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PHYTOCHEMICAL, ANTIOXIDANT AND ANTIMICROBIAL PROPERTIES OF THE WHITE VARIETY OF 'SIBUJING' (*Allium ampeloprasum*)

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ABSTRACT

One of the most popular plants used as a spice by the Maranao Muslims of Marawi City is a white variety of *Allium ampeloprasum*. This plant is not only popular for the preparation of a variety of dishes but also for the treatment of common infections. This study was conducted to determine the phytochemical constituents, antioxidant and antimicrobial properties of ethanolic extracts of the plant variety. Alkaloids, saponins, flavonoids, and steroids were the phytochemicals that were detected in the samples, while tannins, cyanogenic glycosides and anthraquinones were absent. The plant extract showed strong antioxidant activity based on DPPH assay. The antimicrobial assay showed no inhibition of growth of *P. aeruginosa* UPCC1244 and *B. subtilis* UPCC1295 but strong inhibition of *E. coli* UPCC1195, *K. pneumoniae* UPCC1360, *S. typhimurium* UPCC1368 and *S. aureus* UPCC1142. GCMS analysis showed the presence of compounds with biological properties. This study, therefore, indicated that the plant has potential medicinal and antimicrobial properties.

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Introduction

Various medicinal plants have been used traditionally to treat diseases all over the world. More than 80% of the world's population depends on conventional medicine for primary health care needs [1]. Herbs, specifically spices have been consumed for many centuries to preserve foods, and provide medicinal values [2]. Additionally, traditional plants and spices are important in natural agents for controlling health problems and pathogenic diseases. One of these is *Allium ampeloprasum.* It is a genus of monocotyledonous flowering plants that includes many economically important species such as onions (*A. cepa*), french shallots (*A. oschaninii*), leeks (*A. ampeloprasum*), scallions (some *A.* species), and herbs including garlic (*A. sativum*) and chives (*A. porrum*) [3]. Some have been used as an ethnomedicinal plant. The *A. ampeloprasum* from the family of *Amaryllidaceae* belongs to the *Allium* species having true bulbs which have been characterized by herbaceous geophyte perennials [4]. It is a variety locally named 'Sibujing' utilized by the Maranao Muslims as a spice for Maranao cuisine and delicacies. In addition, it is also consumed for traditional dietary application and medical purposes to treat diseases. In folk and Maranao medicine, this plant is diversely used to cure ailments including fever, baby's teething discomfort, infections, and inflammations. Some studies have shown that related species in the *Allium* family are used for the treatment and prevention of many diseases such as cancer, gastritis and gastroduodenal disorders [5],

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risk of gastrointestinal diseases [6], diabetes and cardiovascular illnesses [7], thrombotic diseases [8] and infections from virus, parasites, bacteria and fungi [9]. Therefore, the objective of this paper was to investigate the selected biological properties of the extracts from *A. ampeloprasum,* and determine the phytochemicals, antioxidant and antimicrobial properties including the GC-MS detection of the compounds present to have a better understanding of its ethnomedicinal values.

Materials and Methods

Ethnomedicinal Information

Key informants composed of the local people were interviewed focusing on the folk medical uses of *A. ampeloprasum.* A chain-referral sampling method was employed for the respondents comprised of residents, traditional healers, and the Maranaos. Data were gathered through series of informal interviews and semi-structured questionnaires among the respondents concerned to the origin of knowledge, varieties, and parts used, traditional preparations as well as uses and modes of application. The survey was initiated with an informed prior consent of the respondents. In conducting the interview, the participation of the members of the non-government indigenous people's organization of Marawi City was also sought to help.

Plant material

The white variety of *A. ampeloprasum* grown and harvested was collected directly from the plants growing in a lowland well-drained farm of Marawi City, Lanao del Sur in the 3rd week of July 2017. This variety of the plant was observed to have a fan of slender, green leaves; and its bulb at the base of the plant began to swell when a certain day-length was reached. Photographs of the plant and its parts were captured for taxonomic keys and identification following the *List of Medicinal Plants of Philippines* by Stuart and Santiago as depicted in Figure 1. Confirmation of the identification was done and verified by a botanist and systematist of the Department of Biological Sciences, MSU-IIT.

Fig. 1. Sibujing (*A. ampeloprasum*)

Extraction of Plant Material.

The plant extraction was carried out according to the modified procedures of the key informants. The procedure was done at the Graduate Research Laboratory of the Department. Two kilograms of the bulbs of the onion were soaked and blended using a food processor or mechanical blender with 2.0 L of 99% absolute ethanol. The container was sealed with cheesecloth, shaken and left to stand for three weeks with regular shaking. After three weeks, the suspension was filtered using cheesecloth and Whatman filter paper No. 1 (Whatman, UK). The filtered sample was covered in a container and left to stand for another two weeks. After two weeks, the filtrate was concentrated using a rotary-evaporating machine to a temperature at about 45 °C. The crude extract was collected and allowed to completely dry at room temperature. The obtained viscous crude extracts were stored in storage vials for antimicrobial, free radical scavenging activities, and phytochemical screening. The partition of crude extracts of the *A. ampeloprasum* using hexane was carried out using the standard partition methods described by [10]. The hexane-soluble extract was concentrated in the rotary evaporator and was then stored in the refrigerator until used for GC-MS analysis. The summary of the extraction and partition process have been shown in Figure 2.

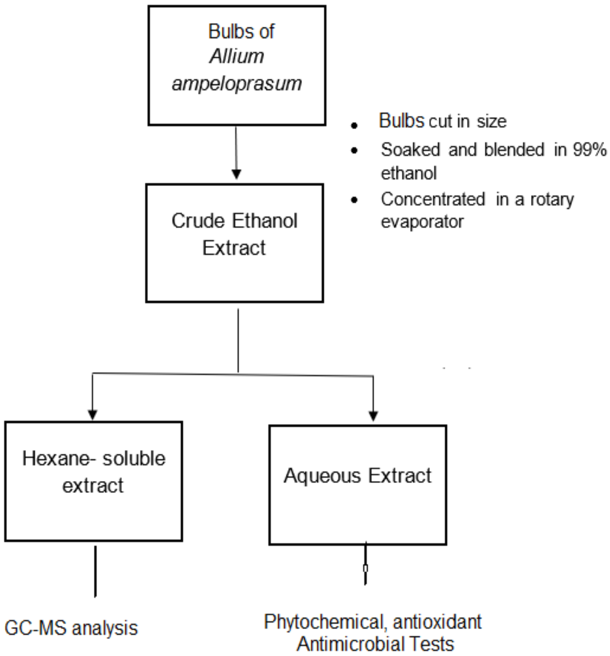

Fig. 2. Schematic diagram of the extraction and partition process.

***In vitro* Antimicrobial Assay**

The *in vitro* antimicrobial assay was screened by the agar well-diffusion method from the Microbiological Research and Services Laboratory in Natural Sciences Research Institute, University of the Philippines in Diliman. The ethanolic extracts were performed against the six (6) selected test microorganisms. The microbial suspensions of Gram-negative bacteria: *Escherichia coli* (UPCC 1195), *Klebsiella pneumonia* (UPCC 1360), *Pseudomonas aeruginosa* (UPCC 1244) and *Salmonella typhimurium* (UPCC 1368) and the Gram-positive bacteria: *Bacillus subtilis* (UPCC 1295) and *Staphylococcus aureus* (UPCC 1143) were prepared in 0.1% peptone broth. The procedure has been outlined in Figure 3.

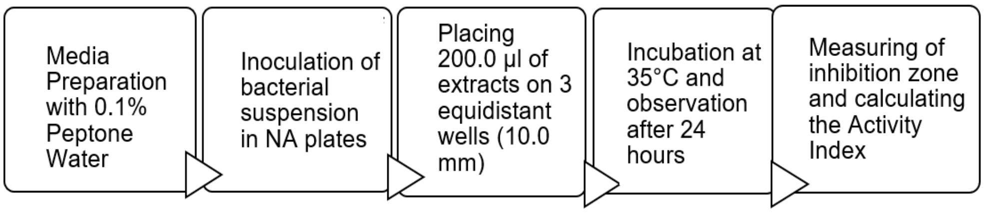

Fig. 3. Diagram of the *in vitro* antimicrobial assay using the agar well diffusion method.

The absolute ethanol was tested to ensure no antimicrobial activities, hence, the negative control. 30 µg of a 6-mm diameter disc of chloramphenicol was used as the positive control. Pre-poured Nutrient Agar (NA) plates about 3.0 mm thick were inoculated with the respective microbial suspension by swabbing the agar surface in the microbial even distribution of the inocula. Three equidistant wells were made using a cork borer (10.0 mm) on the suspension; the swab on applicator stick was submerged, rotated several times and squeezed gently on the inside wall of the tube above the fluid level to remove the excess inoculum from the swab. In the entire agar surface, the swab was streaked over and repeated two more times, rotating the plate 60° each time to guarantee agar plate and a two hundred (200.0) µL portion of the extract was placed in each of the wells. The Pre-poured Nutrient Agar (NA) plates were incubated at 35°C, and observed after 24 hours. All tests were performed in triplicate, and the antibacterial activity was expressed as the average diameter of the clearing zones measured in millimeters (mm). The inhibition zone was calculated by the mean of three replicates produced by the plant extract. The activity index was computed using the following equation:

$$\text{Activity Index} = \frac{(\text{Diameter of well} - \text{Diameter of clearing zone})}{\text{Diameter of well}}$$

Phytochemical Screening and GC-MS Analysis

The phytochemical screening of the ethanolic extract of *A. ampeloprasum* white onion variety was carried out using the standard phytochemical methods described by [11], and was modified according to the Laboratory Manual for the UNESCO Sponsored Workshop on the Phytochemical, Microbiological, and Pharmacological Screening of Medicinal Plants at the

Department of Chemistry, MSU-IIT (Figure 4). A 3-point scale (+ turbid, ++ moderate and +++ heavy) in scoring was based on the Handbook of Philippine Medicinal Plants by de Padua *et al.* [12]. Gas Chromatography-Mass Spectrometry (GC-MS) analysis was performed following the protocol of Chipiti *et al.* [13] with modifications to identify the compounds present in the hexane-partitioned extract. The compounds were identified by direct comparison of the mass spectrum of the analyte at a particular retention time to that of a reference standard found in the National Institute of Standards and Technology (NIST) library. The total GC-MS running time lasted for 45 minutes. At least, 80% similarity index was considered significant [14].

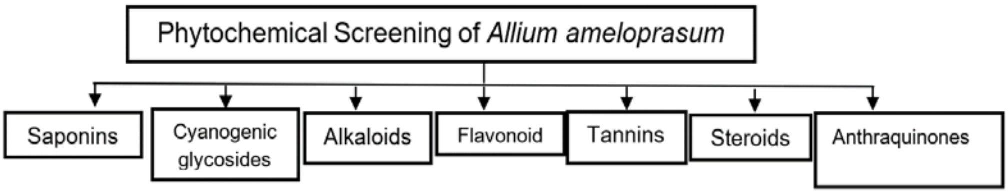

Fig. 4. Schematic diagram of the phytochemical screening of *A. ampeloprasum*

Results and Discussion

Ethnomedicinal Properties

Based on the results of the interviews with the Maranaos, the following were the modes of applications of the different preparations of the plant to cure common ailments (Table 1). The preparations were in the form of decoctions, poultice, and infusions. It could be concluded from the results that the Maranao Muslims use these kinds of preparations of the plant to cure common medical conditions such as cough, skin infections, body pains, spasms, blood pressure problems, blood clotting, sugar level normalization, expectorant, as a diuretic and the treatment of the loss of appetite.

Table 1. Ethnomedicinal Uses of the *A. ampeloprasum* in Lanao del Sur, Philippines.

Traditional Preparation	Parts Used	Modes of Application	Medical Uses
Tincture	Bulb	Oral (drinking)	Purgative Stomach trouble (diarrhea)
Decoction	Leaves and bulb	External: rubbing Oral: drinking the decoction	Inflammations Fever Cough
Direct application	Whole plant	Crushed to make poultice	Treatment of baby's teething discomfort; Skin infection Body pain
Crushing and make infusion	Bulb and root	Drink the infusion	Infection Relaxing spasm Reducing blood pressure Blood clotting and blood sugar levels Expectorant Diuretic Treat loss of appetite

Antimicrobial Properties

The *in vitro* antimicrobial activity of the ethanolic extract of *Allium ampeloprasum* tested against the gram-negative and gram-positive bacteria was assessed using disc diffusion method by getting the growth inhibition zones. The ethanolic extracts were observed to have selective antimicrobial activities and not as strong when compared to a known antibiotic chloramphenicol (Figure 5, Table 2). It was not effective against the gram-positive bacteria *P. aeruginosa* and the gram-negative bacteria *B. subtilis.* The sensitivity of test strains was in, decreasing order: *K. pneumoniae* > *E. coli* > *S. typhimurium* > *S. aureus*. These bacterial organisms have been known as pathogenic causing health issues in humans. The gram-negative bacteria were *E. coli*, facultatively anaerobic bacterium which cause pneumonia, gastrointestinal infections and diarrhea [15], *K. pneumonia*, lactose- fermenting bacterium which cause pneumonia, urinary tract infections and nosocomial infections [16], *S. typhimurium*, a food-borne pathogen which causes gastroenteritis complications [17] and *P. aeruginosa*, a gram-negative opportunistic pathogen which causes nosocomial infections and infections in immunocompromised hosts [18]. The gram- positive bacteria were *B. subtilis*, spore- creating bacterium that cause pneumonia [19] and *S. aureus*, a pyrogenic bacterium which causes skin diseases [20, 21]. The preliminary results appeared to indicate the high potential of antimicrobial activities. Other studies have suggested that other *Allium* species also showed potential against the tested microorganisms [9]. It is important to note that the observed antimicrobial effects of the plant extract carried out by different studies may vary in different regions of the world. This may be due to many factors [22] including the type of solvent used in the extraction methods which has an important role in the process of extracting.

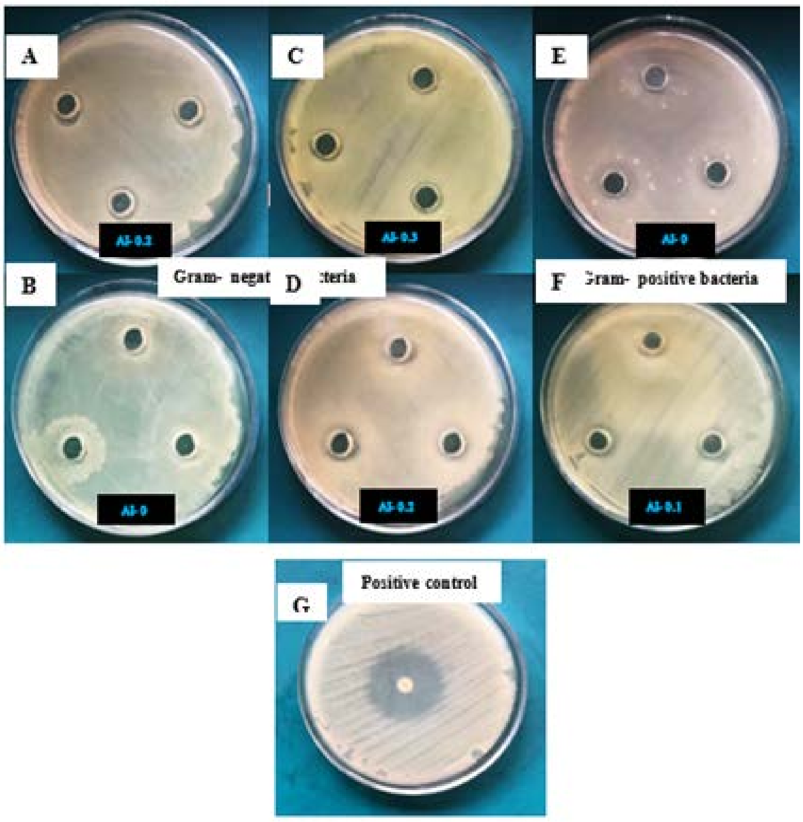

Fig. 5. The Antimicrobial Indices of Gram-negative bacteria: *E. coli* (**A**), *K. pneumoniae* (**B**), *P. aeruginosa* (**C**), *S. typhimurium* (**D**); and Gram-positive bacteria: *B. subtilis* (**E**), and *S. aureus* (**F**) using agar well-diffusion method in three replicates of the *A. ampeloprasum* leaves ethanolic extract; **(G)**, Chloramphenicol used in Gram-negative and Gram-positive bacteria.

Table 2. Computed average of Inhibition Zone (mm) and Antimicrobial Index (AI) from the test organisms in Sibujing extracts.

UPCC Test Organism	Test Sample	Mean	Antimicrobial Index (AI)
Gram-negative bacteria			
Escherichia coli UPCC 1195	'Sibujing' extract	12.33	0.2
Chloramphenicol disc[a]		27	3.5
Klebsiella pneumoniae UPCC 1360	'Sibujing' extract	12.67	0.3
Chloramphenicol disc		38	5.3
Pseudomonas aeruginosa UPCC 1244	'Sibujing' extract	-[b]	-
Chloramphenicol disc		15	1.5
Salmonella typhimurium UPCC 1368	'Sibujing' extract	12	0.2
Chloramphenicol disc		30	4
Gram-positive bacteria			
Bacillus subtilis UPCC 1295	'Sibujing extract'	-	-
Chloramphenicol disc		20	2.3
Staphylococcus aureus UPCC 1142	'Sibujing extract'	11	0.1
Chloramphenicol disc		33	4.5

Legend: [a]6-mm diameter disc, contain 30 µg chloramphenicol
[b]No clearing zone; no inhibition of growth of the test organism

Phytochemical Analysis

The phytochemical analysis of the ethanolic extracts of *A. ampeloprasum* showed to be rich in saponins, flavonoids, and steroids, and a minimal amount was observed for alkaloids. Tests for tannins, cyanogenic glycosides, and anthraquinones yielded no activities (Table 3, Figure 6).

Table 3. Phytochemicals in the ethanolic extracts of *A. ampeloprasum*

Alkaloids	Saponins	Flavonoids	Steroids	Tannins	Cyanogenic-glycoside	Anthraqui-nones
+	+++	+++	+++	-	-	-

Legend: (+) turbid, (++) moderate, (+++) - heavy; **(-)** absent

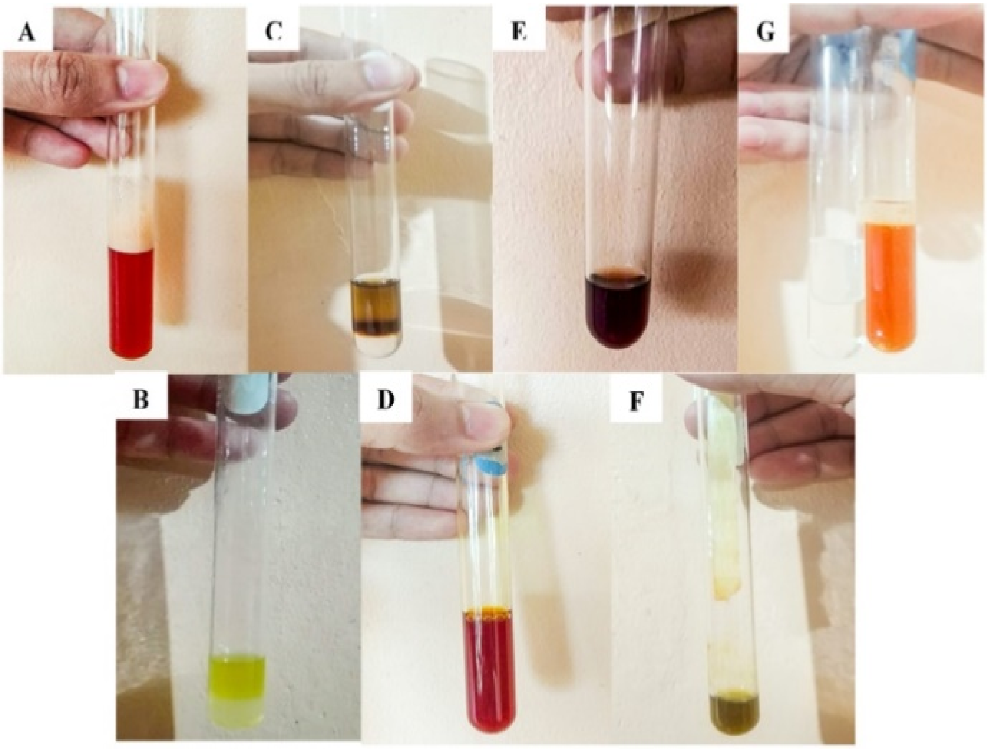

Fig. 6. The Phytochemical Screening Observation of: (+)Saponins (**A**), (+)Flavonoids (**B**), (+)Steroids (**C**), (+)Alkaloids (**D**), (-)Tannins (**E**), (-)Cyanogenic Glycosides (**F**), (-)Anthraquinone (**G**) of *Allium ampeloprasum* ethanolic extract showing (+) if present and (-) if absent as indicated.

Studies have shown that phytochemicals have been described to have different mechanisms such as inhibiting microorganisms, interfering in metabolic processes, signaling transduction pathways and modulating gene expressions [23]. Studies have shown that leaves and other parts of the plant are rich in phytochemical constituents such as alkaloids, flavonoids, and saponins [24]. Alkaloids have been described as a powerful poison showing biological activities such as inflammatory, antimicrobial, cytotoxicity, and pharmacological effects [25]. They have a more significant role against Gram-positive than Gram-negative bacteria due to the complexity of barrier system [26]. Flavonoids also showed to have antimicrobial effects against the variety of pathogenic microorganisms *in vitro* due to the complexity of extracellular and soluble proteins of the bacterial cell wall [27]. They have been reported to have biologically active compounds that are anti-inflammatory and effective against allergic infections and coronary problems [27]. Flavonoids were present in the extract as potent water-soluble antioxidants to neutralize the damage from free radicals which prevent oxidative cell damage, low risk of heart diseases and strong anticancer effects [28, 29]. Saponins with its amphiphilic nature have many applications for lowering cholesterol, antiviral, antibacterial and anticancer properties [30]. These metabolites have been also associated with medical complications such gastroenteritis manifested by diarrhea and dysentery [31]. The administration of saponin compounds with chloramphenicol has been antimicrobial because it inhibits *E. coli* strains [32] since it involves an alteration of normal cell membrane functioning, thus, blocking pathogen entry [30].

The extract was also found to have abundant steroid compounds known important in the regulation of immune responses [28]. These were also reported to have antibacterial properties [25] specifically those associated with membrane lipids that cause liposomes leakage [31]. They have also been considered to have antioxidant [33] properties. While tannins have been known to have antibacterial activity [34], and have been used for therapeutic to some various diseases such as diarrhea [35], these compounds were absent in the extract. Other phytochemicals such as cyanogenic glycosides and anthraquinones were absent in the ethanolic extract of *A. ampeloprasum* indicating none or less toxicity effects of the plant extracts. Cyanogenic glycosides have been secondary metabolites which have been used as flavoring agents in many pharmaceuticals, but foodstuff containing these compounds may cause food poisoning resulting gastric irritations and damage [35].

Antioxidant Properties

The results of the antioxidant property evaluation of the plant extract showed 37.83% antioxidant activity based on DPPH radical scavenging activity when compared to Vitamin C, the positive control (Table 4, Figure 7). While this indicated a weaker antioxidant property when compared to Vitamin C, the results reported were in agreement with other studies where bulbs and bulblets had weak antioxidant activity [36]. Nevertheless, the results still indicated that the white variety of *A. ampeloprasum* still possessed good antioxidant properties.

Table 4. Antioxidant properties of the extract of *A. ampeloprasum*

'Sibujing' Extract	**%I**	**Vit. C Conc.**	**%I**
Concentration (ppm)		(ppm)	
Control	0	0	0
10	3.91	2	60.07
20	7.39	3	83.91
30	9.13	4	87.73
50	11.3	5	91.55
100	23.91	10	94.56
200	33.62	20	94.44
300	37.83		
IC_{50}=361ppm			

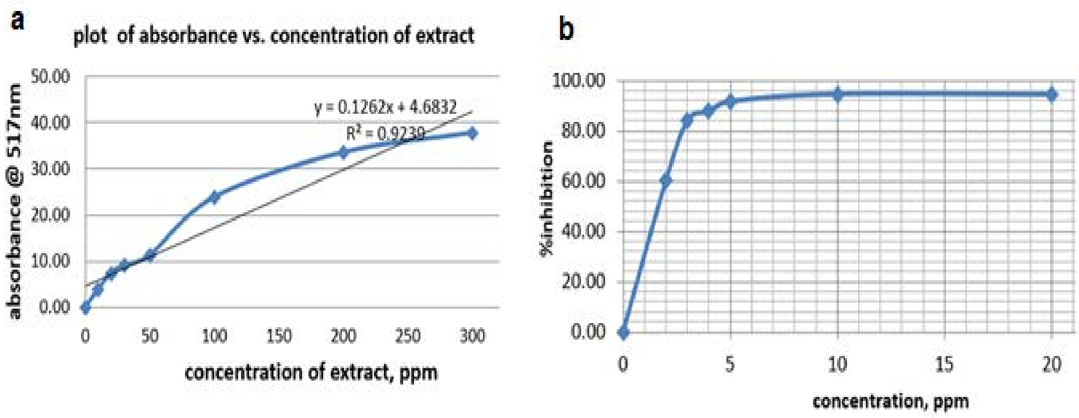

Fig. 7. Plot of absorbance vs concentration of the extract (a) and percentage inhibition vs concentration of Vitamin C (b).

GC-MS Analysis

In the present investigation, unbiased GC-MS analysis of the plant extract identified 19 kinds of compounds (Figure 8 & 9). Three out of these 19 compounds were having similarity index value lower than 80%. Some of the identified compounds were known as antimicrobials (Table 5). The compounds possessed essential oils such as n-eicosane, n-pentadecane, n-hexadecane, n-heptadecane, n-octadecane, octadecanoic acid, ethyl ester, hexadecanoic acid, ethyl ester, 4,6-dimethyldodecane, sulfur-containing compound including dimethyl tetrasulfide and phenolic compound like phenol, 2,4-bis (1,1-dimethylethyl)- may explain the potency for antimicrobial activities of *A. ampeloprasum.* Other compounds in the plant extract had other biological properties. Linoleic acid, ethyl ester is good as antioxidant, and for curing anti-inflammatory such as arthritis [37]. Fatty acids seen in the plant extract such as palmitic, oleic and linoleic acids also reported in other *Allium* species have been known to have pharmacological properties [38]. The fat-soluble compounds such as heptadecane, 4,6-dimethyl dodecane, eicosane and heneicosane have also been known to have pharmacological properties such as lowering blood pressure, being effective in salivary secretions, and respiration-paralyzing actions and also reinforcing kidney, moisture dryness, constipation, treatment of impotence and weakness of loins and knees [39]. The isolation of several cis- 1,2-cyclohexanediol-degrading strains of bacteria such as *Pseudomonas* spp. showed that this compound also had the potential for antimicrobial activities [40]. It has been argued that some essential oils in spices have antimicrobial actions due to the impairment of enzyme for the production of energy in microbial cells [41].

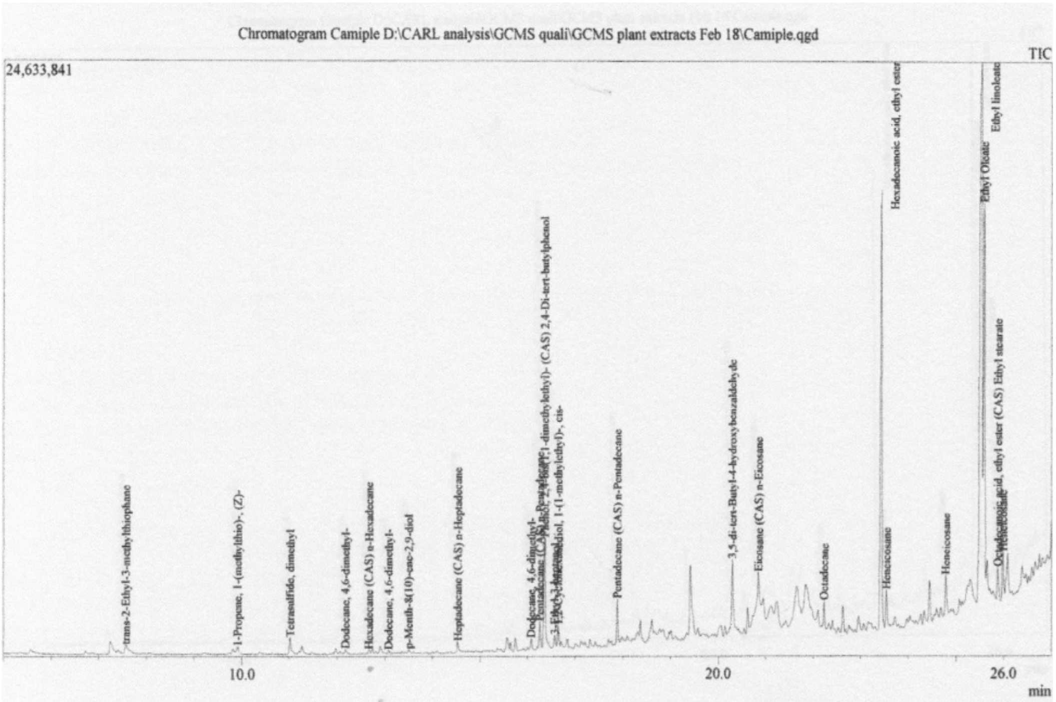

Fig. 8. GCMS analysis of plant extract of *Allium ampeloprasum*

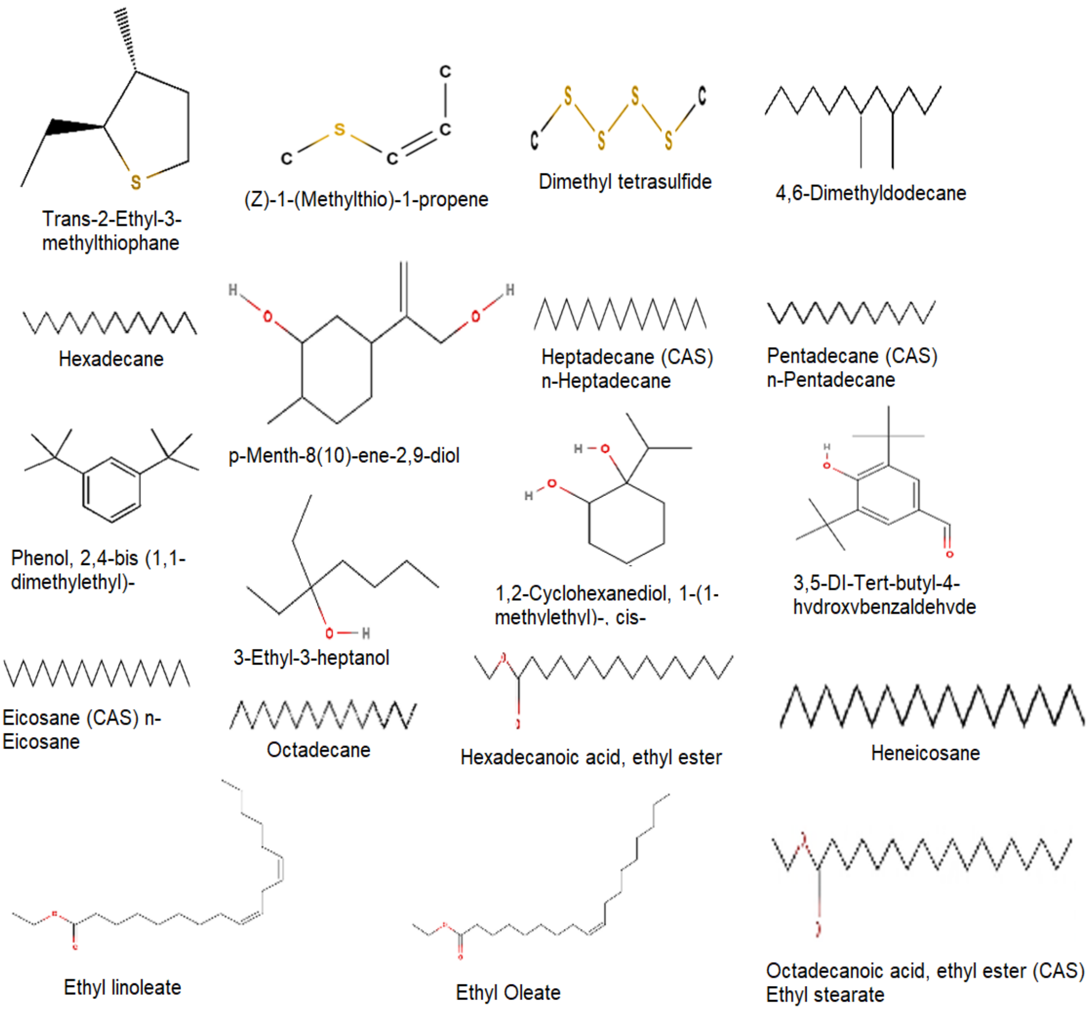

Fig. 9. Chemical compounds in the plant extract

Table 5. Unbiased GC-MS analysis of the plant extract

	Name of Compound	Formula	Similarity Index	Mol. Wt.	Retention Time	Biological Properties
1	Trans-2-Ethyl-3-methylthiophane	$C_7H_{14}S$	80	130	7.57	cardiovascular effects [42]
2	(Z)-1-(Methylthio)-1-propene	C_4H_8S	80	88	9.915	indicative source for medical conditions [43], aroma/ flavor [44]
3	Dimethyl tetrasulfide	$C_2H_6S_4$	93	158	11.02	antimicrobial, antioxidant, thermal effects [45], flavor/pungency [46], induced apoptosis, cytotoxic, anti-cancer, anti-proliferation effects [47], larvicidal [48]
4	4,6-Dimethyldodecane	$C_{14}H_{30}$	94	198	12.18	antimicrobial activity [49], PCM (Phase Change Material [50], flavor [51]
5	n- Hexadecane	$C_{16}H_{34}$	95	226	12.67	antimicrobial activity [49, 52-54], antifungal [53, 55], antioxidant [54, 55], bacterial production of biosurfactants [50]
6	p-Menth-8(10)-ene-2,9-diol	$C_{10}H_{18}O_2$	67	170	13.51	No activity reported[a]
7	n- Heptadecane	$C_{17}H_{36}$	97	240	14.54	antifungal [53, 55, 56], antimicrobial [53], antioxidant, anti-tumor [55]
8	n-Pentadecane	$C_{15}H_{32}$	94	212	16.27	Antimicrobial [53, 54]
9	Phenol, 2,4-bis (1,1-dimethylethyl)-	$C_{14}H_{22}O$	96	206	16.365	Antimicrobial [27, 57], antifungal [58], antioxidant, anti-tumor [57] anti-inflammatory [59]
10	3-Ethyl-3-heptanol	$C_9H_{20}O$	76	144	16.58	antimicrobial, aroma [60]
11	1,2-Cyclohexanediol, 1-(1-methylethyl)-, cis-	$C_9H_{18}O_2$	79	158	16.65	No activity reported
12	3,5-DI-Tert-butyl-4-hydroxybenzaldehyde	$C_{15}H_{22}O_2$	95	234	20.31	Antioxidant [61]
13	n-Eicosane	$C_{20}H_{42}$	96	282	20.86	PMC [50], antifungal [57, 62, 63], Anti- tumor activity [63, 64], antioxidant [64] antibacterial, cytotoxic effects [57, 63], anti- corrosive agents [65]
14	n-Octadecane	$C_{18}H_{38}$	95	254	22.23	antimicrobial, antifungal [52, 53]
15	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	94	284	23.435	Inhibits phagocytosis [54], antioxidant [57, 64] anticancer [64], Hypocholesterolemic [57, 66] Nematicide, Pesticide, Antiandrogenic flavor, Hemolytic, Alpha reductase inhibitor [57], antibacterial [66, 67], antifungal, anti-tumor [67], Lubricant, Flavor, Cosmetic, Perfumery [66]
16	n- Heneicosane	$C_{21}H_{44}$	96	296	23.545	antimicrobial [49, 52], oviposition attractant of mosquito [50], anti-corrosive agents [65]
17	Linoleic acid, ethyl ester	$C_{20}H_{36}O_2$	90	308	25.535	antisclerotic [54], Hypocholesterolemic, Nematicide, Antiarthritic, Hepatoprotective Antiandrogenic, 5-Alpha reductase inhibitor, Antihistaminic, Anticoronary, Insectifuge, Antieczemic, Antiacne [57]
18	9-Octadecenoic acid (Z)-, ethyl ester	$C_{20}H_{38}O_2$	86	310	25.595	lubricant and plasticizer [50], Cancer-preventive, Flavor, Hypocholesterolemic, 5-Alpha reductase inhibitor, Antiandrogenic, Perfumery, Insectifuge, Anti-inflammatory, Anemiagenic, Dermatitigenic, Choleretic [58]
19	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	91	312	25.885	antiviral, antibacterial and antioxidant activities [65]

[a]-no activity reported based on literatures

Conclusion

This study was conducted to determine the phytochemical constituents, antioxidant and antimicrobial properties of ethanolic extracts of the white variety of *Allium ampeloprasum*, and show its potentials as a source of compounds including antioxidants, antimicrobials and other compounds with pharmacological and biological properties; thus, it indicated that the use of the plant as food and medicine by the Maranao people had biological basis.

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