



Optimization Ultrasonic Assisted Extraction (UAE) of Bioactive Compound and Antibacterial Potential from Sea Urchin (*Diadema Setosum*)

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Abstract

Sea urchins can be used in medicine, has potential as a new type of antibiotic to developed in the pharmaceutical field, which is rich in bioactive compounds as a steroid, triterpenoids, saponins, and antioxidant properties. Conventional extraction generally takes a long time, is less environmentally friendly, and potentially triggers bioactive compound damage, so it needs alternative methods such as Ultrasound-Assisted Extraction (UAE). The extracting technology, including ultrasonic-assisted extraction (UAE) and solvent variation (ethyl acetate and methanol) of bioactive compounds from sea urchin (*Diadema setosum*) were optimized and compared. The purpose of this study was to study the application of UAE and solvent variation methods for sea urchin extraction from the Barrang Lompo Island in South Sulawesi. Comparing the maceration and ultrasonic assisted extraction methods. Optimization of extraction with UAE was carried out on the variable of extraction duration and type of solvent. Gas Chromatography-Mass Spectrometry results show that ultrasound-assisted extraction generally produces compounds of CHOLEST-5-EN-3-OL (3. BETA.), palmitic acid, 9-Octadecenoic acid (Z)-, methyl ester, stearic acid, oleic acid, flavonoids, phenols, pentadecanoic acid and batilol and streoid, which has a function as an antioxidant, anti-inflammatory, anti-tumor, anti-cancer agents and antibacterial. The results showed the best results using ultrasound-assisted extraction with a duration of 30 minutes and using solvent ethyl acetate. These results implied that extracts obtained by sonication showed the highest bioactive compounds and antioxidant activity, thus proving that this activity depends directly on the antibacterial properties.



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Introduction

Indonesia, especially in the Barrang Lompo Island, South Sulawesi Province, is a famous area for the diversity of species of invertebrate animals such as starfish (*Asterias vulgaris*), snake star (*Ophioderma superba*), sea urchin (*Diadema setosum*), sea urchin (*Echinus esculentus*) and many more. Sea urchins are small and spiny, has a high selling value and mostly consumed by Japanese people (sushi),¹ South America and France as well as in the United States (Boston, California, New York, British Columbia).² The shell that is known to contain various pigments is polyhydroxylated naphthoquinone (PHNQ) spinochromes³ of antibacterial effect. Sea urchin part gonads have potential as antibacterial because has a compound polyhydroxylated naphthoquinone,⁴ according to research,⁵ extraction polyhydroxylated naphthoquinone from the spines and shell sea urchin *evchinus chloroticus* from New Zealand with six separate macroporous resins as an alternative to organic solvent extraction. The instrument HPLC and GC-MS have been found to be susceptible to degradation in light exposure, with PHNQ aminated being the least stable. Research,⁶ extracting sea urchin *Echinometra mathaei* shell and spine parts by using a solvent diethyl ether. Screening uses HPLC instrument and radical scavenging assay (antioxidant) 1-diphenyl-2-picrylhydrazyl. It is confirmed with the use of photodiode array detector and LC-ESI – MS PHNQ acquired (Spinochrome B and C, Echinochrome A and Spinochrome A). Results show that marine urchin shells and spines, which are mainly disposed of as waste, can act as a new biologically active resource.

The compounds' chemical nature influences the extraction of phenolic and bacterial compounds from sea urchin materials, sample size, method of extraction, time and storage and the occurrence of agents such as proteins and carbohydrates.⁷⁻⁹ Has been reported that the use of aquades solutions of ethanol, methanol, and acetone dramatically improves the extraction of polyphenols compared to a single-compound solvent system.¹⁰ Extraction methods that most reported is maceration with solvents, hot-water extraction, alkaline extraction, resin-based extraction, enzyme-assisted extraction, extractions based on gamma and electron-beam irradiation and extraction using supercritical fluids. Some of these methods can cause a loss

of bioactive compounds due to the use of high temperatures and long extraction times, or, in the case of irradiation, it can represent a health risk if the proper care does not take.¹¹ Sustainable innovative green techniques have been used to reduce time, to consume energy, to increase the efficiency of the extraction and to help preserve the natural environment, by reducing the use of solvents and water in Ultrasound-Assisted Extraction (UAE) and Ultrasound Microwave-Assisted Extraction (UMAE),¹² generation of hazardous substances and fossil energy.¹³⁻¹⁵ Ultrasonic radiation use power 20-100 kHz to extract natural compounds provides high reproducibility, secure handling, low solvent, and energy consumption, low-temperature processing and a smaller loss of bioactive compounds.¹⁶ The extraction techniques can be compared with the microwave-assisted extraction, the ultrasound equipment is cheaper and the operation is easier. In addition, ultrasonic extraction, such as maceration, can be used for the extraction of a wide range of natural compounds with any solvent.¹⁷

Ultrasound can hydrate and facilitate swelling of vegetal tissue. According to research,¹⁰ ultrasound can allow high rates of diffusion across the cell and increase the transfer of mass. On the other hand, cavitation produced by ultrasonic waves can also disrupt the cell, and then release the contents. In research,¹⁸ the reported use frequency of 25 kHz from orange peel using an ultrasonic processor operated can produce higher extraction yields of polyphenols. Sonication is a faster, simpler, and more effective technique than maceration to extract organic compounds.¹⁹ Extraction of a bioactive compound from sea urchin using Ultrasonic Assisted Extraction (UAE). Therefore, the aim of this study was to assess the effect of ultrasonic treatment on the total bioactive compound content of the sea urchin and shell gonad.¹⁸

Material and Methods

Sea Urchin

Sea urchin (*Diadema setosum*) was obtained by trawling off the coast Barrang Lompo Island in Makassar, South Sulawesi, Indonesia. The gonads separated from the sea urchin shell (fig.1), then washed to remove other components and taken to the laboratory by carrying in the coolbox, and stored in the freezer (-20°C) until the gonads

and shell sea urchin processed in Food Science and Instrumental Analysis Laboratory, Chemical Engineering Department, Politeknik Negeri Ujung Pandang, Indonesia.

Chemicals and Tools

All chemicals substances were of analytical grade, methanol (CAS: 67-56-1), aquadestilate (CAS: 7732-18-5), and ethyl acetate (CAS: 141-78-6), supplied by Merck Millipore (Burlington, Massachusetts, United States). The tools used are water bath (Memmert WNB 7 Basic control) Hettich Zentrifugen EBA-20, and Hitachi centrifuge brands, Ultrasonic Assisted

Extraction instrument (Elmasonic P30), Shimadzu GC-MS 2010 brand Gas Chromatography-Mass Spectrometry plus.

Preparation of Extract

Sea urchin was bled to death; tissues and various organs dissected out carefully and collected. Sea urchin divided into intestinal organs, gills, eggs, and body walls (including plates, feet, and spines).²⁰ After removal of the internal organs (shell and gonad) a stream of cold water washed and cut into small pieces.



Fig:1 Sea urchin (*Diadema setosum*): a) Sea urchin harvest in the sea by fishers; b) Sea urchin collected; c) Sea urchin shell, and 4) Sea urchin gonad

Solvent Extraction

The extraction process began with the weighing stage of the sample, two hundred grams of sea urchin (gonad and shells) and 300 ml of methanol, added in a 500 ml glass flask. The mixture put in a water bath (Elmasonic P30 instrument) with a power of 40 kHz at 35°C for 30 min and 60 min to remove bioactive compounds from the sea urchin materials.¹⁷ Same treatment for ethyl acetate solvents. The extract was purified and evaporated at 39°C by a rotary evaporator, working principle of the rotary evaporator not only lies in heating but by lowering the pressure and regulating the velocity at a certain point so that the solvent methanol and ethyl acetate will evaporate and the soluble compounds in the solvent do not follow Evaporate but settlers. The boiling point of methanol and ethyl acetate solvent ranges from 64.7°C and 77.1°C, by heating below the solvent boiling point, so that the compounds in

the solvent are not affected at high temperatures.¹⁷ The high polarity and volatility of methanol and ethyl acetate was used as solvent to enhance yield and concentrate the desired compounds.²¹ Methanol and ethyl acetate were dissolved into the sea urchin.²² The solvent will evaporate perfectly when the evaporation process on the rotary evaporator until obtained the solvent that has not dripped again on the round base flask and can also be seen with the more potent substances present in the sample round base flask So that the bubbles are formed on the surface of substances.²³ The yield of sea urchin (%) is the ratio of the resulting sea urchin (g) compared to the sample weight used in the process (g). The formula calculates the calculation of the yield:

$$\text{Yield (\%)} = \frac{\text{Weight of sea urchin (g)}}{\text{Weight of starting material (g)}} \times 100\%$$

Table 1: Ultrasonic Assisted Extraction with Variations of Solvents and Time Extraction

Ingredients	Sea urchin (Shell)		Sea urchin (gonad)	
	Methanol (min)	Ethyl acetate (min)	Methanol (min)	Ethyl acetate (min)
Sea urchin	30	30	30	30
(<i>Diadema setosum</i>)	60	60	60	60

The Steps of Ultrasonic-Assisted Extraction (UAE)

Elmasonic Instrument P30 is paired with a cooling coil operated heating system (Fisher Scientific Inc St Louis, USA); and a cooling system for chillers; and water pumps (HJ-111 versions, 250 L/h submersible pumps, Sunshine Inc., Zhejiang, China). Ultrasonic waves are produced locally from micro cavitations around the material to be extracted so that the material is heated and the extract compounds are released. Double effects are generated, i.e. the cell wall screed to release the compound content in and the local heating in liquids and to increase the diffusion of the extracts. Variables selected temperatures of 30°C, 40°C, and 50°C; power settings of 30%, 50%, and 70 %; and high durations of 10 min, 20 min, and 30 min. Cinetic energy is transferred to all parts of the fluid, followed by the formation of cavitation bubbles on the wall or surface, thereby increasing the mass transfer

between the solid-liquid surfaces. The mechanical effect is to increase the penetration of the fluid into the cell membrane wall, support the release of the cell components and increase the transfer of mass.²⁴ Research²⁵ states that ultrasonic cavitation generates broken power that breaks the cell wall mechanically and improves material transfer. The variety of solvents used for extraction in Table 1.

Analysis of Bioactive Compounds (Gas Chromatography-Mass Spectrometry)

In a Shimadzu GCMS-QP 2010 Plus SHIMADZU instrument under computer control at 70 eV, the bioactive compound was determined using GC-MS analysis.²⁶ About 1 µl of the methanol extract injected into the GC-MS using a microsyringe, and complete scanning in 45 min. Starting from the phase selection of the motion or commonly called the gas carrier specified according to the analysis of the sample to be performed. In this research, gas

carrier He (Helium) with Principal level is 99.99%, and temperature maintained at 70-280°C or UHP (Ultra High Purity) type. Then the gas carrier is channelled through Stainless Steel tubing, and the pressure value of the gas carrier entered in Gas Chromatography is controlled by the AFC (Advanced Flow Controller) through the analysis software used

by GC-Solution. The column used Elite 1 (100% dimethyl polysiloxane) to isolate components^{27,28}. The Advanced Flow Controller (AFC) controls the flow going into the column. Compounds were then identified by comparing their spectra of the spectral mass libraries of the Wiley and NIST/EPA/NIH.

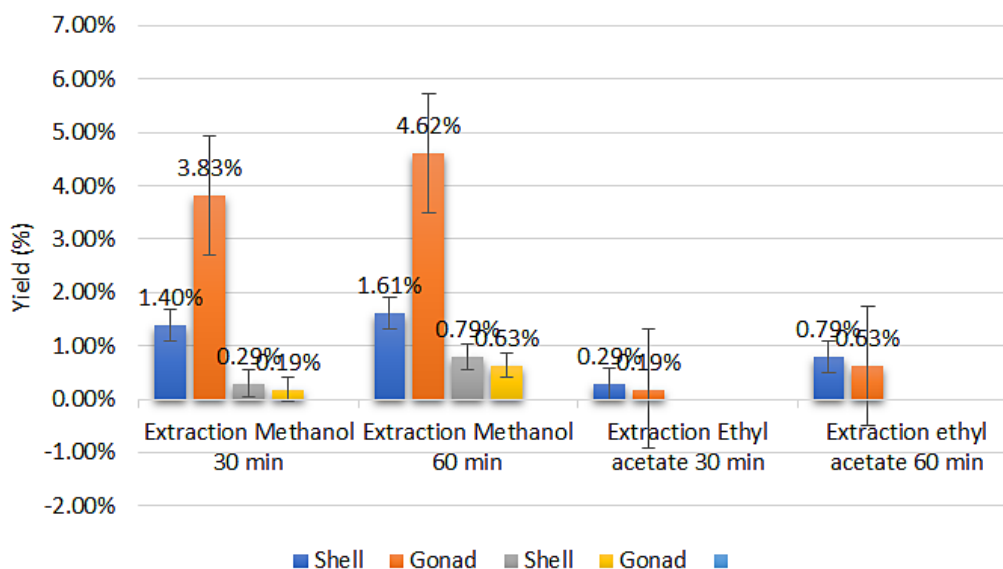


Fig. 2: The yield of sea urchin: a) Total yield per solvent; b) Values were presented as mean + SD (n=12)

Results

Extraction Yield of Sea Urchin

The yield of each sample was recorded on the dry basis of sample weight from 0.18 to 4.61% using ultrasonic-assisted extraction method and methanol or ethyl acetate solvents, as shown in Fig. 2. Maximum extraction yields of 1.61% and 4.61% were obtained from shell and gonad sea urchin, respectively, with methanol solvents at 60 min.

Unlike the above, the minimum yield is achieved by using ethyl acetate solvent at 30 min was 0.29% and 0.18% for shell and gonad sea urchin (*Diadema setosum*), respectively. Methanol solvents are capable of extracting components derived from alkaloids, phenolic, rubberonoid, tannin, amino acids, sugars, and glycosides. Besides, that methanol can destroy cell walls, so that components

to break down and dissolve in methanol solvents.^{29,30} Standard deviation (SD) test results indicate that SD value is smaller than the mean value, so data is not significant ($P < 0.05$), mean the yield amount does not affect the number of bioactive compounds produced by each solvent in a sample of a sea urchin gonads and shell.

The Bioactive Compound of Sea Urchin Shell with Methanol Solvent ExtractiSon

GC-MS test has performed showing the presence of 50 idealized compound components in methanol extract of sea urchin shell (Fig. 3), and 20 compounds identified in methanol extract of sea urchin gonads (Fig. 4). Some of the compounds detected at methanol extract of sea urchin shell, and gonad contains antibacterial substances. These antibacterial compounds include pentadecanoat acid, palmitic acid, stearic acid, ethyl cyclohexane, Tetradeconoic

acid (Stearic acid), Phthalic Acid, 1-Eicosanol, and Ergosterol. Another study done by³¹ informed that the white Oyster mushroom alcohol extract contains palmitic acid, 9,12-octadecadienoic acid, methyl ester (E, E)-18.55, 14.17-acid Oktadekadenolat, cholestatic-3, 7, 1, 25-tetraacetic extracts, methyl ester (E, E)-5.59, ethyl ester-3.84, (3a, 5a, 7a, 12a)-55.20 is potentially an antibacterial agent. The analysis of the chemical components of the sea urchin shell and gonad methanol extract using GC-

MS showed several active compounds that serve as antioxidant, antitumor, anticancer, analgesic agent and anti-inflammatory drugs. These active compounds include arachidonic acid, batilol, methyl ester, methyl ester, 9-octadecenoic acid (Z) -, tetradecanoic acid, and oleic acid. Analysis results also show that antibacterial components and anti-inflammatory drugs have the highest content, among other compounds. The antibacterial part detected is Cholest-5-EN-3-OL (3. Beta.) containing 39.51%.

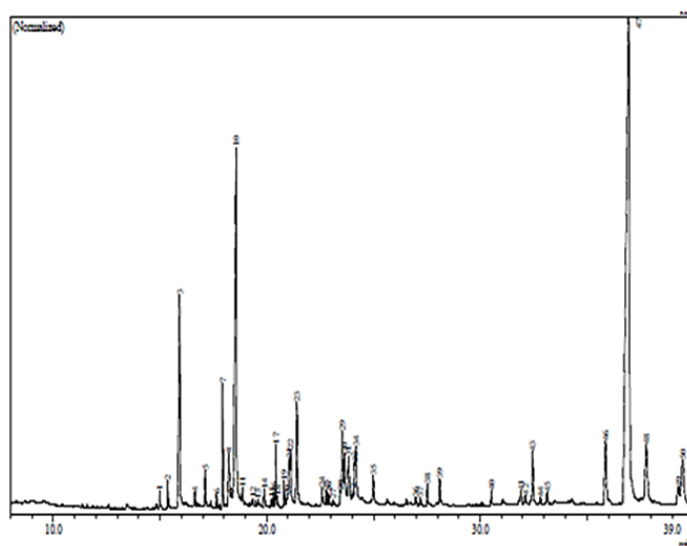


Fig. 3: Sea urchin shell chromatogram of methanol extract

A Bioactive compound of sea urchin gonad with methanol solvent extraction

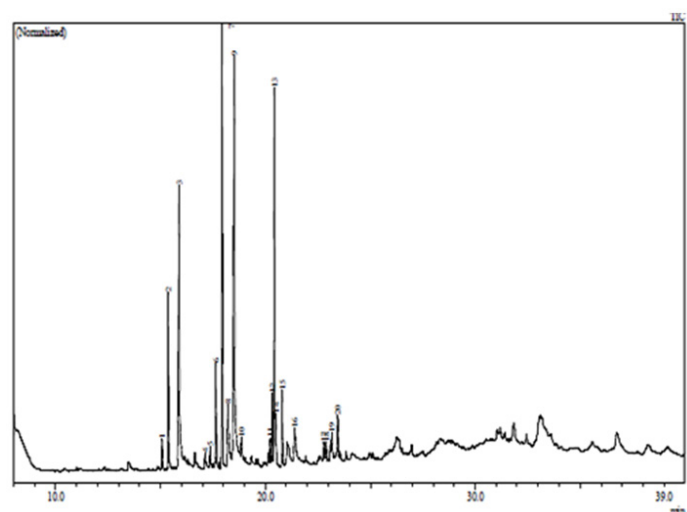
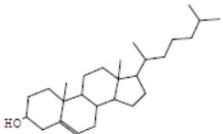
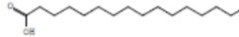
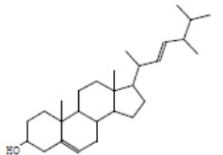
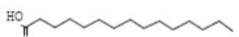
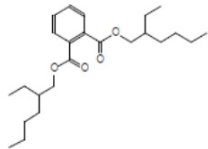
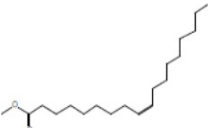
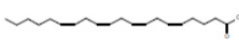
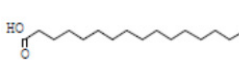
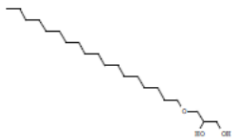
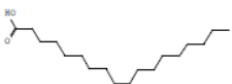


Fig. 4: Sea urchin gonad chromatogram of methanol extract

Table 1: Major phytochemicals identified by a sea urchin shell methanol extract

No.	Phytochemical component	Area (%)	Formula	Molecular weight (g/Mol)	Structure of the chemical	Pharmacological measures
1.	CHOLEST-5-EN-3-OL (3. BETA.)	39.51	$C_{27}H_{46}O$	386		Antibacterial activity and anti-inflammatory
2.	n-Hexadecanoic acid (Palmitic acid)	14.87	$C_{16}H_{32}O_2$	256		Anti-carcinogenic effects, Antioxidant, nematisida ³² Antibacterial activity ³³
3.	Ergosta-5,22-dien-3-ol	3.14	$C_{28}H_{46}O$	398		Anticyanobacteria ³⁴ , Antibacterial activity ³⁵
4.	Pentadecanoic Acid	0.31	$C_{15}H_{30}O_2$	242		Antibacterial activity ³⁶
5.	1,2-BENZENE DICARBOXYLIC ACID (Phthalic Acid)	0.10	$C_{24}H_{38}O_4$	390		Antibacterial activity ³¹ , Antioxidant, nematocides
6.	9-Octadecenoic acid (Z) -, methyl ester ³⁷	1.15	$C_{19}H_{36}O_2$	296		Antioxidant, anti-tumor, anti-cancer agents ³⁸
7.	Arachidonic acid	2.29	$C_{20}H_{32}O_2$	304		Antioxidant, anti-tumor, anti-cancer agents, Analgesic effects
8.	Palmitic Acid	0.19	$C_{16}H_{32}O_2$	256		Anti-carcinogenic effects, Anti-oxidative, nematisida, Antibacterial activity ³³

9.	Batilol	0.64	$C_{21}H_{44}O_3$	344		Antioxidant, anti-tumour, anti-cancer agents, Analgesic effects
10.	Oleic Acid	0.97	$C_{18}H_{34}O_2$	282		Antioxidant, anti-tumour, anti-cancer agents

A Bioactive compound of sea urchin gonad with methanol solvent extraction

Table 2: Major phytochemicals identified by a sea urchin gonad methanol extract

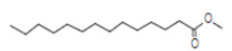
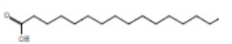
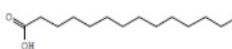
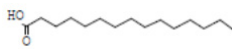

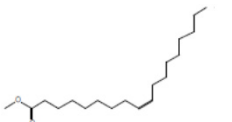

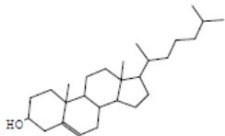
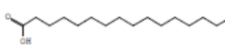
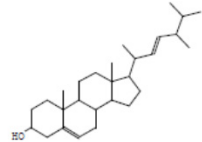

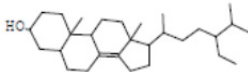
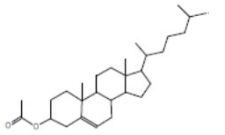
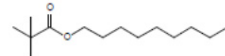

No.	Phytochemical component	Area (%)	Formula	Molecular weight (g/Mol)	Structure of the chemical	Pharmacological measures
1.	Tetradecanoic Acid, Methyl Ester	5.38	$C_{15}H_{30}O_2$	242		Anti-carcinogenic effects, Antioxidant, nematisida Antibacterial activity
2.	n-Hexadecanoic acid (Palmitic acid)	23.26	$C_{16}H_{32}O_2$	256		Anti-carcinogenic effects, Antioxidant, nematisida Antibacterial activity
3.	Tetradecanoic acid (Stearic acid)	15.63	$C_{14}H_{28}O_2$	228		Antibacterial activity
4.	Pentadecanoic	0.90	$C_{15}H_{30}O_2$	242		Antibacterial activity
5.	5,8,11-Eicosatrienoic acid, methyl ester	0.78	$C_{21}H_{36}O_2$	320		Antioxidant, Anti-Inflammatory
6.	9-Octadecenoic acid (Z) -,	14.31	$C_{19}H_{36}O_2$	296		Antioxidant, anti-tumor, anti-cancer agents
7.	1-Eicosanol	0.62	$C_{20}H_{42}O$	298		Antibacterial activity

Table 3: Major phytochemicals identified by a sea urchin shell ethyl acetate extract

No.	Phytochemical component	Area (%)	Formula	Molecular weight (g/Mol)	Structure of the chemical	Pharmacological measures
1.	Cholest-5-EN-3-OL (3. beta.)	46.24	C ₂₇ H ₄₆ O	386		Antibacterial activity and anti-inflammatory
2.	n-Hexadecanoic acid (Palmitic acid)	2.66	C ₁₆ H ₃₂ O ₂	256		Anti-carcinogenic effects, Antioxidant, nematisida, Antibacterial activity
3.	Ergosta-5,22-dien-3-ol	5.30	C ₂₈ H ₄₆ O	398		Anticyanobacteria, Antibacterial activity
4.	Pentacosane	25.83	C ₂₅ H ₅₂	352		Antioxidant, anti-tumor, anti-cancer agents, anti-virus
5.	Stigmast-8 (14)-EN-3.beta.-OL ³⁹	0.65	C ₂₉ H ₅₀ O	414		Anti-bacterial, anti-fungi, Antibacterial activity and anti-inflammatory
6.	Cholest-5-EN-3-YL acetate	0.84	C ₂₉ H ₄₈ O ₂	428		Antibacterial activity and anti-inflammatory
7.	2,2-Dimethylpropanoic acid, nonyl ester	0.18	C ₁₄ H ₂₈ O ₂	228		Antioxidant
8.	Pentatriacontane	0.60	C ₃₅ H ₇₂	492		Antioxidant,

The bioactive compound of sea urchin shell with ethyl acetate solvent extraction

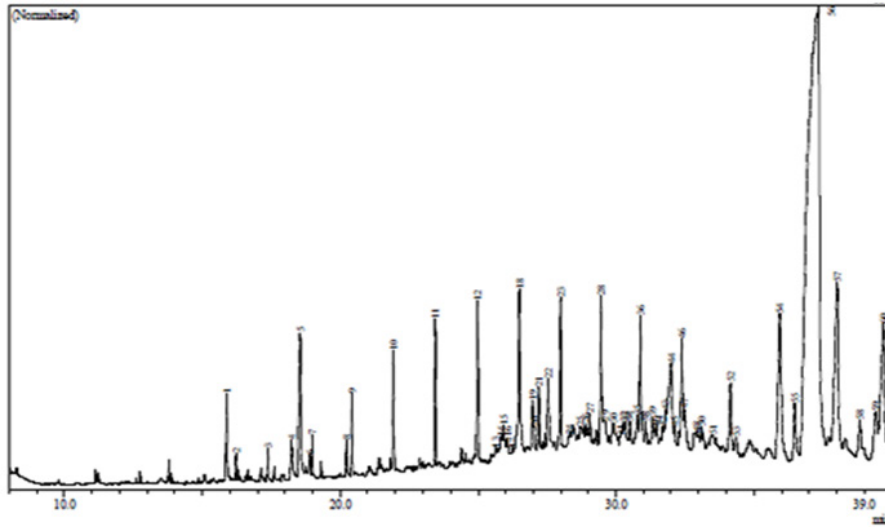


Fig. 5: Sea urchin shell chromatogram of ethyl acetate extract

A Bioactive compound of sea urchin gonad with ethyl acetate solvent extraction

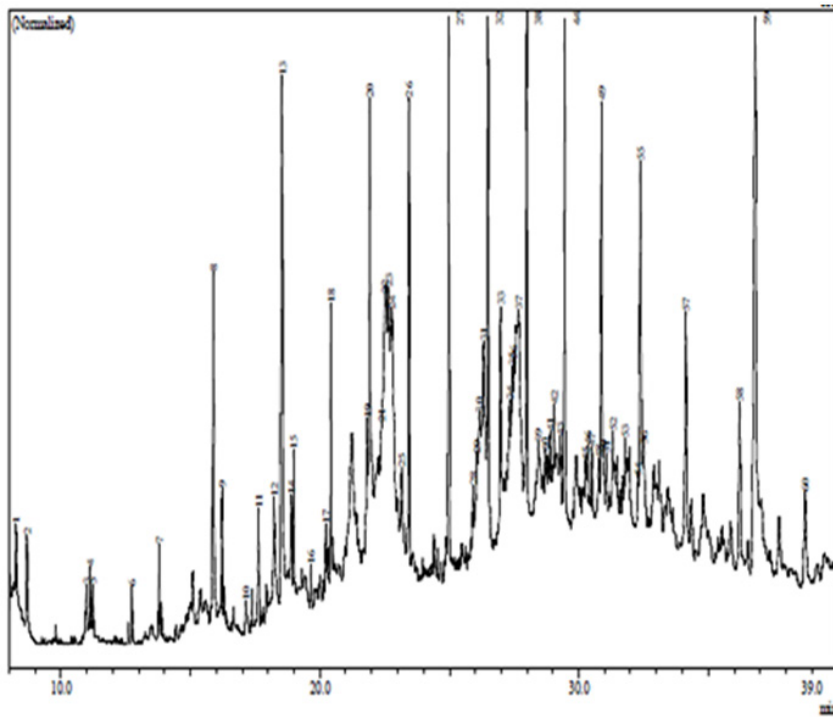
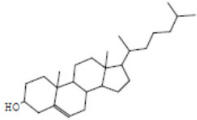
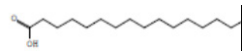
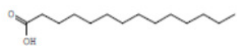

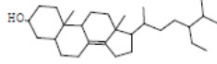
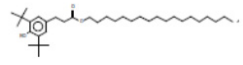
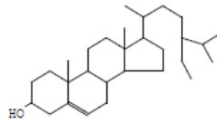
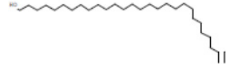


Fig. 6: Sea urchin gonad chromatogram of ethyl acetate extract

Table 4: Major phytochemicals identified by a sea urchin gonad methanol extract

No.	Phytochemical component	Area (%)	Formula	Molecular weight (g/Mol)	Structure of the chemical	Pharmacological measures
1.	Cholest-5-EN-3-OL (3. beta.)	9.17	C ₂₇ H ₄₆ O	386		Antibacterial activity and anti-inflammatory
2.	n-Hexadecanoic acid (Palmitic acid)	6.05	C ₁₆ H ₃₂ O ₂	256		Anti-carcinogenic effects, Antioxidant, nematisida, Antibacterial activity
3.	Tetradecanoic acid	3.30	C ₁₄ H ₂₈ O ₂	228		Antibacterial, antifungi40
4.	Pentacosane	15.48	C ₂₅ H ₅₂	352		Antioxidant, anti-tumor, anti-cancer agents, anti-virus
5.	STIGMAST-8 (14) -EN-3. BETA.-OL	3.10	C ₂₉ H ₅₀ O	414		Anti-bacterial, anti-fungi, Antibacterial activity and anti-inflammatory
6.	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, octadecyl ester	5.19	C ₃₅ H ₆₂ O ₃	530		Antioxidant
7.	Gamma. -Sitosterol	2.81	C ₂₉ H ₅₀ O	414		Anti-hypoglycemic,
8.	Octacosanol	0.35	C ₂₈ H ₅₈ O	410		Anti-fatigue, Anti-Parkinson effect, anticancer, anti-tumour

The GC-MS test showed the presence of 60 idealized compound components in the sea urchin extract of ethyl acetate (Fig. 5), and 60 compounds identified in the extract of ethyl acetate from sea urchin

gonades (Fig. 6). Some compounds found in ethyl acetate extract of sea urchin shell and gonad contain antibacterial and anti-fungal substances. These antibacterial compounds include pentadecanoat

acid, Cholest-5-EN-3-OL (3. BETA.), Stigmast-8 (14) -EN-3.BETA.-OL, palmitic acid, stearic acid, ethyl cyclohexane, tetradecanoic acid (stearic acid), phthalic acid, pentacosane, and ergosterol. The analysis of the chemical components of the sea urchin shell and gonad ethyl acetate extract using GC-MS showed several active compounds that serve as an antioxidant, antitumour, anticancer, analgesic agent and anti-inflammatory drugs. These active compounds include 9-octadecenoic acid (Z) -, methyl ester, pentacosane, 2,2-Dimethylpropanoic acid, p-Anisic acid, benzenepropanoic acid, 3,5-bi's (1,1-dimethyl ethyl) -4-hydroxy-, pentatriacontane, n-hexadecanoic acid or palmitic acid, Cholest-5-EN-3-YL acetate, and octadecyl ester. Some of the octacosanol compounds amounting to 0.81% (total amount) function as Anti-fatigue and Anti-Parkinson's effects. As well as a small fraction of 2.81% of the gamma.-sitosterol A compound can function as an antihyperglycemic. Analysis results also show that antibacterial components and anti-inflammatory drugs have the highest content, among other compounds. The most significant content is Cholest-5-EN-3-OL (3. beta.) or 46.24% steroid as an antibacterial agent.

Conclusion

This study resulted in data types of bioactive compounds, quantities, benefits and phytochemical

characteristics of sea urchin gonads and shells. Sea urchin (*Diadema setosum*) was obtained by trawling off the coast Barrang Lompo Island in Makassar, South Sulawesi, Indonesia. Contains chemical constitutions that are useful for different herbal formulations as anti-tumour, anti-cancer, anti-inflammatory, analgesic, anti-fatigue, anti-Parkinson effect, and anti-hypoglycemic. And potentially as a preservative in food and beverages because it is as antibacterial and antifungal.

Suggestions

Need to do further research to uses ultrasonic microwave-assisted extraction in extracting the sea urchin

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Conflict of Interest

The authors do not have any conflict of interest.

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