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Bioactive Compounds From Majapahit Fruit (*Crescentia cujete*) As a Potential Natural Antibacterial

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Abstract : The source of active ingredients that are commonly used in treating or preventing diseases, and are thought to play a beneficial role in health, for example are plants. Majapahit (*Crescentia cujete*) plant is one of such plants, whose leaves, barks, fruits, or roots have medicinal and preservative properties. These studies are needed to ascertain the compound of Majapahit fruit as a Natural Antibacterial and Identification of active ingredients using various characterisation techniques. The methanol, ethyl acetate, and, n-hexane extract of these fruit were prepared, and agar diffusion method with paper disc to investigate antimicrobial activity. Identification of active ingredients with; Phytochemicals Test, UV-VIS Spectrophotometry, FTIR spectrophotometer and Gas Chromatography-Mass Spectrometry (GC-MS). Results of the phytochemical test revealed the presence of flavonoids, saponins, , and triterpenoid in the Majapahit fruit. The methanol extract was the most effective (17.29 mm) in antibacterial activity. Ultraviolet spectrum is with maximum peaks at 407.0,- 396.9 nm and 313.0, - 219 nm, result of FTIR spectroscopy are wave numbers 3662 cm^{-1},- 518 cm^{-1} that indicates of; OH^- bending, -CH- (SP3), OH^-, -C=C-, $-CH_2-$, $-CH_3-$, and -C=CH-. The GC-MS chromatogram indicating the presence of 12 phytochemical constituents from 5 peaks highest. Majapahit (*Crescentia cujete*) fruit which implies that the extracts have a potential natural antibacterial. The phytochemical test revealed the presence of flavonoids, saponins, and triterpenoids, and The bioactive constituents of the plant extract were analysed by FTIR, UV-VIS spectrophotometry and GC-MS spectrometry, and they revealed varied and wide compounds.
Keywords: Majapahit (*Crescentia cujete*) fruit, antibacterial, flavonoids, saponins,triterpenoids.

Introduction

Medicinal plants abound around the world, and in Indonesia, it is reported that out of about 30,000 plant species, about 9,600 species have medicinal properties [1]. Despite this abundance, only about 300 Indonesian medicinal plant species have been (industrially) explored for their beneficial properties[1.] These beneficial properties are conferred on these plants by their bioactive ingredients, and plants are a green technology that can be used in treating or preventing diseases, preserving foods and controlling food spoilage. They are, therefore, valuable in food and healthcare systems.

Majapahit (*Crescentia cujete*) plant is one of such plants, whose leaves, barks, fruits, or roots have medicinal and preservative properties. Various parts of the plant have been used as a purgative, diuretic, analgesic, or anti-inflammatory drug possibly because they contain alkaloids, flavonoids, saponins, phenolics tannins, steroids, vitamins, carbohydrates, organic acids, amino acids and mineral [2,3,4,5] Specific bioactive compounds in the plant include β-sitosterol, estigmastrol, α- and β-amirina, apigenins, and carotenoids [2,6] These constituents are also responsible for the antibacterial properties of the plant, having been proven to effectively inhibit the growth of *Staphylococcus aureus, Enterococcus faecalis, Streptococcus pneumoniae, Streptococcus pyogenes, Escherichia coli, Candida albicans,* and *Ralstonia solanacearum*[7,8] Although these are foodborne organisms or organisms with healthcare concerns, the effectiveness of Majapahit plant for specific organisms that are responsible for diseases, and spoilage and health concerns in seafood, such as *Vibrio* spp., have not been extensively studied.

Various studies on antimicrobial properties of plants have been reported.[9] studied patikan kerbau (*Euphorbia hirta*), and found it to be effective against bacteria *Vibrio alginolitycus* and *Aeromonas hydropila*. Extracts of plant *Acumata Alstonia* were shown to have antibacterial properties against *Vibrio harveyi* [10], which was also inhibited [11] by the leaf extracts of kopasanda (*Chromolaena odorata* L.).[12] showed the extracts of seaweed *Caulerpa* spp. to be most active against *Pseudomonas bacteria*, and bacteria *Vibrio* spp. was inhibited [13] by other seaweed species (*Gelidium* spp., *Sargasum polycistum* and *Eucheuma cottoni*).[5] investigated antimicrobial activities of certain species of the family Biogenaceae (*Kigelia aficana, Jacaranda mimosifolia, Millingtonia hortensis, Tabebuia argentia, Dolichandron* spp. and *Haplophragm* spp.), and possibly because of their potentials, these authors advised more studies on the species of the family Bignoniaceae. Majapahit plant, is a specie of the family Bignoniaceae, and [14,] showed that the leaf extracts had a greater inhibition zone than the fruit and bark extracts against bacteria *Vibrio alginolyticus*, possibly because of differences in the bioactive constituents of these parts. However,[14] did not comprehensively investigate the differences in the constituents of these plant parts. The solubility of these constituents in polar and non-polar solvents will influence the concentrations of the plant constituents in the extracts, and consequently, the extents of their medicinal and preservative properties [2,15,16].

The common solvents used in extracting plants constituents include ethanol, chloroform, benzene, water, n-hexane, methanol, and ethyl acetate [17, 18,19,20]. [18] extracted the leaf and bark of Majapahit plant with ethanol and chloroform, and found the ethanol extracts to be more effective against *Staphylococcus aureus* and *Escherichia coli*.[19] investigated the antimicrobial properties of *Sonneratia lanceolate* against *Vibrio harveyi* by using water and methanol extracts of the plant. These authors found the water extracts to be more effective. Antifungal activities of *Aegle marmelos* leaf extracts in hexane, benzene, chloroform, ethyl acetate, methanol, water, and ethanol were investigated [20] and chloroform, methanol, water, and ethanol extracts had the least minimum inhibition concentration and were more effective. [5] extracted the stem, bark and leaves of *Cresentia cujete* using water and ethanol. These authors concluded that the aqueous extracts of the stem and bark were more effective against *Mycobacterium tubercolosis* than the alcoholic extracts of the plant parts. Despite its potential beneficial properties for health and food, studies on the constituents of Majapahit plant are limited, and the differences between the various parts have not been comprehensively examined, nor possible differences in the effectiveness of extracting solvents. These studies are needed to ascertain or classify Majapahit fruits as a medicinal plant with potential natural antibacterial and food preservative properties. The present study was aimed along these lines using novel characterisation techniques.

Material and Methods

Materials

Majapahit fruit was obtained from Tuban, Indonesia. The fruit was correctly identified as *Cresentia cujete* L. by the Indonesian Centre for Research in Biology, Bogor, Indonesia. TLC plates were a product of Merck (Darmstadt, Germany). All chemicals, reagents and solvents used were pure and/or of analytical grade.

Preparation of raw materials and extraction

The fruit was cleaned, cut into small pieces and air-dried, pulverized in a blender (HR2102, Phillips, Indonesia) to a fine (passed through a 250-µm sieve) powder. About 50 g of the powder was soaked in 250 mL of methanol for about 72 hr in a glass jar, before the supernatant was separated from the pulp. The supernatant

was then evaporated in a rotary evaporator under vacuum at about 45°C to obtain a concentrated extract with no trace of the solvent. Other solvents investigated following the same procedure (with or without vacuum) were water, ethanol, acetone, ethyl acetate, diethyl ether, n-hexane, and chloroform. These solvents were chosen in order to examine the best that maximises the beneficial properties of the Majapahit plant.

Phytochemicals test

The phytochemical test procedure of [21] was used, with about 20 mg of the powder dissolved in 25 mL of 96% ethanol to detect alkaloids, flavonoids, saponins, tannins, triterpenoids and steroids. The TLC followed standard procedure, and the plates were activated in the oven at a temperature of 100 - 105^{o}C for 30 min.

Antibacterial test

The extracts were tested against *Vibrio harveyi* at a concentration of 10 mg/mL using the agar diffusion method with paper disc [22,11] Sterile paper discs (Oxoid, Basingstoke RG24 8PW, UK) were dipped in the extracts for 15 - 30 min. before placing the paper disc in the Mueller-Hinton Agar (MHA) media that had been inoculated with the bacteria. Measurements were made after an incubation period of 24 hr at 37^{o}C by observing the presence or absence of clear zones formed around the paper disc [23,11] *V. harveyi* is a well-known food poisoning bacteria found in marine animals [24,25] and it was chosen because, one of the aims of the long-term research under which the present study falls, was to find a green technology for preservation of seafood.

Identification of active ingredients

UV-VIS Spectrophotometry

A known volume (0.1 mL) of the extracts was diluted to 10 mL with appropriate solvents, and the solutions were measured at wavelengths of 200 – 400 nm using a UV-VIS spectrophotometer (Varian Type Cary 50, Agilent, Santa Clara, CA 95051, USA).

Fourier Transform Infra-Red Spectroscopy (FTIR)

Infrared spectroscopy was applied to confirm functional groups in the extracts. A drop of each extract was mixed with KBr to make pellets (2g), which were subsequently analyzed in an FTIR spectrophotometer model (FTIR 1000 SCIMITAR, California, USA) at wavenumbers 4000 - 800 cm^{-1}.

Gas Chromatography-Mass Spectrometry (GC-MS)

The extracts were analysed by GC-MS (GC-MS QP2010, Shimadzu, Kyoto 604-8511, Japan), using helium as the carrier gas. The injection temperature was 320^{o}C, and thermal gradients were applied from 700^{o}C - 300^{o}C within 4 hr with a gradient rate of 100^{o}C/hr.

Result and discussion

Phytochemical profile

The phytochemical test revealed the presence of flavonoids, , saponins, and triterpenoid, in the Majapahit plant (Table 1). Flavonoids are reported to be the most natural, widely spread components in plants [22,9,11,10] and their presence in the fruit is not surprising.

Tabel 1. **Result for phytochemical screening in Majapahit fruit (*Crescentia cujete*)**

Compound	**Observation**	**Inference**
Flavonoids	Orange	Present
Saponins	Little foam	Present
Tanins	Brown	Absent
Steroids	Brown	Absent
Triterpenoids	Brown	Present
Alkaloids	Orange Cloudy	Absent

Antibacterial activity

Solvents vary in polarities, and consequently, the abilities to dissolve bioactive constituents of the Majapahit plant fruit. With differences in the dissolved constituents, the extracts might vary in their overall beneficial properties, and Table 2 shows that the efficacy of the extracts was solvent dependent as expected. As highlighted above [18] found the ethanol extracts of the leaf and bark of Majapahit plant to be more effective than the chloroform extracts on *Staphylococcus aureus* and *Escherichia coli*. Similar solvent differences were reported by [19,20] amongst others. In the present study, it appears that methanol was the most effective, while n-hexane was ineffective.

Table 2. Extract of Majapahit fruit (*Crescentia cujete*) inhibition zone against *V. harveyi*

Samples	Zone of Inhibition (mm)		
	n-Hexane	Ethyl acetate	Methanol
Majapahit fruit	2.4[c]	14.66[b]	17.29[a]

*Values with the same letters are not significantly ($p > 0.05$) different. The zone of inhibition of erythromycin (positive control) was 26.4 mm

Table 1 shows the presence of flavonoids, saponin and triterpenoid in the fruits, and these have antibacterial properties, and the presence of these compounds could explain the results in Table 2. However, different solvents differently solubilize various compounds of the flavonoid group. Flavonoids are phenolic structures containing one carbonyl group complexes with extra cellular and soluble protein and bacterial cell wall, and they exhibit antibacterial activities through these complexes [26]. Flavonoids inhibit the growth of bacteria or microorganisms by interfering with their cell wall permeability, and the consequent disruptions cause other compounds such as saponins, triterpenoids, phenolics, alkaloids, and tannins to penetrate and damage the cell wall [27] It was thought that because flavonoids are polar compounds, they easily penetrate the cell walls of microorganisms.

Triterpenoids are a diverse group of secondary metabolites that are associated with a variety of biological activities [28] The main groups of triterpenoids are represented by tetracyclic and pentacyclic derivatives[29] Important elements that play a role in the antibacterial activity of triterpenoids relate to chemical compositions that include functional groups and hydroxyl groups of phenolic triterpenoids as well as the number of single components. However, when triterpenoids are used as single compound, they are less effective as an antibacterial. The concentrations are important too [26] because at low concentrations, triterpenoids only affect enzymes involved in energy production while at high concentrations, triterpenoids can lyse membranes. Bacterial species of *Mycobacterium* are supposed to be sensitive to triterpenoids[30] . Triterpenoids showed bacteriostatic effects against *Staphylococcus aureus* [29]. Triterpenoids reportedly exhibit antimicrobial activity against gram-positive bacteria, block cell divisions by inhibiting DNA synthesis and macromolecular synthesis in *Bacillus subtilis*. The inhibition of macromolecular synthesis could be due to the damage to cell membranes[31] influence of cell morphology, enhancement of detergent-induced lysis, and autolysis of isolated cell walls.

Saponins are glycosides occurring widely in plants, and are complex compounds of condensation products of a sugar with an organic hydroxyl compound, which when hydrolyzed would produce sugars (glikon), non-sugar (aglycone) and foam [32] Saponins compose of two groups, triterpenoid saponins and steroidal saponins, which are respectively fat- and water-solubles. When concentrated on the cell membrane, they lower the surface tension and works as an antimicrobial to destabilize bacterial cell membranes causing bacterial cells to die through lysis [33,15,32] As surface active agents, they interfere with or alter the permeability of cell walls, thereby facilitating the entry of toxic materials to or leakages of vital constituents from the cells [34,32] showed the crude saponin extracts prepared from *Sorghum bicolor* posses antimicrobial property, and were active against *Staphylococcus aureus,* a pathogen, which has been implicated in several human and animal infections. This is similar to reports by[33] that saponins from *Hibiscus sabdariffa* were also active against *Escherichia coli, Klebseilla pneumoniae, Bacillus cereus, and Micrococcus luteus*. In order to fully understand the measured antibacterial activities of the Majapahit fruit, specific information on the extracts was obtained using the following techniques.

Analysis compounds of the extracts majapahit fruit by UV-VIS Spectrophotometry

There were 47 wavelength spectra from 200 to 800 nm (Fig. 1), with maximum peaks at 407.0 - 396.9 nm and 313.0 - 219 nm. The occurrence of a strong absorption in the ultraviolet spectrum showed the presence of a number of C = C double bonds in the compounds from the Majapahit fruit. This strong uptake showed a phenol compound and its derivatives.

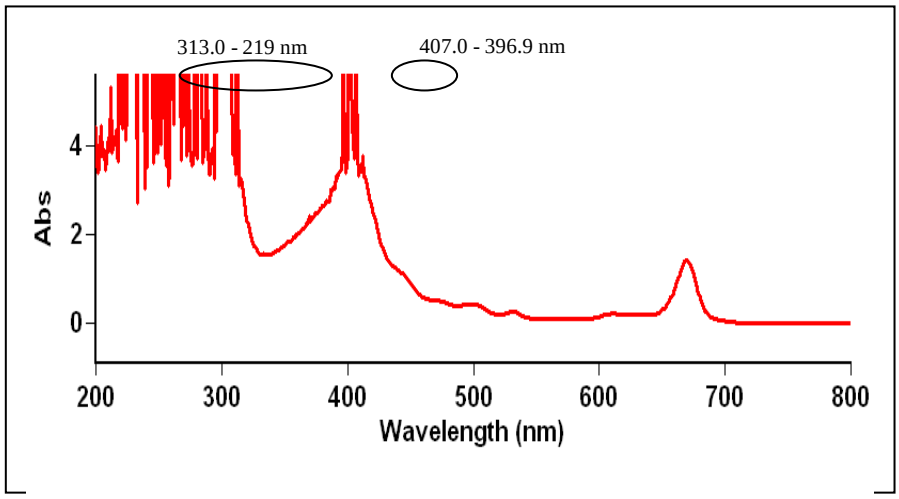


Figure 1. A typical spectrum of the compound fractions of Majapahit fruit

From published results, the possible compounds that correspond to the prominent wavelengths of maximum absorption from the extracts of the Majapahit fruit, are summarized in Table 3.

Table 3.Possible compounds from the prominent wavelength from the extracts of Majapahit fruit

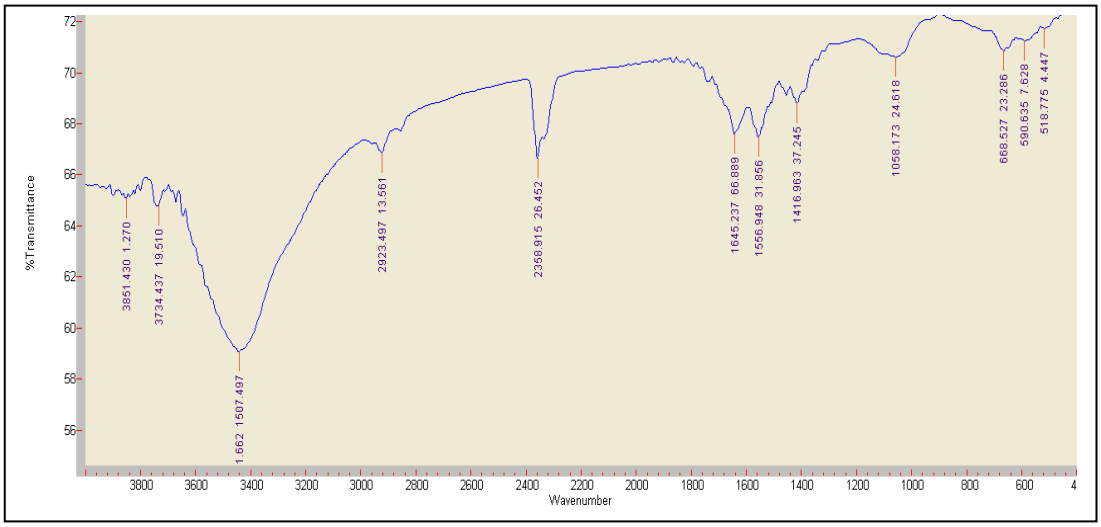
No	wavelength result of UV-VIS	Wavelength Range based on the literature	Compounds	PubChem CID
	269.0	269.0	Ezetimibe	150311
	223,0	223,0	Fosinopril	55891
	288.0	288.0	Gemcitabine	60750
	257.1	257.1	ketoconazole	47576
	284.1	284.1	Rabeprazole	5029
	253.0	253.0	repaglinide	65981
	308.0,	308.0,	rofecoxib	5090
	293	293	Vincmine	15376
	271.0	271.0	Aceclofenac; Paracetamol	71771;1983
	280.0	280.0	Ibuprofen; Paracetamol	3672;1983
	294	294	Levamisole; oxyclozanide	26879; 16779
	313.0	313.0	Aceclofenac; tizanidine	71771; 5487
	219	219	Amitriptyline	2160
	247.8	247.8	Diflucortolone valerate Isoconazole nitrate	33140
	271.0	271.0	Hydrochlorothiazide Olmesartan Medoxomil	9940864
	295.0	295.0	Ofloxacin; Tinidazole	4583; 5479
1	274, 271, 267, 261, 250.9, 247.9, 245	240 – 290	Tramadol hydrochloride	63013
		240 - 285	Ibuprofen (IB);Pseudoephedine	3672; 7028

		210 -280	Hydrochlorothiade; Quinaphril (QA)	3639; 54892
2	237,5, 245	235 - 245	Letrozole	3902
		210 - 280	Hydrochlorothiade; Quinaphril (QA)	3639; 54892
3	232	227 – 232	Cabergoline	54746
		210 - 280	Hydrochlorothiade; Quinaphril (QA)	3639; 54892
4	274, 271	271 - 275	Acerclofenac;Paracetamol	71771; 1983

Source: [35,36]

Analysis compounds of the extracts majapahit fruit by FTIR

FTIR spectroscopy showed (Fig. 2) the presence of OH^- bending with a wide ribbon shape at the absorption wavenumbers 3662 cm^{-1}, absorption at 2923 cm^{-1}, with a weak ribbon shape that indicates -CH- (SP3) aliphatic stretching. Absorptions at 1645 $cm^{-1}$and 1556 cm^{-1} indicated a -C=C- aromatic with a moderate ribbon shape. Wavenumber 1058 cm^{-1} showed a primary OH^-, while wavenumbers 1416, 688, 590, and 518 respectively indicated $-CH_2-$, $-CH_3-$ scissoring, -C=CH- out of plane, and mono-branched alkanes ($-CH_3-$) that formed a weak ribbon.



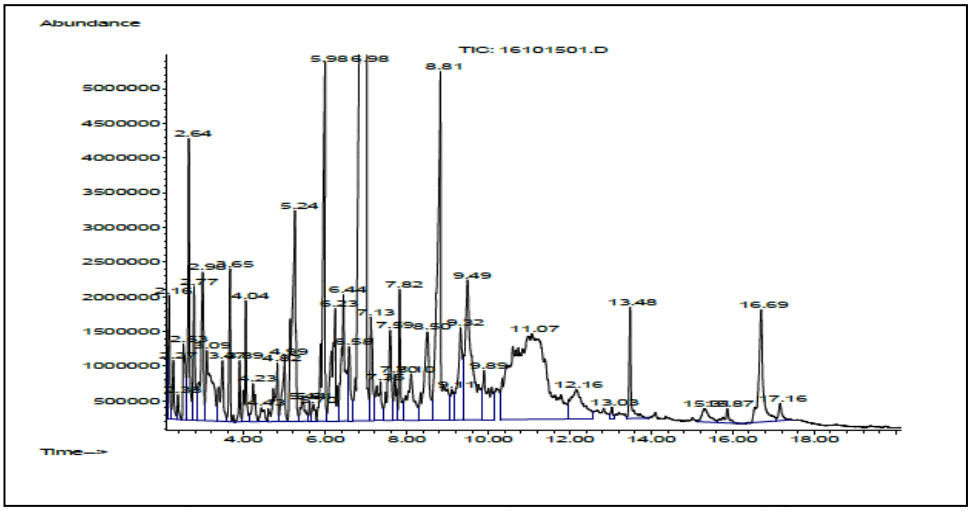
Based on Fig. 2, the suspected compounds from the extracts Majapahit fruit are summarised in Table 4

Table 4 Absorption band, wave number , the type of vibration and FTIR results ribbon shape extracts fruit majapahit

Absorption band	Wavenumber (cm^{-1})	Reference (Harborne, 1994)	Type of vibration	Shape ribbon
1	3851			
2	3734			
3	3662	3500 – 3200	-OH bending	splay
4	2923	3000 – 2700	-C-H (SP^3) stretching aliphatic	Weak
6	1556			Moderate
7	1416	1470 – 1450	$-CH_2$, $-CH_3$ scissoring	Weak
8	1058	~ 1050	-OH primer	Moderate
9	688	690 – 590	-C=CH out of plane	Weak
10	590			Weak
11	518	~ 570 – 540	Monobranched alkanes ($-CH_3$)	Weak

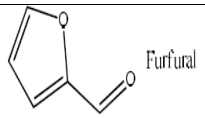
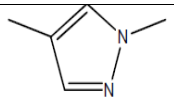
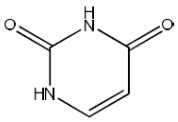
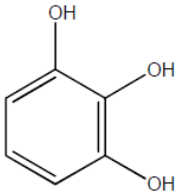
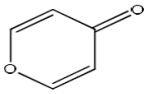
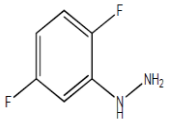
Analysis compounds of the extracts majapahit fruit by GC-MS

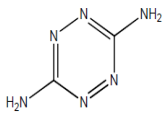
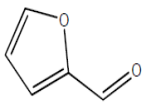
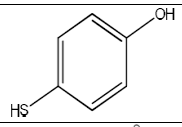
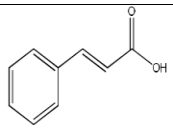
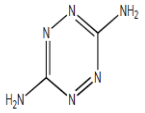
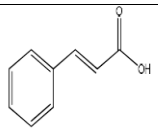
Figure 3 shows a typical spectrum from the GC-MS test, while Table 5 summarises the suspected compounds and their structures. There were 45 diverse compounds in the Majapahit plant, with four prominent compounds having peaks at 2.64, 5.98, 6.98, and 8.81.



The majapahit fruit extract showed 5 peaks highest in the GC-MS chromatogram indicating the presence of 12 phytochemical constituents. Through comparison with mass spectra 12 phytochemical constituents were characterized and identified.

Table 5. The prominent compounds in the majapahit fruit

No	RT	Area	Compound name	Chemical formula	Compound structure	PubChem CID
1	2.21	2.64	Furfural	$C_5H_4O_2$		7362
			Pyrazole,1,4-dimethyl, 3,5-dimethyl-1H-pyrazole	$C_5H_8N_2$		6210
2	5.12	5.24	2,4(1h,3h)-pyrimidinedione	$C_4H_4N_2O_2$		1174
			1.2.3 Benzenetriol	$C_6H_6O^3$		10787
3	5.81	5.98	4H-Pyran-4-one,	$C_5H_4O_2$		7968
			2,5 difluorophenylhydrazine	$C_6H_6F_2N_2$		10920925

			1,2,4,5-tetrazine-3,6-diamine	$C_2H_4N_6$		49863143
4	7.12	6.98	Furancarboxaldehyde	$C_5H_4O_2$		7968
			4-mercaptophenol	C_6H_6OS		240147
5	8.89	8.81	2-propenoic acid, 3-phenyl	$C_9H_8O_2$		444539
			1,3,5-Triazine-2,4,6-triamine	$C_2H_4N_6$		61176
			Trans-Cinnamic acid	$C_9H_8O_2$		444539

Conclusion

Majapahit (*Crescentia cujete*) fruit were extracted using methanol more effective against *V. harveyi*, which implies that the extracts have a potential natural antibacterial. The phytochemical test revealed the presence of flavonoids, , saponins, and triterpenoid, and The bioactive constituents of the plant extract were analysed by FTIR, UV-VIS spectrophotometry and GC-MS spectrometry, and they revealed varied and wide compounds.

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