4, 5- Trimethoxy-. beta.-methyl-. beta.-nitrostyrene, 2-Methyl-3,5-dinitro-benzyl alcohol, 2-Amino-3,5-dibromopyrazine, Benzyl Benzoate, 2-Hydroxy-1-isoindolinone, 2,6-Lutidine, l-Methionine, 3,5-Dimethylbenzaldehyde, thiocarbamoylhydrazone and Quinoline which is identified with the retention time 2.569, 3.24, 4.12, 5.245, 6.332, 6.975, 8.743, 9.831, 10.833, 11.221, 12.327, 13.149 and 13.651 minutes, respectively. All these bioactive compounds are responsible for several acute and chronic diseases (Table 3, Fig. 2).

The ethanolic extract of *S. nigrum* contains 12 volatile bioactive compounds, among this only Imidazole (2.9), 2,5-Cyclohexadien-1-one (4.621), Propanephosphonic acid (7.07), (9-Oxo-9,10-dihydroacridin-4-yl)acetic acid (9.717), 1-Benzazirene-1-carboxylic acid (10.37) and Caffeine (10.682) molecules showed good biological properties such as Antioxidant, Antiparasitic, Immunostumulant, Anticancer, Antimicrobial and Hypercholesterolemia activity, respectively (Table 4, Fig. 3).

Table 2: GC-MS analysis of *A. calamus*

Peak No.	R. Time	Name	Molecular Formula	Molecular Weight (g/mol)	Pharmacological Properties	Reference
1	3.25	1-Propenylaziridine	C_5H_9N	83.134	Unidentified	-
2	4.12	Propylure	$C_{18}H_{32}O_2$	280.452	Sex pheromone	Jacobson 1969
3	4.252	Imidazo[4,5-e][1,4]diazepine-5,8-dione, 1,4,6,7-tetrahydro-1,4,7-trimethyl-2-nitro-	$C_{12}H_{12}N_2O_2$	216.24	Unidentified	-
4	4.649	Thymol	$C_{10}H_{14}O$	150.221	Antidiabetic activity	Saravanan *et al.,* 2016
5	5.642	2-Propen-1-one	$C_{16}H_{13}BrO_2$	317.182	Unidentified	-
6	6.077	Caryophyllene	$C_{15}H_{24}$	204.357	Hypercholesterolemic	Baldissera *et al.,* 2017
7	6.834	2,5-Cyclohexadien-1-one	C_6H_6O	94.113	Anbiotics	NCBI, 2019a
8	7.439	2,5-dimethyl-4-[(2,4,5-trimethylphenyl)imino]-	$C_{17}H_{19}NO$	253.345	Antioxidant	Kumar *et al.,* 2019
9	7.675	Chalcone	$C_{15}H_{12}O$	208.26	Anti TB	
10	10.077	N-Methyl-1-adamantaneacetamide	$C_{13}H_{21}NO$	207.317	Antioxidant activity	Singh *et al.,* 2017
11	10.256	[1,2,4]Triazolo[1,5-a]pyrimidine-6-carboxylic acid, 4,7-dihydro-7-imino	$C_8H_9N_5O_2$	207.193	Unidentified	-
12	10.937	3-Methylindole-2-carboxylic acid, 4,5,6,7-tetrahydro-, ethyl ester	$C_{12}H_{17}NO_2$	207.273	Unidentified	-
13	11.476	N-(2-Acetylcyclopentylidene) cyclohexylamine	$C_{12}H_{21}N$	179.307 g/mol	Unidentified	-
14	12.299	Silicic acid	H_4O_4Si	96.113	Biodegratation properties	Sabourin *et al.,* 1998

Table 3: GC MS analysis of *C. halicacabum*

Peak No.	R. Time	Name	Molecular Formula	Molecular Weight (g/mol)	Biological Properties	Reference
1	2.569	Propane	C_3H_8	44.097	Anticancer activity	Kumagai *et al.*, 2013
2	3.24	Dichloroacetic acid	$CHCl_2COOH$	128.936	Anticancer activity	James *et al.*, 2016
3	4.12	D-Carvone	$C_{10}H_{14}O$	150.221	Antidiabetic activity	Rentzsch *et al.*, 2012
4	5.245	2,5-Cyclohexadien-1-one	C_6H_6O	94.113	Unidentified	-
5	6.332	3,4,5-Trimethoxy-.beta.-methyl-.beta.-nitrostyrene	$C_{12}H_{15}NO_5$	253.254	Unidentified	-
6	6.975	2-Methyl-3,5-dinitrobenzyl alcohol	$C_8H_8N_2O_5$	212.161	Antibacterial activity	NCBI, 2019
7	8.743	2-Amino-3,5-dibromopyrazine	$C_4H_3Br_2N_3$	252.897	Anticancer activity	Whelligan *et al.*, 2019
8	9.831	Benzyl Benzoate	$C_{14}H_{12}O_2$	212.248	Anticancer activity	Marie *et al.*, 2013
9	10.833	2-Hydroxy-1-isoindolinone	$C_{12}H_{15}NO_2$	205.257	Unidentified	-
10	11.221	2,6-Lutidine	C_7H_9N	107.156	Against Urinary Tract Infections	Hu *et al.*, 2012
11	12.327	l-Methionine	$C_5H_{11}NO_2S$	149.208	Antioxidant activity	Wu *et al.*, 2018
12	13.149	3,5-Dimethylbenzaldehyde thiocarbamoylhydrazone	$C_{10}H_{13}N_3S$	207.295	Unidentified	-
13	13.651	Quinoline	C_9H_7N	129.162	Antioxidant activity	Vogt *et al.*, 2018

Table 4: GC-MS analysis of *S. nigrum*

Peak No.	R. Time	Name	Molecular Formula	Molecular Weight (g/mol)	Biological Properties	Reference
1	2.9	Imidazole	$C_3H_4N_2$	68.079	Antioxidant and Anticancer	Changqin *et al.,* 2017
2	3.638	-(2-Hydroxyphenyl)(7H)triazolo[e]benzofurazan	$C_{12}H_7N_5O$	359.64	Unidentified	-
3	4.252	Methyl 6,6,8,8,10,10-hexamethyl-3-oxo-2,5,7,9,11-pentaoxa-6,8,10-trisilatridecan-13-oate	$C_{12}H_{28}O_8Si_3$	384.6024	Unidentified	-
4	4.621	2,5-Cyclohexadien-1-one	C_6H_6O	94.113	Antiparasitic	Bottini *et al.,* 2007
5	5.481	Methyl 6,6,8,8,10,10-hexamethyl-3-oxo-2,5,7,9,11-pentaoxa-6,8,10-trisilatridecan-13-oate	$C_{12}H_{28}O_8Si_3$	384.6024	Unidentified	-
6	7.07	Propanephosphonic acid	$C_3H_9O_3P$	124.076	Immunostumulant activity	Bazhanova et al., 2012
7	7.722	1-Tripropylsilyloxydodec-9-yn	$C_{12}H_{22}O$	182.307	Unidentified	-
8	9.367	2-Methyl-6-(5-methyl-2-thiazolin-2-ylamino)pyridine	$C_{10}H_{13}N_3S$	207.295	Unidentified	-
9	9.717	(9-Oxo-9,10-dihydroacridin-4-yl)acetic acid	$C_{15}H_{11}NO_3$	253.257	Anticancer	Kennedy et al., 2015
10	10.37	1-Benzazirene-1-carboxylic acid	$C_{13}H_8N_2O_2$	224.219	Antimicrobial	Guo et al., 2018
11	10.682	Caffeine	$C_8H_{10}N_4O_2$	194.194	Hypercholesterolemia	Avarave et al., 2022
12	11.221	N,N-Diethyl-N'-phenylethylenediamine	$C_{12}H_{20}N_2$	192.306	Unidentified	-

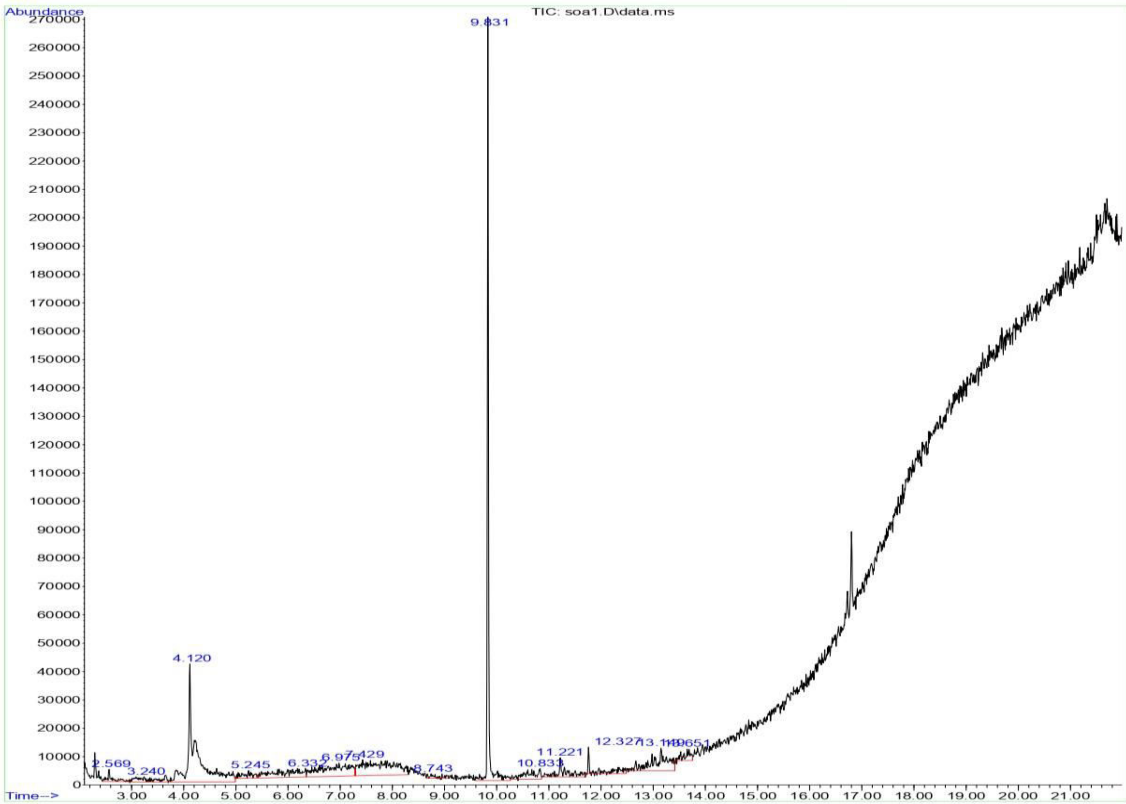

Fig. 1: Gas chromatogram of *A. calamus.*

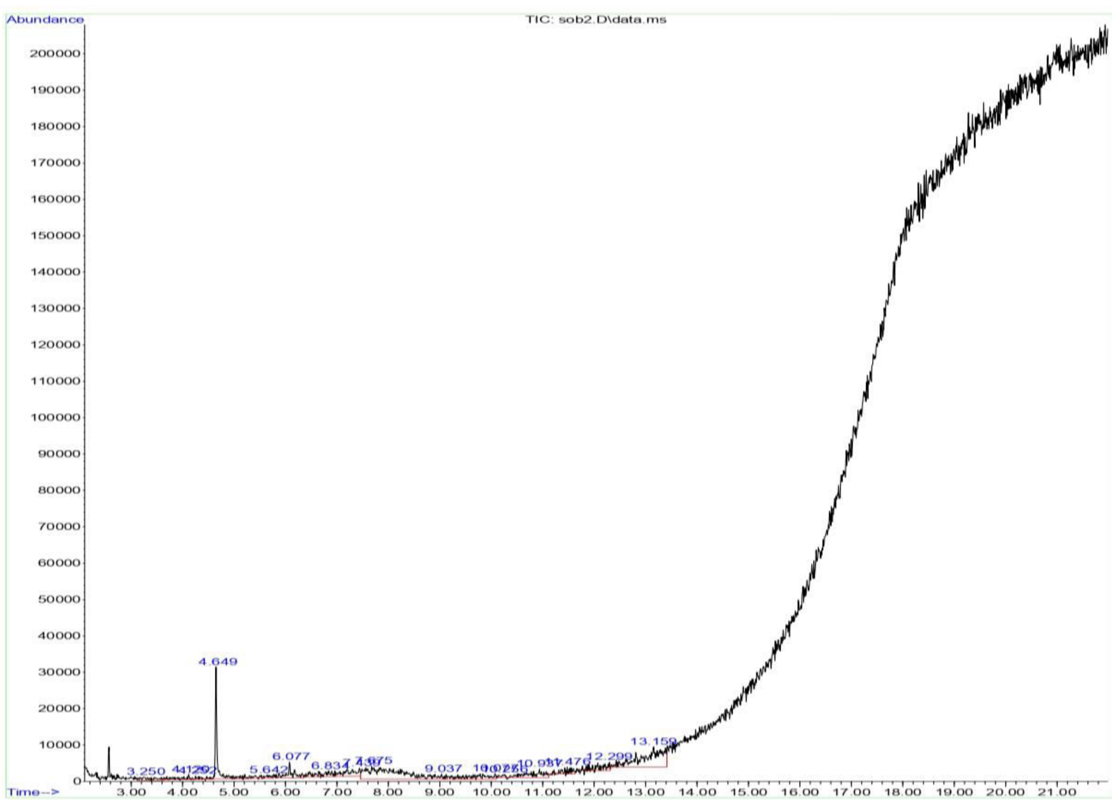

Fig. 2: Gas chromatogram of *C. halicacabum.*

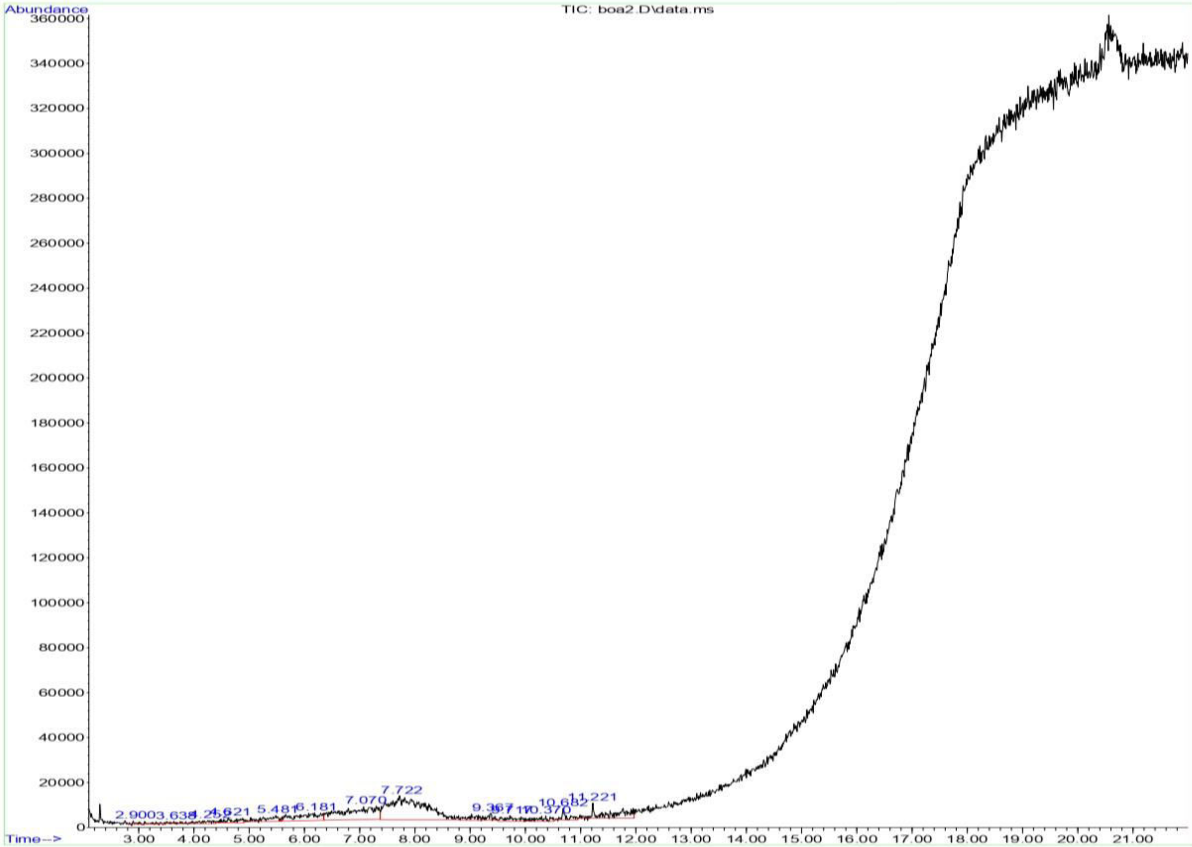

Fig. 3: Gas chromatogram of *S. nigrum.*

Hence, the present study enumerates new secondary metabolites from different medicinal plant using phytochemical and GC-MS analysis. These secondary metabolites from natural sources could be effective compounds in the pharmaceutical applications especially in aquaculture industry for formulation of herbal fish feed/ fish feed additives that can be used to promote growth performance, disease resistance, and reproduction in fresh water fishes. The present study revealed that bioactive compounds from *A. calamus, C. halicacabum* and *S. nigrum* might play an important role when mixed with feed to control bacterial disease in fish. Further detailed studies are necessary before applying herbal therapy by herbal feed in field condition.

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