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PHYTOCHEMICAL SCREENING OF DIFFERENT EXTRACTS OF ARGEMONE MEXICANA L. AND ITS ANTIBACTERIAL AND ICTHYOTOXICITY ACTIVITY

Pushpalatha M[1], Vignesh A[1], SathyaNarayan[2], Venkatesh R[2].

[1]PACHAIYAPPAS COLLEGE OR MEN, KANCHIPURAM - 631 502.

[2]PACHAIYAPPAS COLLEGE, CHENNAI – 600030.

*CORRESPONDING AUTHOR E-MAIL ID: DRMPL2014@GMAIL.COM

ABSTRACT

To study the phytochemical profile of acetone and methanol extract of A. mexicana. The study was designed to investigate the phytochemicals potentials in A. mexicana. To evaluate the ethyl acetate and chloroform extract of A. mexicana using GC – MS. In this background, the present study intended to evaluate the toxic influence, activity range and potency of A. Mexicana. This study aims to evaluate the antimicrobial potential of extract of A. Mexicana which were found abundance in and around Kanchipuram Dt., Evaluate the toxicity of the underexploited A. Mexicana in freshwater fish Ctenopharyngodon Idella. The objectives of this work is together the information on the antimicrobial properties of compounds from A.mexicana leaves, their extraction and there major applications.

KEYWORDS: A. Mexicana, GC-MS. ANTIBACTERIAL AND ICTHYOTOXICITY ACTIVITY

1. INTRODUCTION

The use of plant has long been part of local cultures and traditions as source of food, medicines and other derivable products. The role of indigenous knowledge in the identification, conservation and utilization of plant species cannot be overemphasized. Though these plants and their derivatives are believed to be nontoxic compared to their synthetic counterparts, they may contain a number of harmful ingredients on their secondary metabolites which may have deleterious side effects including mutagenic potentials.

Bacterial infection causes high rate seaweeds of pernarbw Brazil of mortality in human population and aquaculture organisms. Preventing disease out breaks or treating the disease with drugs or chemicals tackles these problems Now a days, the use of antibiotics increased significantly due to heavy infections disease and the pathogenic bacteria becoming résistance to drugs is common due to discriminate use of antibiotics it becomes a greater problem of giving treatment against resistant pathogenic bacteria (EL-shourg et al., 2017).

1.1 Importance of secondary metabolites

Now a day's seaweeds are considered a novel source of bioactive compounds and produce a great variety of secondary metabolites exhibiting broad spectrum of biological activities (Khotimehenko et al., 2002). Many types of seaweeds have been screamed extensively to isolate natural drugs or biological active substance from different habits around the world. Several solvent like with different polarities eg: hexane, chloroform, ethanol, methanol, acetone and water were used to extract the antimicrobial material from such seaweeds. Compounds with antiviral, antifungal, bacteriostatic and bactericidal activities have been detected in green, brown and red seaweeds with comparable promising results with authentic antibiotics.

The major chemical substances of interest in these surveys have been the polyphenols and tannins, however, other diverse groups of naturally occurring phytochemicals such as alkaloids and saponins have also been reported. The natural active compounds classes or secondary metabolites as alkaloids, saponins, tannins and others have attracted researchers to investigate their chemical, toxicological and pharmacological features.

The alkaloids represent a group of natural products that has had a major impact throughout history on the economic, medical, political and social affairs of humans. They are a diverse group of low molecular weight nitrogen-containing compounds derived mostly from amino acids. These secondary metabolites are found in about 20 % of plant species and they classified as true alkaloids, A wide range of biological activities of alkaloids have been reported: emetic, anti-cholinergic, antitumor, anti-diuretic, sympathomimetic, antiviral, antihypertensive, hypnoanalgesic, antidepressant, miorelaxant, antitussigen, antimicrobial and anti-inflammatory. However, the alkaloids and other natural compounds have complex activities and it is necessary to analyze pharmacological activities in the general tissues, linking the structure with the activity presented. It is common to find pharmacological results where a single experimental model generalizes a biological answer, but these can't be accepted because all the pathologies

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in question are also complex and it is necessary to investigate specific experimental models (Abulude, 2007).

Many metabolites isolated from marine algae possess bioactive effects. The discovery of metabolites with biological activities, from macro algae, has increased significantly in the past three decades; on the other hand, seaweeds have recently received significant attention for their potential as natural antioxidants. Marine organisms are a rich source of structurally novel and biologically active metabolites (Perry et al., 1991). Secondary or primary metabolites produced by these organisms may be potential bioactive compounds of interest in the pharmaceutical industry. Some seaweed has the valuable medicinal value components such as antibiotics, laxatives, anticoagulants, anti-ulcer products and suspending agents in radiological preparations. Fresh and dry seaweeds are extensively consumed by people especially living in the coastal areas. In the marine ecosystem's seaweeds are directly exposed and are susceptible to ambient microorganisms such as bacteria, fungi and viruses (Ranganayaki et al., 2014).

Some microbes are sociable but most of them are very pathogenic, Escherichia coli, Staphylococcus aurous and salmonella are causes diseases like food borne gastroenteritis, urinary tract infections and upper respiratory complications (Jawetz et al., 1985 and Leven, 1987). Traditionally, seaweeds have been used in the treatment of various infectious diseases. Screening of antimicrobial compounds from seaweeds is vital and increasing demand for therapeutic drugs (Prasad et al., 2010).

Marine sessile organisms, such as algae, sponges and corals, have developed physiological adaptations, including the synthesis of bioactives which confer defense against grazers and/or the installation of epiphytes and fouling organisms. Parasites have a potential ability to attain wide variety of physiological and biochemical adaptations to survive within the specialized environment of the host (Barret, 1994). Secondary metabolites produced by endophytes usually produce the enzymes necessary for the colonization of plant tissues. It was demonstrated that most endophytes are able to utilize, at least in vitro, most plant cell components. Most of investigated endophytes utilize xylan and pectin, show lipolytic activity and produce non-specific peroxidase, chitinase and gluconase.

1.2. Antimicrobial activities in A. mexicana

The study of biologically active compounds from natural sources has always been of great interest to scientists looking for new sources of useful drugs for treating infectious diseases. Infectious diseases caused by bacteria, fungi, viruses, and parasites remain a major threat to public health, despite tremendous progress in human medicine. Their impact is particularly great in developing countries because of the relative unavailability of medicines and the emergence of widespread drug resistance (Nirater et al., 2015). Bacteria cause serious infections in humans as well as other animals. For example, it was found that Staphylococcus aureus (S. aureus) causes superficial skin lesion and food poisoning Pseudomonas aeruginosa (P. aeruginosa) is a nosocomial pathogen accounting for a significant percentage of hospital-acquired infections and health care centers because there are little effective antimicrobial agents against it (Abu-Shanab et al., 2004).

The discovery, development and clinical use of antibiotics during the 20th century decreased substantially the morbidity and mortality from bacterial infections. Marine bacteria being a heterotrophy with simple cell multiplication process, which can be cultivated in large amounts inexpensively. This has prompted the present study, to accesses the possible utilization of associated bacteria as resources, to meet the sufficient supply of desired metabolites. The present investigation was initiated to screen the antibacterial efficacy of A.mexicana plant extract associated bacterial population against the pathogens. In recent years, there are numerous reports of macro algae derived compounds that have a broad range of biological activities such as antibacterial, antifungal, antiviral, antineoplastic, antifouling, anti inflammatory, antitumor, cytotoxic and antibiotic activities. Presently plant phytocompounds constitute commercially important resources which are providing valuable ideas for the development of new drugs against cancer, microbial infections and inflammations.

1.3 Studies of toxicity

Botanicals have broad-spectrum activities and can be extracted in commercial qualities (Olaifa et al., 1987). They are biodegradable (Kela et al., 1989) less severe than synthetic chemicals (Ahmed and Grainge, 1986), and easily reversed in fish subjected to chronic concentrations (Onusiriuka and Ufodike, 1998). People in the Lower Amazon basin in Brazil have used the leaves of the small herb 159 Ichthyothere terminalis as a fish poison for many years (Cascon, 1965). The leaves are incorporated into baits prepared with locusts or manioc flour, and the baits are thrown into the water to be swallowed by fish. The active ingredients in the herb leaves contain ichthyothereol and ichthyothereol acetate.

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The importance of ethno botanical studies as cost-effective means of locating new and useful plant compounds shows that commercial synthesis of drugs cost more than extractions from plants. The use of botanicals has been found to aid fish cropping greatly as it saves time of fishing and increase easy handling of even stubborn fish like Gymnarchus, Heterotis and Clarias (Burkill, 1985). The herbivorous or predaceous fishes in culture pond are distributing worldwide, causing serious threat for culturing shrimps/prawns, especially in nursery ponds. The predatory fish species prey on cultured stock whereas the competitors compete for food, space and oxygen. These species are extremely harmful when present in sufficient numbers. Moreover, some of the herbivorous/predaceous fishes are vectors for many shrimp parasites including microsporidian (Flegel et al., 1992).

Marine secondary metabolites are organic compounds produced by microbes, sponges, seaweeds, and other marine organisms. The host organism biosynthesizes these compounds as non-primary or secondary metabolites to protect themselves and to maintain homeostasis in their environment. Some of these secondary metabolites offer avenues for developing cost-effective, safe and potent drugs. Secondary metabolites from a wide variety of benthic organisms have been reported to deterfeeding by natural predators (Paul, 1992; Pawlik, 1993; Hay, 1996).

In France, extract studies with ethanol/water draw up from dried entire plant of G. foliifera showed toxicity in humans when treated with oral dose and cytotoxicity studies (Bhakuni et al., 1992). G. coronopifolia and G. edulis were also toxic to humans (Nagai et al., 1996 and Yotsu-yamashita et al., 1993). Carbohydrate, heparin (Kamat et al., 1992), agar, manauealide A, manauealide B , manauealide C, palmitic, palmitoleic, oleic, lauric and myristic acids (Parekh et al., 1984) steroids and alkaloids malyngamide were found in these species.

1.4. ARGEMONE MEXICANA

Argemone mexicana L. (papaveraceae) prickly poppy weeds of most cropping systems. Argemone is from Greek ‘argena’ meaning ‘Cataract of the eye’, and Mexicana combines ‘mexico’ with the Latin suffix ‘ana’, belonging to suggesting the country of origin (Hasan Ali et al.,2016). A. mexicana widely distributed in many tropical and sub-tropical countries. This plant is commonly found everywhere by roadsides as well as in fields of India

A. mexicana occurs in Southern India at an altitude of 800m. It is an annual, herbaceous and seed propagated herb; growing up to 150cm with a slightly branched taproot system. The stem is erect, branched and extremely prickly. Leaves are alternate, without petioles and with spiny margins. Flowers are solitary, 2.5-5cm diameter and yellow in colour. Fruits are prickly and oblong. Seeds are very numerous, nearly spherical, covered in a fine network of veins and brownish black colour (Hasan Ali et al., 2016).

CLASSIFICATION

Kingdom	:	Plantae
Division	:	Magnoliophyta
Class	:	Magnoliopsida Dicotyledons
Subclass	:	Magnoliidae
Order	:	Papaverales
Family	:	Papavaraceae
Genus	:	Argemone
Species	:	Mexicana

Figure. 1 Argemone mexicana

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The fresh Argemone mexicana Linn (Fig: 1). was collected from Ayyenpettai village, Kanchipuram Dt., the species was identified by the Dr. P. Jayaraman Director of Institute of herbal Botany, Reg. No. PARC/2020/ 4208.

1.5. CTENOPHARYNGODON IDELLA

Ctenopharyngodon idella commonly known 'Grass carp' is identified by the body which is moderately compressed laterally. Its mouth is terminally located on a wide head and eyes are small and low on the head. It is olive-brown on the dorsal side, with silver sides and a white belly. It's a large herbivorous freshwater fish species of the family cyprinidae native to eastern Asia. An exotic fish first introduces in Bangladesh from honking. It was successfully breed in 1980. C. idella is widely distributed in almost all the freshwaters, grass carp is a good source of protein and highly growing species if gets proper food weight will be 4.5 kg/year. C. idella is of high economic value as it is available throughout the year and fetches a very high price locally. Feed on higher aquatic plants and submerged grasses; takes also detritus, insects and other invertebrates, it is much suited for aquaculture. C. idella is economically important for the weaker section of the people as it yields high income for the fisher folk.

Figure: 2 Ctenopharyngodon idella

GRASS CARP-SCIENTIFIC CLASSIFICATION	
Kingdom	**Animalia**
Phylum	**Chordata**
Class	**Actinopterygii**
Order	**Cypriniformes**
Family	**Cyprinidae**
Subfamily	**Squaliobarbinae**
Genus	**Ctenopharyngodon**
Species	**C. idella**
Binominal name	**Ctenopharyngodon idella**

2. Materials and methods

2.1 Extraction method

After identification leaves were thoroughly washed with running tap water 2-3 times and finally washed with distilled water followed by shade-dried for seven days. 100gms of powdered A. mexicana was taken for fraction method and initiated with methanol solvent, followed by the concentrated methanol extracts were further subjected to partial fractionation with solvents of increasing polarity viz., Acetone, Chloroform, Ethyl acetate and Aqueous. Methanol extracts were distributed in the non-polar solvent, dissolved portion, is called Methanol Fraction (MF), was filtered and concentrated by removing the solvent

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under reduced pressure. The residue thus obtained was fractionated subsequently with medium polar solvents such as chloroform (CLF), ethyl acetate (EAF), polar solvent, methanol (MF) and aqueous.

2.2 Phytochemical analysis

Phytochemical analysis of the organic extract was carried out according to the general method of Harbone (1998). Basic phytochemical screening was carried out using simple chemical tests to detect the presence of secondary plant constituents such as alkaloids, tannins, flavonoids, saponins, triterpenes, sterols, phenols, glycoside, reducing sugar and soluble carbohydrate in the sample. The methods used were those outlined by Harbone (1998) and (Ekwueme, et al., 2011) except otherwise stated.

2.3 Quantitative Phytochemical Analysis

2.3.1 Test for Saponins

The extract (1g) was macerated with 10 ml of petroleum ether and decanted into a beaker. Another 10 ml of the petroleum ether was added into the beaker and the filtrate evaporated into dryness. The residue was dissolved in 6 ml of ethanol. The solution (2 ml) was put in a test tube and 2 ml of chromagen solution added into it. It was left to stand for 30 min and the absorbance was read at 550 nm.

2.3.2 Test for Alkaloids

The extract (1 g) was macerated with 20 ml of ethanol and 20% H_2SO_4 (1:1 v/v). The filterate (1 ml) was added to 5 ml of 60% H_2SO_4. After 5 min, 5 ml of 0.5% formaldehyde in 60% H_2SO4 was mixed with the mixture and allowed to stand for 3 hr. The absorbance was read at 565 nm.

2.3.3 Test for Tannins

The extract (1 g) was macerated with 50 ml of methanol and filtered. To the filterate (5 ml), 0.3 ml of 0.1N ferric chloride in 0.1N HCl and 0.3 ml of 0.0008 M of potassium ferricyanide were added and the absorbance read at 720 nm.

2.3.4 Test for Flavonoids

The extract (1 g) was macerated with 20 ml of ethyl acetate for 5 min and filtered. To the filterate (5 ml), 5 ml of dilute ammonia was added and shaken for 5 min. The upper layer was collected and the absorbance read at 490 nm.

2.3.5 Test for Terpenoids

The extract (1 g) was macerated with 50 ml of ethanol and filtered. To the filtrate (2.5 ml), 2.5 ml of 5% aqueous phosphomolybdic acid solution was added and 2.5 ml of concentrated H_2SO_4 was gradually added and mixed. The mixture was left to stand for 30 min and then made up to 12.5 ml with ethanol. The absorbance was taken at 700 nm.

2.3.6 Test for Steroids

The extract (1 g) was macerated with 20 ml of ethanol and filtered. To the filtrate (2 ml), 2 ml of chromagen solution was added and the solution left to stand for 30 min. The absorbance was read at 550 nm.

2.3.7 Test for Glycosides

The extract (1 g) was macerated with 50 ml of distilled water and filtered. To the filtrate (1 ml), 4 ml of alkaline pirate solution was added. The mixture was boiled for 5 min and allowed to cool. The absorbance was read at 490 nm.

2.3.8 Test for Carbohydrate

The extract (1 g) was macerated with 50 ml of distilled water and filtered. To the 1 ml of the filtrate, saturated aqueous solution of picric acid was added and absorbance read at 580 nm.

2.3.9 Test for Phenols

The extract (1 g) was macerated with 20 ml of 80% ethanol and then filtered. The filtrate (5 ml) was added to 0.5 ml of folinciocalteus reagent and allowed to stand for 30 min. Then 2 ml of 20% sodium carbonate was added and absorbance measured at 650 nm.

2.4. GC-MS analysis

GC-MS is a combination of two different analytical techniques Gas Chromatography and Mass Spectrometry. It is a versatile tool to separate, quantify and identify unknown (volatile) organic compounds and permanent gases. By combining sensitivity and a high resolving power, complex mixture can be analysed.

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GC-MS analysis of the crude of algal extracts were carried out on Agilent technologies (6890 N), JEOL GCMATE II which comprised of an auto sampler and gas chromatography interfaced to a mass spectrometer (GC-MS) instrument exploying the following condition: capillary column – 624 ms (30 m x 0.32 mm x 1.8 m) operating in an electron mode at 70 eV; helium (99.999 %) was used as carrier gas at a constant flow of 1.491 ml/min and injection volume of 1.0 ml, injector temperature was 140° C Mass spectra were taken at 70 eV.

2.5. Anti-bacterial activity (Well-diffusion method)

The antibacterial activities of all extracts were carried out by well diffusion method. The concentrations of the test compounds were taken in DMSO and used in the concentration of 10, 25 and 50 µg. The target microorganisms were cultured in Mueller–Hinton broth (MHB). After 24 hr the suspensions were adjusted to standard sub culture dilution. The Petri dishes containing Mueller Hinton Agar (MHA) medium were cultured with diluted bacterial strain. Well made of diameter 6 mm was pre-sterilized and was maintained in aseptic chamber. Each concentration was injected to the sterile well papers. Then the prepared wells were placed on the culture medium. Standard drug streptomycin (20 µg) was used as a positive reference standard to determine the sensitivity of each microbial species tested. Then the inoculated plates were incubated at 37 ºC for 24 hr. The diameter of the clear zone around the well was measured and expressed in millimeters as its anti-bacterial activity.

2.5.1 Organism used

S.no	Organisms	Type
1.	Vibrio harveyi	Gram-negative

2.6

Icthyotoxicity

Fingerlings (1.5-2.0 cm) of freshwater fish Ctenopharyngodon idella were used for evaluating the ichthyotoxic potential. Five fingerlings each were introduced in experimental and control glass bowls containing 1,000 mL freshwater and chosen concentrations of extract. Immediate reflex changes and mortality were observed continuously for six hours at 1 hr interval for the next 12 hr. After 24 hr of exposure, the number dead and live fish were counted. The acute toxicological reflexes were observed and recorded (Indap and Pathare, 1998) and (Joseph Selvin and Aaron Premnath Lipton, 2004).

2.7. Experimental setup. Figure 3a-3c

Fig. 3a. Control

Fig. 3b. Icthyotoxic activity in chloroform extracts of A. mexicana

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Fig. 3c. Icthyotoxic activity in ethyl acetate extracts of A. mexicana

Statistical analysis

Analysis of variance (ANOVA) was carried out using SPSS program (version. 15). Means and standard deviation of three replicates were calculated. The results were considered significant at $p < 0.05$.

3. RESULT

3.1 Preliminary screening of active Phytochemicals

Quantitative phytochemical screening of A. mexicana was conducted to find out the types and quantity of secondary metabolites existing in the extract. The secondary metabolites accessed were tannins, alkaloids, carbohydrates, flavonoids, phenols, saponins, steroids, terpenoids, proteins, coumarins and glycosides are present can be seen in (Table. 1).

Table: 1. Phyto-chemical screening of chloroform and ethyl aetate extract of the A. mexicana

S. No	Amino acids	A.mexicana Chloroform Extract Yield % (mg)	A.mexicana Ethyl acetate Extract Yield % (mg)
1	Alkaloids	4.33	12.11
2	Flavonoids	20.44	8.11
3	Saponin	0.13	1.44
4	Terpenoids	2.78	5.45
5	Carbohydrates	12.98	22.78
6	Coumarin	0.99	0.56
7	Protein	67.9	34.9
8	Phenols	2.78	2.08
9	Steroids	8.45	4.53

3.3. GC-MS analysis of A. mexicana in Chloroform extracts

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The composition and identification of the main compounds and its biological activity present in the chloroform extract of A. mexicana is shown in (Table. 4 & 5). Fourteen compounds were identified by GC-MS. The main compounds were 1-Dodecene, 1-Pentadecene, 1-Heptadecene, 1-nonadecene, n-Hexadecanoic acid, n-Tetracosanol-1, Phytol, Linoleic acid ethyl ester, Ethyl (9Z,12Z)-9,12-octadecadieno, 1-Heptacosanol, 1-Hexacosanol, 1(2H)-Isoquinolinone,3-(2-Etheny), 1-Triacontanol (Fig. 6)

Table: 2. GC-MS of Analysis A. mexicana Chloroform extract

S. no	R.Time	Area %	Name	Molecular formula	Molecular weight (g/mol)
1	8.522	2.99	1-Dodecene	$C_{12}H_{24}$	168.319
2	15.739	5.85	1-Pentadecene	$C_{15}H_{30}$	210.4
3	23.438	8.75	1-Heptadecene	$C_{17}H_{34}$	238.5
4	30.699	10.53	1-nonadecene	$C_{19}H_{38}$	266.5
5	35.814	2.99	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.43
6	36.682	20.31	n-Tetracosanol-1	$C_{24}H_{50}O$	354.65
7	39.305	1.74	Phytol	$C_{20}H_{40}O$	296.539
8	40.348	6.36	Linoleic acid ethyl ester	C20H34O2	306.48
9	40.468	5.09	Ethyl (9Z,12Z)-9,12-Octadecadieno	C20H36O2	308.5
10	41.108	13.34	1-Heptacosanol	C27H56O	396.73
11	44.794	9.68	1-Hexacosanol	C26H54O	382.71
12	44.960	1.86	1(2H)-IsoQuinolinone,3-(2-Etheny	C_9H_7N	129.16
13	51.809	3.84	1-Triacontanol	C30H62O	438.81

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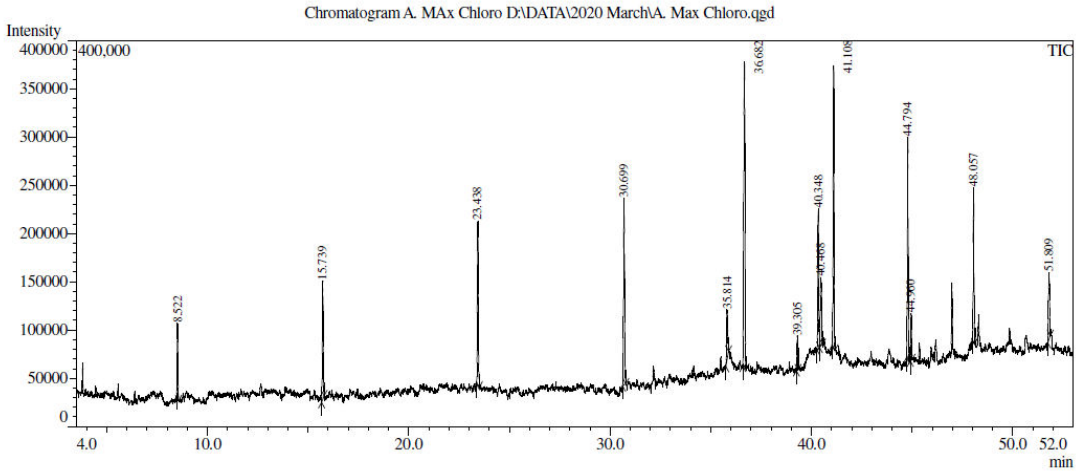
Table: 3. Activity of phytocompounds identified in A. Mexicana Chloroform extract by GC-MS of analysis

S.No	Compound Name	Biological activity
1	1-Dodecene	Surfactant
2	1-Pentadecene	Anti microbial and anti oxidant
3	1-nonadecene	Anti a bacterial and strong anti fungal activity
4	n-Hexadecanoic acid	Anti-inflammatory, anticancer effects and antioxidant properties
5	n-Tetracosanol-1	Anti bacterial
6	Phytol	Antimicrobial, resistant gonorrhea, joint dislocation
7	Linoleic acid ethyl ester	Hypocholesterolemic, nematicide, insectifuge, anti-eczemic, anti-acne
8	Ethyl (9Z,12Z)-9,12-Octadecadieno	Anti viral activity and cytotoxicity
9	1-Heptacosanol	Anti microbial activity
10	1-Hexacosanol	Anti fungal and anti microbial activity
11	1(2H)-Isoquinolinone,3-(2-Etheny	Anti tumor activity
12	1-Hexacosanol	Anti fungal and anti microbial activity
13	1-Triacontanol	Potent plant growth regulator

Figure. 4. Phytocompounds identified from Chloroform extract of A. mexicana by GC-MS



3.4 GC-MS analysis of A. mexicana in Ethyl acetate extracts

The composition and identification of the main compounds and its biological activity present in the ethyl acetate extracts of A. mexicana is shown in (Table. 6 & 7). Fourteen compounds were identified by GC-MS. The main compounds were Hexadecanoic acid,methyl ester, 9-Octadecenoic acid (Z)-,methyl ester, Myristic acid glycidyl ester, Glycidyl palmitate, Hexatriacontane, Octadecane, Methyl dimethylhexadecadienoic acid, Tetracosane, Octadecane, 5-Methyl-z-5-docosane, Hexatriacontane, Tetrapentacosane, Hexacontane, 2-Methylpentacosane, Dotriacontane and 2,6-Dodecadien-1-ol, 3,7,11-trimethyl(Fig: 7)

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Table: 4. GC-MS of analysis A. mexicana Ethyl acetate extract

S. no	R.Time	Area%	Name	Molecular formula	Molecular weight (g/mol)
1	42.216	1.44	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270
2	44.615	2.31	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296
3	45.348	1.35	Myristic acid glycidyl ester	$C_{17}H_{32}O_3$	284
4	46.308	2.10	Glycidyl palmitate	$C_{19}H_{36}O_3$	312
5	46.729	2.06	Hexatriacontane	$C_{36}H_{74}$	507
6	47.021	1.49	OCTADECANE	$C_{18}H_{38}$	254
7	47.060	1.13	Methyl dimethylhexadecadienoic acid		
8	47.680	5.83	Tetracosane	$C_{24}H_{50}$	338
9	48.335	1.34	Octadecane	$C_{18}H_{38}$	254
10	48.436	2.40	5-Methyl-z-5-docosane	$C_{23}H_{46}$	322
11	48.751	13.75	Hexatriacontane	$C_{36}H_{74}$	507
12	50.019	23.10	Tetrapentacontane	$C_{25}H_{52}$	352
13	50.946	1.25	Hexacontane	$C_{60}H_{122}$	843
14	51.118	1.38	2-Methylpentacosane	$C_{26}H_{54}$	366
15	51.562	35.49	Dotriacontane	$C_{32}H_{66}$	450
16	51.741	3.58	2,6-Dodecadien-1-ol, 3,7,11-trimethyl	$C_{15}H_{28}O$	224

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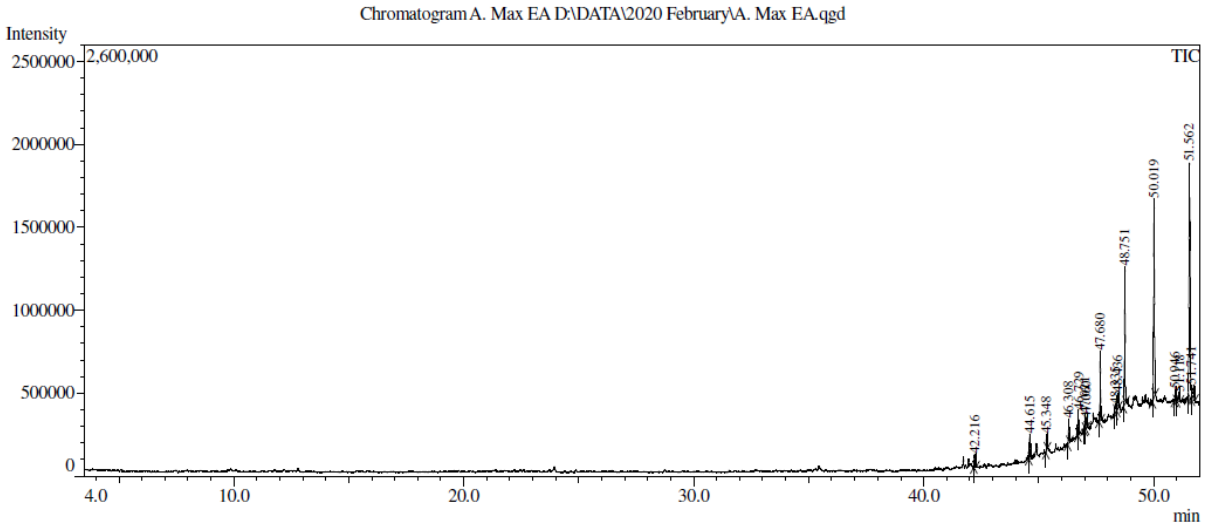
Table: 5. Activity of phytocompounds identified in A. Mexicana Ethyl acetate extract by GC-MS of analysis

S.No	Compound Name	Biological activity
1	Hexadecanoic acid, methyl ester	Anti fungal, anti oxidant, nematicide and antimalarial property
2	Myristic acid glycidyl ester	
3	Glycidyl palmitate	Preparation of lysophosphatidic acids which inhibit apoptosis
4	Hexatriacontane	Anti inflammatory and analgesic activity
5	OCTADECANE	Anti fungal
6	Tetracosane	Cytotoxic against cancer
7	Octadecane	Anti fungal
8	Hexatriacontane	Anti inflammatory,analgesic activity Anti microbial activity
9	Tetrapentacontane	Anti cancer, anti inflammatory, hepatoprotective and anti viral activities
10	Dotriacontane	Anti microbial activity

Figure: 5. Phytocompounds identified from Ethyl acetate extract of A. mexicana by GC-MS



3.4 Antimicrobial activity of A. mexicana

The antimicrobial effective of the plant extract was examined using the well diffusion assay which is mainly used to test the sensitivity of bacterial strains towards antibiotics with a clear zone around the well reflects the bacterial sensitivity toward antibiotics (Table: 12) and (Figure: 10 (a to d)). Antimicrobial activity of A. mexicana extract against Gram positive (Staphylococcus aureus) bacteria revealed an antimicrobial activity against the test microorganisms. The zone of inhibition of plant extract against gram positive bacteria was measured. The result indicated that A. mexicana extract of Methanol showed effective antibacterial activity in Staphylococcus aureus followed by lower inhibition in the order of acetone > ethyl acetate>chloroform and aqueous. The extracts of selected plant A. mexicana was potentially good source

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(Figure: 8) of antibacterial substance with broad range of activities in retarding and preventing in the growth of Gram positive (Staphylococcus aureus) selected common bacteria.

Table: 6. Size of inhibition zone of extract with different solvent of A. mexicana against bacteria

Samples	Concentration (µl)	Vibrio harveyi
Ethyl acetate	10	-
	20	-
	30	-
	40	2
	Control	18
Chloroform	10	-
	20	-
	30	-
	40	-
	Control	20

Control – Gentamycin (20mg/ml)

Figure: 6. Antibacterial activity of different extracts of A. mexicana

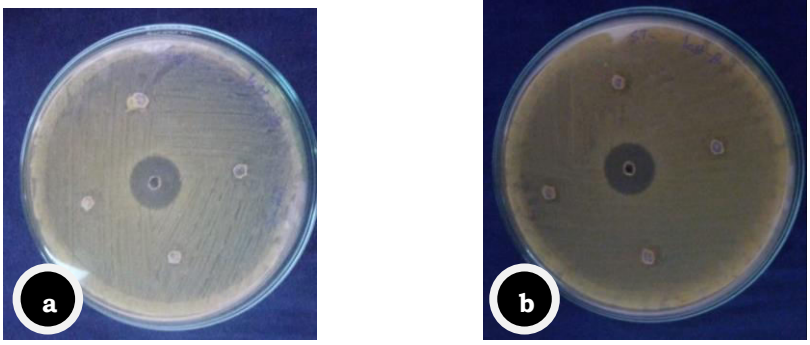

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Table: 7. General behavioral changes observed in C. idella exposed to A. mexicana extracts

Stages	Behavioral changes
Stage I:	a) Increased in ventilatory frequency
Initial signs	b) Erratic/rapid movements
Stage II:	a) Inclined towards one side
Secondary signs	b) Loss of swimming activity
Stage III:	a) Rapid surface respiration
Advanced signs	b) Inclined to bottom
	c) Start of sporadic uncontrollable
	swimming with non directional bursts

Antifeedant activity (ichthyotoxicity profile) of A. mexicana leaves extracts is presented in (Table: 6). C. idella exhibited same sort of behavioral changes as listed in (Table: 5). Initially the fishes exhibited erratic movements and then inclined towards one side. Later, they rapidly went for surface respiration followed by settling at bottom or rapid swimming activity with non-directional bursts, which culminated in dwelling at bottom and mortality (Indap and Pathare, 1998). Chloroform extract of A. mexicana was toxic at 10mg/ml. A. mexicana extract ethyl acetate was less toxic as 100% mortality was observed in 10mg/ml in 10hr and less toxicity i.e., lower the 20% of mortality was observed in 4mg/ml and 2mg/ml (Table: 6).

Table: 8. Ichthyotoxicity profile of A. mexicana **extracts to C. idella fingerlings**

	Concentration (mg/ml)	Mortality (%)	Time of death (h)
Chloroform	10	100	2
	8	81±0.03	6
	6	40	10
	4	10	12
	2	0	Nil
Ethyl acetate	10	100	4
	8	79±1.03	6
	6	36	11
	4	08	17
	2	0	Nil

Mean ± SD

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5. Discussion

Ichthyotoxic plants have been widely used in harvesting of fishes. Local and artisanal fishermen still prefer to use these ichthyotoxic plants to obtain fish from small bodies of water since they are presumably cheaper and more accessible. A decline in fish stock population was reported in South America due to indiscriminate use of these ichthyotoxic plants for fishing [15].

Piscicidal activity of the ethyl acetate and chloroform extracts of A. mexicana were studied and the results are given in Table 6. In the present study, the A. mexicana extracts were assessed to find out whether the bioactive phytochemicals was capable to produce toxicity to the test fish, Ctenopharyngodon idella. It was noticed that A. mexicana extracts were able to produce toxicity to the Grass crap. None of the extracts of H. ovalis was able to produce mortality to the test fishes. Four concentrations like 25µl, 50µl, 75µl, 100µl were used in the study and the results reveal that the guppy fishes died at higher concentration. It was observed that all the extracts of H. pinifolia was able to produce mortality to guppy fishes. The 50µl, 75µl, 100µl of methanol, acetone, ethyl acetate of H. pinifolia showed toxicity after one hour. Toxicity was dose dependent. 25µl of methanolic, ethanolic, acetone and ethyl acetate extracts of H. pinifolia exhibited toxic effects to the model fish only after 24 hours. All the concentrations of the ethanolic extracts also showed mortality after 24 hours.

The respective control blanks of the solvents did not posses any toxic effects and the fish survived for long duration. All the four concentrations of the ethanol, acetone and ethyl acetate extracts of T. hemprichii did not show toxicity to guppy fish. The methanolic extract at 25µl, 50µl and 75µl possessed toxicity and showed mortality after 24 hours while the 100µl of the same extracts showed toxicity after one hour. None of the extracts of H. ovalis was toxic to guppy fishes and the fishes survived at even the higher concentrations for longer duration.

It was found that all the extracts impart same sort of behavioral changes. They were studied in four 162 stages and in the initial stage guppy fishes showed rapid movement with high stress and surface gasping. In the second stage slow swimming and violent movement of the gill chambers were noted, whereas in the third stage there was loss of equilibrium and occasional paralysis. In the last stage, they were inclined to the bottom in an inverted posture and mortality was recorded. The chloroform extracts were not used in the study because the solvent blanks showed immediate toxicity to guppy fishes and hence the extracts could not be used to test the toxic potential against the fishes.

Conclusion

Implicitly, the findings suggest that Tephrosia vogelii, Adenia cissampeloides and Asystasia vogeliana leaves extract have acute toxic effect on farmed African catfish (C. gariepinus) and could prove to be potent fishing stocks. However, holistic measures should always be taken considering the effect that it could exert on other aquatic inhabitants and systems.

CONFLICTS OF INTEREST

The authors declared no conflicts of interest.

COMPLIANCE WITH ETHICS REQUIREMENTS

This article does not contain any studies with human or animal subjects.

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