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IDENTIFICATION OF BIOACTIVE COMPOUNDS AND ANTIMICROBIAL ACTIVITY OF MARINE CLAM *ANADARA GRANOSA* (LINN.)

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ABSTRACT
Comprehensive inventory of the bioactive compounds produced by *Anadara granosa* during full growth has been established by whole tissue using organic solvents system and subjecting in to gas chromatography mass spectrum (GC-MS) twelve bioactive compounds of which ester, furan, acid and ketone were identified. The most representative bioactive compounds of *Anadara granosa* were employed in the antibacterial activity against pathogenic bacteria and the study pertaining to the identification of active principles and the antimicrobial activities.

KEY WORDS: *Anadara granosa;* bioactive compounds; Gas chromatography and mass spectrum; pathogens.

INTRODUCTION

Anadara granosa, belongs to the family Arcidae. It is popularly known as blood clam due to the presence of haemoglobin which gives red colour to the meat (Narashiman, 1988). It is widely distributed in the indopacific, from Eash Africa to Polynesia, North Japan and South to Northern and Eastern Australia (Poutiers, 1988) and also widely distributed along the Indian coast and form a fishery of considerable magnitude of Bay of Bengal the ocean was a vast potential for a huge number of novel chemicals that may useful for finding drugs with greater efficacy and specificity for the treatment of many human diseases (Bergman and Feeney, 1951, Faulkner, 2001). The marine organism have to with stand extreme variation in pressure, salinity, temperature etc., and these environmental valuables have facilitated the organism to produced varied chemicals of unique features. So far more than 10,000 compounds have been isolated from marine organisms (Proksch *et al*., 2002) with hundred of near compounds still being discovered every year (Proskch *et al*., 2002). Extensive Biochemical studies have been carried out in whole body tissue of *Anandra granosa*. Since there are no reports on the Biochemical aspects related with antimicrobial activity against various pathogens.

MATERIALS AND METHODS

Marine clam *Anadara granosa* were collected from Muthupet estuary (Latitude 10°20 N, Longitude 79°32 E), South East Coast of India and identified using the standard reference (Sathiyamoorthy, 1952) and also confirmed with zoological survey of India. 50 gm sample were dissected out and cleaned in distilled water. Extracts are homogenized with organic solvent a mixture of (1:1 v/v) pentane: diethyl ether. The upper organic phase were separated, dried over anhydrous sodium sulphate, and concentrated at 42°C to 1 ml and at 20°C for antibacterial assay and GC-MS analysis.

Bacterial strain and culture condition

Antibacterial activity of marine clam was determined against four bacterial strain viz. *Staphylococcus aureus, Streptococcus faecalis, Escherichia coli, Pseudomonas aeruginosa* these pathogens were isolated from patients of Raja Mirasudar Hospital, Thanjavur, South India.

Preparation of bacterial culture

Nutrient broth medium was prepared and sterilized in an autoclave at 151lb and the presence of four bacterial species were inoculated in the nutrient broth and incubated at 28 ± 2°C for 24 hrs. On this 24 hrs old bacterial broth cultures were inoculated by using a sterile colony swab.

Gas chromatography – Mass spectrum (GC-MS)

Volatile component were identified by GC-MS using GC Clarus 500 Perkin Elmer (Turbo mass gold – Perkin Elmer) equipped with column Elite – 1 (30m × 0.25 mm × 1) nmds carrier gas (Hon rate, 1.0 ml/min) Helium was used and infection volumes were 1m. The column temperature was maintained initially at 400°C for 3.5 min followed by increase to 600°C at a rate of 50°C/min from 60° to 120°C at a rate of 60°C/min and from 120 to 230°C. The electron impact energy was 70 ev and the ion source temperature as set at 2300°C. Electron impact (EI) Mass spectra were recorded in the 45-450 a Mu range at 1s intervals.

RESULTS

Antibacterial activity was tested against four bacteria among the bacterial pathogens.

The antibacterial activity of clam extract is summarized in Table 1 the level of activity which was measured by inhibition zone, varied between 10 to 16 mm. The zone of inhibition showed highest activity against *Pseudomonas aeruginosa* (Fig.1).

TABLE 1. Zone of inhibition (mm) of clams crude extract against 4 pathogenic bacteria

S.No.	Pathogen	Zone of inhibition	
		Clams extract (mm)	Standard (mm)
1.	*Staphylococcus aureus*	16	20
2.	*Streptococcus faecalis*	13	17
3.	*E. coli*	10	14
4.	*Pseudomonas aeruginosa*	16	17

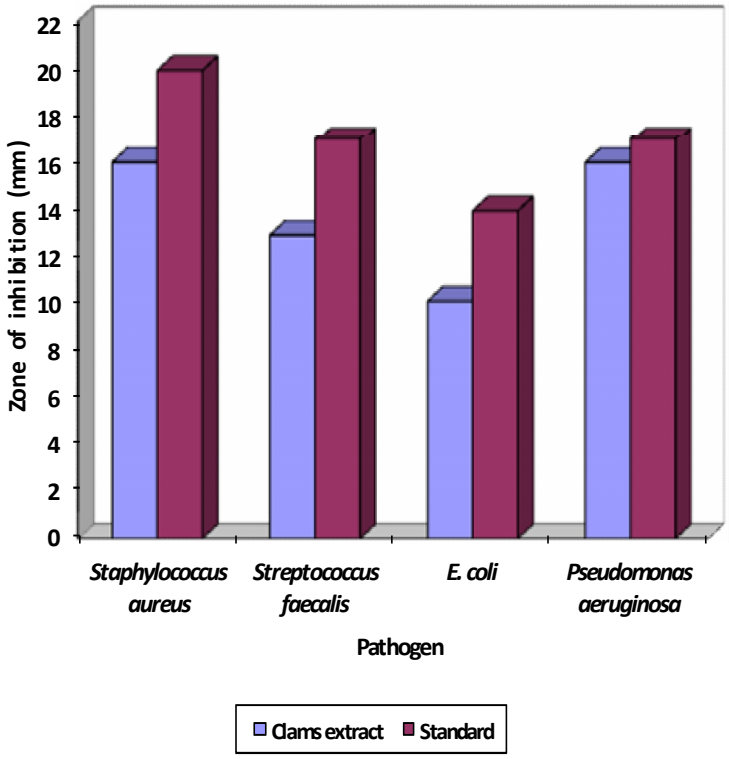

FIGURE1. Effect of clams extract against four pathogens

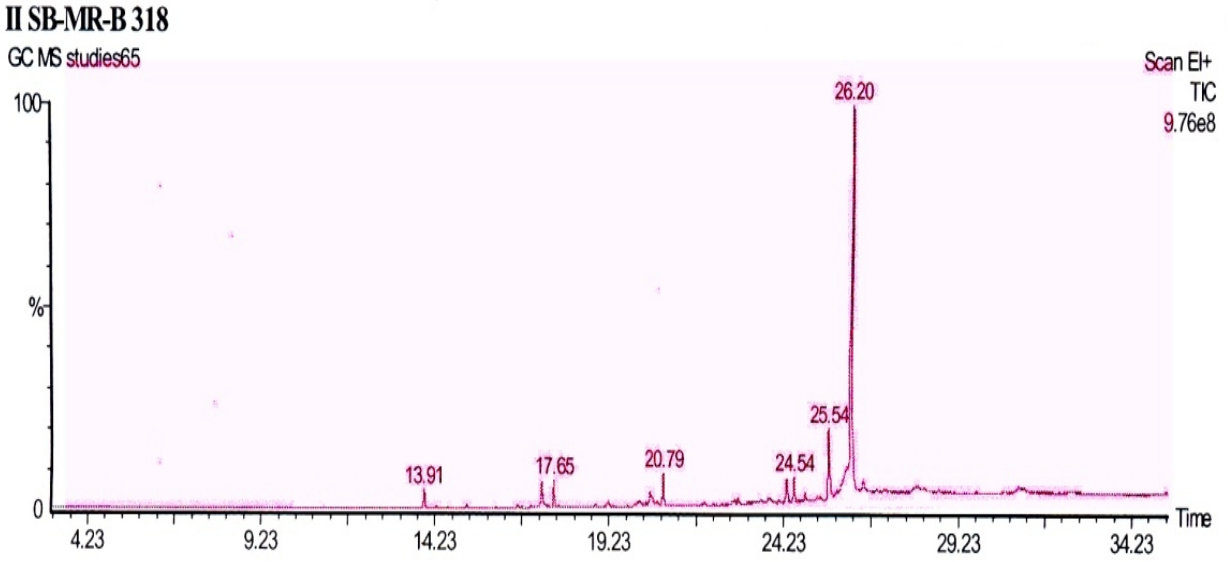

Figure 2. Chromatogram – Gas Chromatography and Mass Spectra for *Anadara granosa*

Identification of bioactive compounds

A typical chromatogram from clam extract obtained in the GC/MS system under programmed temperature condition is shown in Fig.2.

Twelve bioactive compounds were detected. They are Furan compound, Palmitic acid, Fatty acid esters, Stearic acid, Ketone, Phenol compound and Plasticizer compound. Results of clam GC-MS analysis showed that *Anadra granosa* has a complex fatty acid ester compound. From which 4, 7, 10, 13, 19. Doca sahexaenoic acid has antidiabetic, antiasthma, anticancer and anti heart diseases properties Retention time 25.54 phenol, 2-4 bis (1-phenylethyl) has analgesic Anasthedic, Antioxidant antiseptic, antibacterial, antitumor and cancer preventive properties (Table 2).

TABLE 2. Bioactive composition in clam *Anadara granosa*

S.No.	RT	Name of the compound	Molecular formula	MW	Peak area %	Compound nature	Activity**
1.	13.91	3-Phenyl-2,3-dihydrobenzo[b] furan-2-ol	$C_{14}H_{12}O_2$	212	2.15	Furan compound	Antimicrobial
2.	16.60	Methyl 5-(2-phenylpropionyl) hexanoate	$C_{16}H_{22}O_3$	262	0.45		
3.	17.29	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	4.31	Palmitic acid	Antioxidant Hyporcholesterolemic Nematicide Pesticide Lubricant antiandrogenic Flavor Hemolytic
4.	17.65	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	2.70	Fatty acid ester	-do-
5.	19.75	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	298	0.36	Fatty acid ester	No activity reported
6.	20.42	Octadecanoic acid	$C_{18}H_{36}O_2$	284	3.59	Stearic acid	No activity reported
7.	20.79	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312	3.42	Fatty acid ester	No activity reported
8.	24.33	Methanone, (1,4-dimethyl-7-(1-methylethyl)-2-azulenyl)phenyl-	$C_{22}H_{22}O$	302	3.17	Ketone	No activity reported
9.	24.54	Methanone, [2-(1-methylethyl) Phenyl]phenyl-	$C_{16}H_{16}O$	224	2.96	Ketone	No activity reported
10.	24.86	4,7,10,13,16,19- Docosahexaenoic acid, methyl ester, (all-z)-	$C_{23}H_{34}O_2$	342	0.95	Fatty acid ester	Antidiabetic Anti asthma Anticancer Anti heart disease
11.	25.24	Phenol, 2,4-bis (1-phenylethyl)-	$C_{22}H_{22}O$	302	9.57	Phenolic compound	Analgesic, Anesthetic, Antioxidant, Antiseptic, Antibacterial, Antiviral Diuretic Cancer preventive, Fungicide, Rodenticide Emetic, Vasodilator
12.	26.20	1,2-Benzenedicaryboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	66.36	Plasticizer compounde	Antimicrobial Anti fouling

DISCUSSION

In the present study, GC-MS analysis and antibacterial activity *Anadara granosa* showed 100 per cent success in the identification of bioactive compounds responsible for antimicrobial and anticancer activities. Whole tissue extract of *Anadara granosa* showed broad spectrum of antibacterial activity (Table 1). The secretion of hypobranchial gland of murcid Rapana rapiformis, was found to inhibit eight bacteria (Murugan *et al*., 1991). Sincerely the hypobranchial gland of *C. ramosus* inhibited a broad spectrum of activity against ten bacterial strain (Kagoo and Ayyakkannu, 1992). Many classes of bioactive compounds exhibiting antitumor, antileukemia, antibacterial and antiviral activities have been reported worldwide.

The sterol composition of clam *Anadara granosa* is shown in table 2 among 6 sterols identified in this study 4, 7, 10, 13, 19 – Docosahexaenoic acid is the major sterol component, which were similar to those observed in the bivalve *Macoma balthica* from the Baltic sea (Pazos *et al*., 1992). These sterol composition of *Anadara granosa* was very similar to that of *Ascallop* sp. *Placopecten magallanicus* and *Pectan maximus* and the pacific oyster *Crassostrea giga* (9,10) (Idler *et al*., 1971, Dunstan *et al*., 1931) a study of the occurrence and determination of sterols in marine bivalves provides valuable information on their nutrient requirements and the efficiency of seed production in hatcheries (Napolitana *et al*., 1993). Sterol composition may prove to be useful molecular biomarker; bivalves

incorporate the sterols present in sea water basically from the microalgae and sediments (Kanazawa *et al*., 2001). It was assumed that not a single compound might be responsible, but a synergism of several compounds might on line on the antibacterial activity. Hence no further attempts on purification were made.

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