RESEARCH ARTICLE

Phytochemical Profiling, Antimicrobial Activity and Gas Chromatography Mass Spectrometry Analysis of *Allium odorum* L. Collected from Ema market, Manipur

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ABSTRACT

Allium odorum (*A. odorum*) L. which is locally known as *Maroi nakupi* (Chinese chives) belongs to the Alliaceae family and is generally used as condiments/vegetables/spice or as a medicinal herb in Manipur since time immemorial. It can be eaten either as raw/fresh/ or as cooked/ boiled besides consuming as salads and soups. This plant which is similar to normal grass in appearance, is an important perennial, draught resistant, fast-growing and quickest income-generating herb. In view of the various health benefits, we aimed to study the phytochemical screening, its antimicrobial activities and gas chromatography-mass spectrometry **(**GC-MS) analysis of *A. odorum* L.collected from Ema market Manipur. Preliminary phytochemical screening shows the presence of most of the phytochemicals in both aqueous and methanolic extracts of *A.odorum* L.except amino acids, carbohydrates, steroids and terpenoids. Cardiac glycosides were found to be present in aqueous extract but absent in methanolic extract. Both the phenolic and flavonoid content of *A. odorum* L.were higher in methanolic extract than aqueous extract. Percentage DPPH scavenging activity of aqueous and methanolic extracts was comparable with standard ascorbic acid with methanolic extract showing higher scavenging activity than aqueous extract. Reducing power for both the aqueous and methanolic extracts showed a slight higher in the methanolic extract. Similar trend was noted in total anti-oxidant activity where methanolic extract showed higher activity than aqueous extract. Antimicrobial Screening for both the methanolic and aqueous extracts of *Allium odorum* L.showed that both the extracts were found to have antibacterial activity against *E.coli* and *P.aeruginosa* but not against *B.subtilis* and *S.aureus.* The extracts showed no antifungal activity against any of the test fungi. The methanolic extract exhibited more antibacterial activity when compared with the aqueous extract. It was also observed that both the methanolic and aqueous extract showed antibacterial activity against gram negative bacteria only. GC-MS analysis is the first step towards understanding the nature of the bioactive compounds and this study leads to the identification of a number of compounds. Isolation of individual phytochemical constituent and subjecting it to biological activity will definitely give fruitful results. Furthur studies for identification of the bioactive components responsible for higher anti-oxidant activity and exploitation for largescale production for used in pharmaceutical industries will be our next target. The present study provides a baseline data for future studies geared towards the therapeutic benefits of *A.odorum* L.

Keywords: *Allium odorum* L.**,** Antimicrobial activities, Chinese chives, Cholesterol, Phytochemical, Spice.

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INTRODUCTION

A. odorum L. is an important perennial herb. It belongs to the family Alliaceae and is locally known as *Maroi nakupi* (Chinese chives).[1] It can be eaten either as raw/fresh/cooked or boiled besides consuming as salads and soups. It plays an important role right from the kitchen for providing colour, taste and flavour coupled with acting as an important ingredient in manufacturing of herbal medicines. This herb which looks similar to grass is an important perennial, draught resistant, fast growing and quickest income generating plant. It is available throughout the year and can be harvested within the shortest duration of 15–20 days during summer/rainy season and 25–30 days during winter. It is one of the cheapest and comparatively easiest to

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cultivate though not commonly grown in any other part of India.

A. odorum L. contributes not only in flavors, colorants and preservatives for their culinary properties but also is being used for their potential health benefits. It can be eaten both in dry and in fresh form. This herb is used traditionally in various folklore medicine as diuretic and as wormicide in infants suffering from tape, thread, and roundworms. It can be served both by steaming or boiling in water for those who are suffering from urinary troubles. This herb is conventionally used to treat fungal or bacterial infection on head, locally known as "Samjabi" by applying its crushed juice until it is cured.[2] The leaf decoction is taken for urinary disorder, and the leaf paste is applied on head for improving hair growth.[3] In addition, the green leaves contain antioxidants such as carotenes, zea-xanthin, and lutein. In view of its various health benefits, we aimed to study the phytochemical screening, its antimicrobial activities, and GC-MS analysis of *A. odorum* L. collected from Ema market, Manipur.

MATERIALS AND METHODS

Plant Sample

A. odorum L.was collected from Khwairamband Bazar, Ema Market, Imphal-West district of Manipur, Northeast India. Identification of the sample was done by L Somarjit Singh, Associate Professor, Department of Botany, Imphal College, Imphal. Stem and leaves of the plant were washed with tap water and then rinsed with distilled water, shade dried and ground into fine powder.

Soxhlet Extraction

A total of 40g of powdered *A. odorum* L. was extracted separately using 400mL of methanol and double distilled water by soxhlation until the solvent became colorless in the soxhlet extractor's main chamber. The extract was evaporated to dryness and crude extract was obtained. The crude extracts were screened for the phytochemical constituents (Figure 1).

Figure 1: *Allium odorum* L. a) and b) Fresh c) Powdered form

Phytochemical screening was carried out for aqueous and methanolic extracts of *A. odorum* L. using standard protocol.[4-8]

Determination of total phenolic content

The total phenolic content in aqueous and methanolic extracts of *A. odorum* L.was determined with Folin-Ciocalteu reagent.[9-13]

Estimation of total flavonoid content

Total flavonoid content in the sample extracts were estimated by Aluminium chloride colorimetric method.[12-15]

Determination of free radical scavenging assay

The free radical scavenging capacities of aqueous and methanolic extracts of *A. odorum* L. were determined using DPPH assay.[12,13,16]

Estimation of reducing power

Reducing power of various concentrations (30–150µg/mL) of the crude aqueous and methanolic *A. odorum* L**.** extracts were determined by Ferric reducing anti-oxidant power assay. Increased absorbance of the reaction mixture indicates increase in reducing power.[12,13,17]

Determination of total antioxidant activity

The phosphomolybdenum method was used to evaluate the total antioxidant activity of the extract.[12,13,18]

Antimicrobial Screening

Antimicrobial assay

Test organisms

The test bacteria used were the gram-positive organisms *Staphylococcus aureus* (MTCC 737), and *Bacillus subtilis* (MTCC 441), and the Gram negative bacteria *Escherichia coli* (MTCC 738) and *Pseudomonas species* (MTCC 424).The test fungi used were *Fusarium oxysporum* (MTCC 227), *Trichoderma viride* (MTCC 793) and *Aspergillus Niger* (MTCC 281). All the reference strains were procured from microbial type culture collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India.

Media

Nutrient Agar (NA) and Nutrient Broth (NB) were used for bacterial culture and Potato Dextrose Agar (PDA) and Potato Dextrose Broth (PDB) were used for fungal culture in each experiment.

Antimicrobial Agents

Streptomycin (10 µg) and Kanamycin (30 µg) discs were used as positive control for bacteria and fungi respectively and sterile distilled water was used as negative control.

Preparation of Extract of Different Concentration

Stock solution of each of the crude extracts was prepared with N, N-Dimethyl sulfoxide (DMSO) making a concentration of 40 mg/mL . From the stock, three different dilutions were made to obtained 2 mg/50 µL 1 mg/50µL and 0.5 mg/50µL for both the aqueous and methanolic extracts.

Antimicrobial Activity

Antimicrobial activity was done by using Kirby-Bauer method.[19] A loopful of freshly grown test organisms (both bacteria and fungi) were inoculated on NB (for bacteria) and PDB (for fungal) in an orbital shaker (150 rpm, 30°C). The test bacteria were incubated for 24 hrs while the test fungi were incubated for 48–72 hrs.0.1 mL each of the bacterial and fungal broths were spread uniformly with a sterile L shaped spreader on NA and PDA plates, respectively. Wells were punched with the help of a cork borer (6 mm diameter) on the media plates. Then 50 µL each of 500 µg, 750 µg and 1000 µg concentrations were put in each well in triplicates for both the extracts and kept at 40C for 1-hour for proper diffusion of the test organisms. It was then incubated for 24-48 hrs at 300 C. The zone of inhibition (in mm diameter) were measured (as mean of the triplicate readings) using a scale (18) and taken as the activity against the test organisms. The zone of inhibition was graded according to Kang SN *et al*.[20] (Table 1). Streptomycin (10 µg) and Kanamycin (30 µg) discs were used as a positive control for bacteria and fungi, respectively and sterile distilled water as a negative control.

Gas Chromatography and Mass Spectrometry Analysis

Gas chromatography (GC) analysis was carried out at Advanced Instrumentation Research Facility (JNU) New Delhi. This technique is best for the identification of various phytochemicals of plant. The equipment was GC-MS QP-2010 ultra. The carrier gas used in GC-MS program was helium 1 mL/minute (split ratio = 10:0). The initial oven temperature program is 70°C, and final temperature is 280°C, hold time 23 minutes, ion source temperature is 230°C and interface temperature is 270°C, solvent cut time 4.50 minutes, Detector Gain Mode: Relative to the tuning result, Detector Gain +0.00 VK, threshold 1000, start time 10 minutes, end time 45.0 min, Event time 0.20 sec, Scan speed 3333, start m/z 40.00 AND end m/z 650.00 (Figure 2 and 3).

Table 1: Grading of zone of inhibition

Diameter of ZOI	*Antimicrobial activity*
6–8 mm	No antimicrobial activity
8.1–9 mm	Slight antimicrobial activity
9.1–12 mm	Moderate antimicrobial activity
12.1–15 mm	Clear antimicrobial activity
> 15 mm	Strong antimicrobial activity

RESULTS AND DISCUSSION

Phytochemical constituents in aqueous and methanolic extracts of *A.odorum* L. are listed in Table 2. Preliminary phytochemical screening shows the presence of most of the phytochemicals in both aqueous and methanolic extracts of *A.odorum* L. except amino acids, carbohydrates and steroids and terpenoids. Cardiac glycosides were present in aqueous extract but absent in methanolic extract.

The total phenolic content in aqueous and methanolic extracts of *A. odorum* L. was found to be 3.68 ± 0.02 mg/g (GAE) and 3.91 ± 0.01 mg/g (GAE) respectively indicating a higher total phenolic content in methanol extract than aqueous extract (Table 3). This phenolic compound have wide bioactivity including antioxidant properties/ activity which is due to the presence of the hydroxyl group and is responsible for the radical scavenging effect mainly due to redox potential.[21]

The total flavonoid content in aqueous and methanolic extract was found to be 8.01 ± 0.02 and 2.86 ± 0.02, respectively (Table 3). It was found to be higher in methanolic extract than the aqueous extract of the plant. The antioxidant property of flavonoids is that they are potent free radical scavengers due to presence of double bond in hydroxyl positions in their molecule.

The DPPH assay showed that an increase in concentration increases the free radical scavenging activity for the reference standard, ascorbic acid and crude aqueous and methanolic extracts of *A.odorum* L. The assay indicates a dose dependent manner for standard and both the extracts of the plant sample as illustrated in Figures 4 and 5. Percentage DPPH scavenging

Table 2: Phytochemical constituents present in the whole plant extract of *Allium odorum* L.

		Allium odorum	
Phytochemical constituents	*Test*	*Aqueous extract*	*Methanolic extract*
Amino acids	Ninhydrin test	-	-
Alkaloids	Hager's test	+	+
Carbohydrates (reducing sugar)	Benedict's test	-	-
	Fehling's test	-	-
Flavonoids	Alkaline reagent test	+	+
	Lead acetate test	+	+
Phenolic compounds	Lead acetate test	+	+
Steroids and Terpenoids	Ferric Chloride test	+	+
Saponins	Salkowski's test	-	-
Tannins	Froth test	+	+
	Lead acetate test	+	+
Cardiac glycosides	Ferric chloride test	+	-
Oil	Keller-killiani test	+	+

Table 3: Total phenolic and total flavonoid content in crude extracts of *Allium odorum* L.

Sample	*Total phenolic content in mg /g of extract (in GAE)*		*Total flavonoid content in µg/100g of dried extract (in QE)*	
Allium odorum.L	Aqueous	Methanol	Aqueous	Methanol
	3.68 ± 0.02	3.914 ± 0.01	8.01 ± 0.02	12.86 ± 0.02

* Assays were performed in triplicate. Values are expressed as means ± SD

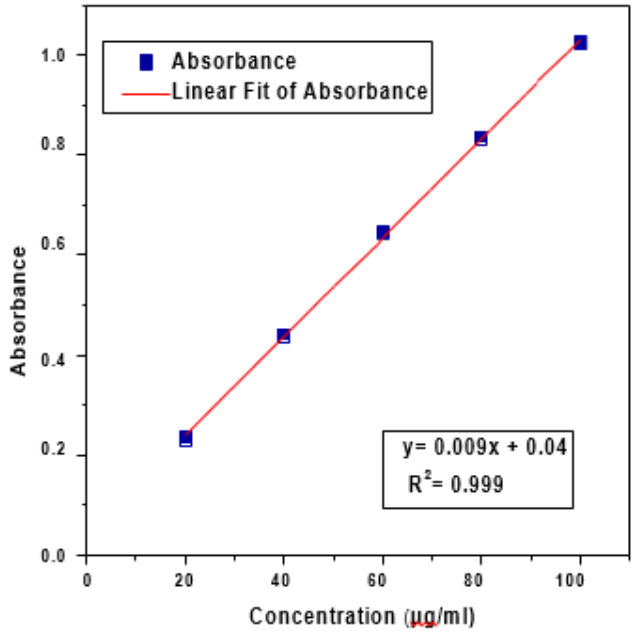


Figure 2: Standard curve of Gallic acid

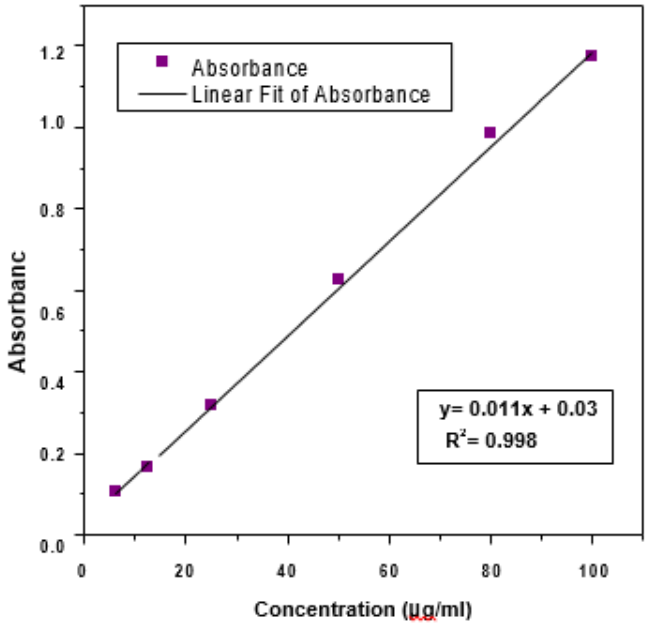


Figure 3: Standard curve for Quercetin

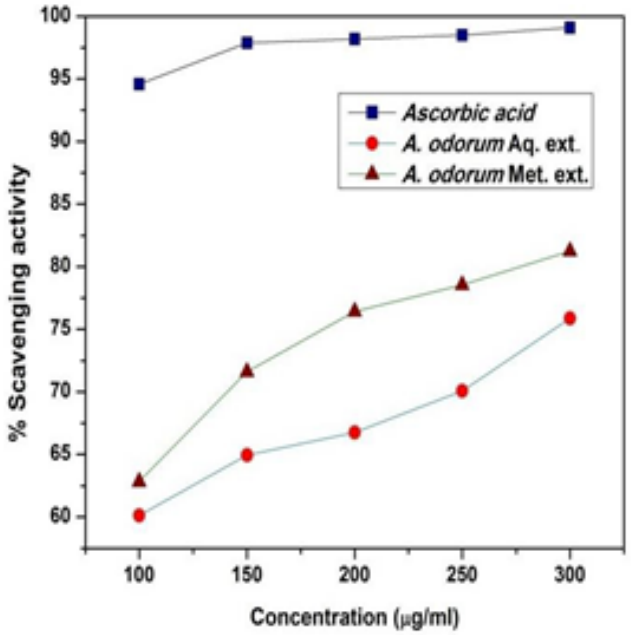


Figure 4: DPPH scavenging activity shown standard and crude extracts of *A.odorum* L.

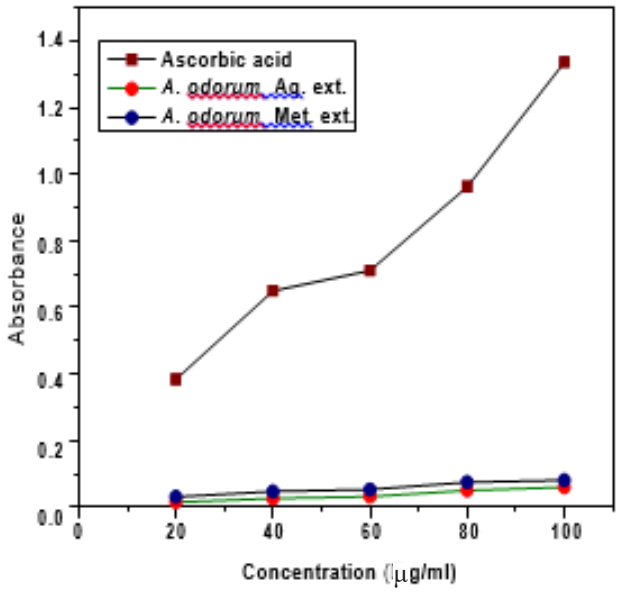


Figure 5: Reducing power shown by standard by and crude extracts of *A.odorum* L.

activity of aqueous and methanolic extracts was comparable with standard ascorbic acid and methanolic extract showed higher scavenging activity than aqueous extract. It was observed that 100 µg/mL was required to scavenge 60% of DPPH radical in aqueous extract while the same amount was required to scavanged 63% of DPPH radical in methanolic extract. At the highest concentration (250µg/mL) used for the study, the DPPH scavenging activity of the standard, aqueous and methanolic extracts of *A.odorum* L. were 97.88%, 70.09% and 81.26% respectively. DPPH method measures electron donating activity of other compounds in the mixture and hence

provides an evaluation of antioxidant activity due to free radical scavenging. Determination of the free radical scavenging capacity or antioxidant potential of the test sample shows its effectiveness, prevention and repair mechanism against many health related disorders and diseases such as infections, diabetes, arthritis, cancer, AIDS, etc.[22] The presence of many phytochemicals in *A. odorum* L. such as phenol, flavonoids, alkaloids etc may also help in preventing against free radicals.

Reducing power assay indicates an increasing order for standard as well as for both the extracts of plant sample as shown in Figure 5. Reducing power is associated with antioxidant activity and serves as a significant reflection of the antioxidant activity. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes so that they can act as primary and secondary antioxidants.[23] It results in the reduction of Fe3+ to Fe2+ by donating an electron.By measuring the formation of Pearls Prussian Blue at 700 nm, it is possible to determine the concentration of ferrous ions. Reducing power noted in aqueous and methanolic extract was almost equal with methanolic extract showing a slight higher in reducing power in methanolic extract than the aqueous extract of the plant. Increased absorbance of the reaction mixture indicated increased reducing power of the extracts.[24]

A similar trend was noted in total antioxidant activity where methanolic extract showed higher activity than aqueous extract. At highest concentration used for the study, total antioxidant activity shown by methanolic extract was 17.1 ± 0.02µg/mL AAE of extract while that of aqueous extract was 8.7 ± 0.02 µg/mL AAE of extract. Total antioxidant activity shown by various concentrations (20–100 µg/mL) of crude aqueous and methanolic extracts of *A.odorum* L. is shown in Table 4.

Antimicrobial Screening of both the methanolic and aqueous extracts of *Allium odoroum* L. showed that both the extracts were found to have antibacterial activity against *E.coli* (MTCC 738) and *P.aeruginosa* (MTCC 424) but not against *B.subtilis* (MTCC 441) and *S.aureus* (MTCC 737). The extracts showed no antifungal activity against any of the test fungi. The methanolic extract exhibited more antibacterial activity when compared with the aqueous extract. Slight antibacterial activity was found against *E coli* at 500 µg/50µL and 750µg/50µL but the activity becomes moderate when the concentration increases to 1000 µg/50µL. At the maximum concentration it was found that the activity against *Pseudomonas* was strong for methanolic extract but moderate for aqueous extract. Thus the activity against both the strains increased with the increase in concentrations of the extracts.It clearly indicates that the methanolic extracts of *A.odorum* L.were more potent against gram-negative bacteria then the gram-positive bacteria. Aqueous extract *of A. odorum* L.showed lesser antibacterial activity when compared to methanolic extract which showed moderate to strong activity. It was also observed that both the methanolic and aqueous extract showed antibacterial activity against gram-negative bacteria only. Different solvents have been reported to have the capacity to extract different phytoconstituents depending on their solubility or polarity of the solubility or polarity in the solvent. Methanolic extracts have been reported to have higher solubility for more phytoconstituents, consequently showed higher antibacterial activity.[25]

GC analysis result leads to the identification of a number of compounds from the GC fractions of the methanolic extract of *A.odorum* L. and these compounds were identified with mass spectrometry attached to GC. The chromatogram (Figure 8) showed the presence of 43 compounds. Among these the 10 major compounds present are 3-Heptyn-2-ol, 2-methyl-; DL-Proline, 5-oxo- methyl ester; 2,5-dimethyl-1,5-Heptadiene-

Table 4: Total antioxidant activity of *Allium odorum* L.

Concentration (µg/mL)	*Total antioxidant activity in µg/mL of extract (in AAE)*	
	Allium odorosum	
	Aqueous	*Methanol*
20	3 ± 0.02	5.4 ± 0.01
40	3.9 ± 0.01	10.2 ± 0.02
60	6.6 ± 0.02	12.3 ± 0.03
80	8.1 ± 0.03	16.5 ± 0.02
100	8.7 ± 0.02	17.1 ± 0.02

Assays were performed in triplicate. Values are expressed as means ± SD

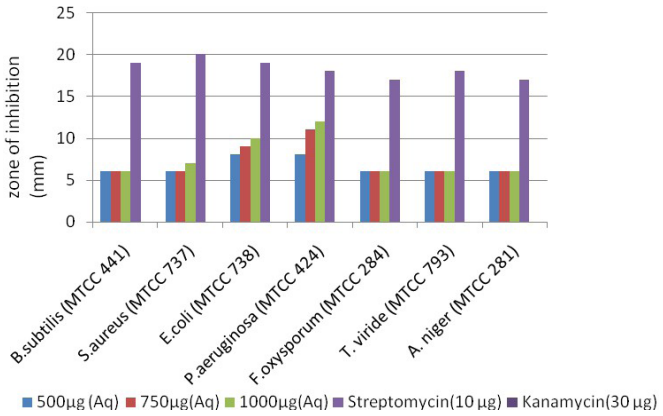


Figure 6: Antimicrobrial activity of aqueous extract of *Allium odorum* L. against the test organisms

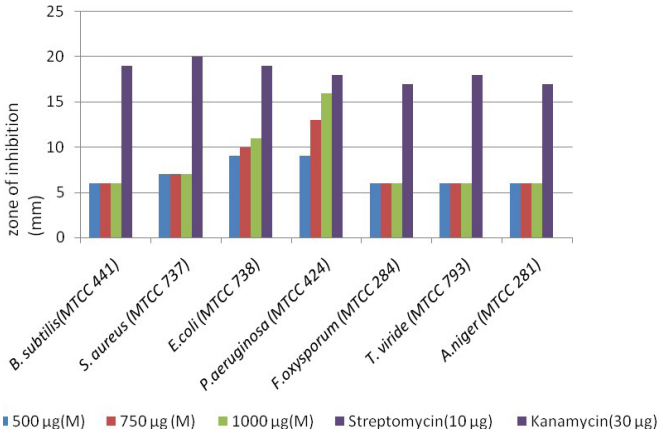


Figure 7: Antimicrobial activity of methanolic extract of *Allium odorum* L. against the test organisms

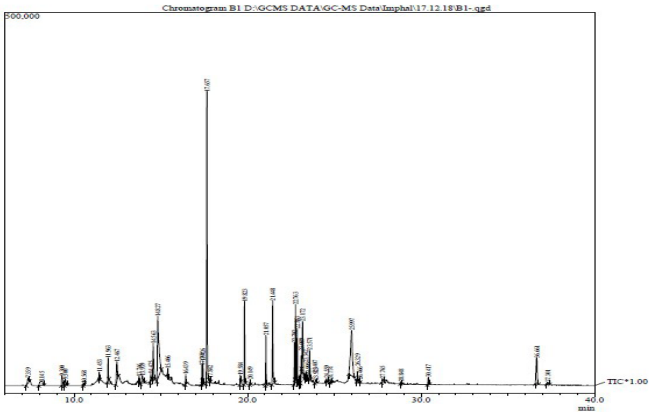
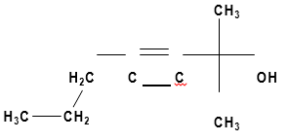
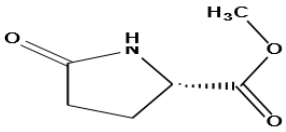
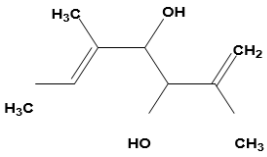
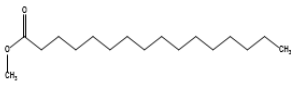
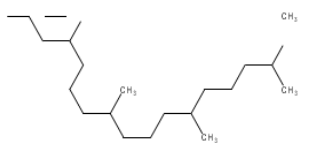
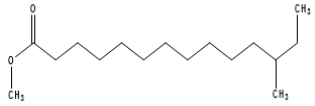
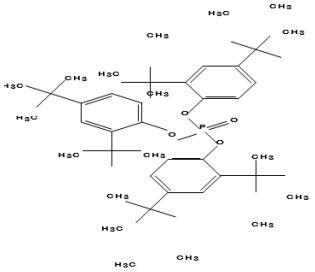


Figure 8: GC-MS Analysis of methanolic extract of *Allium odorum* L.

3,4-diol; Hexadecanoic acid, methyl ester; Hexadecanoic acid; 5,8-Octadecadienoic acid, methyl ester; Phytol; Tetradecanoic acid, 12-methyl-, methyl ester; Tris(2,4-di-tert-butylphenyl) phosphate; Palmitic acid vinyl ester. Among the 10 major compounds 6 of them viz : (DL-5-oxo-Proline, methyl ester; 2,5- dimethyl-1,5-Heptadiene-3,4-diol; Hexadecanoic acid, methyl ester; Hexadecanoic acid; 5,8- Octadecadienoic acid, methyl ester; Phytol; Tetradecanoic acid, 12-methyl-, methyl ester) have biological activities while 4 of the compounds viz: (2-methyl-3-Heptyn-2-ol; 2-methyl-3- Heptyn-2-ol; Tris(2,4-di-tert-butylphenyl) phosphate; Palmitic acid vinyl ester) have no reports on its activities (Table 5). The compounds hexadecanoic acid and phytol have antioxidant activities. Also both the compounds Hexadecanoic acid methyl esters

Table 5: Major components of the methanolic extract of *Allium odorosum* by GC-MS

Sl. No	*Name of the Compound*	*R T*	*Area %*	*MW (g/mol)*	*MF*	*Structure*	*Activity**
1	2-methyl-3-Heptyn-2-ol	14.563	3.54	112.172	$C_8H_{14}O$		No activity reported.
2	DL-5-oxo-Proline, methyl ester	14.827	11.62	143.1406	$C_6H_9NO_3$		As an osmoprotectant , agonist of glycine receptors, catalyst for aldol condensation, essential component of collagen, significant role in the immune system and strengthening of heart muscles.[27], for mental fatigue and memory improvement.[28]
3	2,5-dimethyl-1,5-Heptadiene-3,4-diol	17.657	19.27	156.225	$C_9H_{16}O_2$		No activity reported.
4	Hexadecanoic acid, methyl ester	21.057	2.56	270.450	$C_{17}H_{34}O_2$		Antibacterial and antifungal.[29] Anti- inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematicide, insectifuge, antihistamine, antieczemic, antiacne, alpha reductase inhibitor, antiandrogenic, antiarthritic, anticoronary.[30]
5	Hexadecanoic acid	21.448	5.86	256.43	$C_{16}H_{32}O_2$		Anti-inflammatory,[31] Antioxidant, hypocholesterolemic nematicide, pesticide, anti androgenic flavor, hemolytic, 5-Alpha reductase inhibitor,[32] potent mosquito larvicide.[33]
6	5,8-Octadecadienoic acid, methyl ester	22.763	4.03	294.479	$C_{19}H_{34}O_2$		Anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematicide, insectifuge, antieczemic, anticancer,[34] antiarthritic insectifuge, antihistaminic, anticoronary.[30]

7	Phytol (3,7,11,15-tetramethylhexadec-2-en-1-ol)	22.863	3.23	296.539	$C_{20}H_{40}O$		Anticancer, antioxidant, anti-inflammatory, diuretic, antitumor, chemopreventive, antimicrobial, use in vaccine formulations.[35]
8	Tetradecanoic acid, 12- methyl-, methyl ester	22.995	0.51	256.43	$C_{16}H_{32}O_2$		Decreases fibrinogen levels and prolongs prothrombin time and also decreases serum triglyceride levels.These characteristics hint at a potential role of the formulation for the treatment of ischemic stroke.[36]
9	Tris(2,4-di-tert-butylphenyl) phosphate	25.997	10.79	662.936	$C_{42}H_{63}O_4P$		No activity reported.
10	Palmitic acid vinyl ester	36.661	4.00	282.468	$C_{18}H_{34}O_2$		No activity reported.

*Source: Dr Dukes Phytochemical and Ethnobotanical Database (Online database)

and phytol have antimicrobial activities The presence of antimicrobial compound phytol could be responsible for antibacterial activity against *E.coli* and *P.aeruginosa* as this compound inactivates the protein and enzymes present in the microorganisms.[26]

CONCLUSION

Preliminary phytochemical screening shows the presence of most of the phytochemicals in both aqueous and methanolic extracts of *A.odorum* L.except amino acids, carbohydrates and steroids and terpenoids. Cardiac glycoside was present in aqueous extract but absent in methanolic extract. Both the phenolic and flavonoid content of *A. odorum* L.were higher in methanolic extract than aqueous extract. The present study showed methanolic extract of *A.odorum* L.has higher antioxidant activity than aqueous extract which may be due to higher flavonoid and phenolic content in the methanolic extract which would justify its traditional practice. Percentage DPPH scavenging activity of aqueous and methanolic extracts was comparable with standard ascorbic acid and methanolic extract showed higher scavenging activity than aqueous extract. Reducing power noted in aqueous and methanolic extract was almost equal with methanolic extract showing a slight higher in reducing power in methanolic extract than aqueous extract of the plant. Similar trend was noted in total antioxidant activity where methanolic extract showed higher activity than aqueous extract. Antimicrobial Screening of both the methanolic and aqueous extracts of *A. odorum* L. showed that both the extracts were found to have antibacterial activity against *E.coli* and *P.aeruginosa* but not against *B.subtilis* and *S.aureus.* The extracts showed no antifungal activity against any of the test fungi. The methanolic extract exhibited more antibacterial activity when compared with the aqueous extract. It was also observed that both the methanolic and aqueous extract showed antibacterial activity against gram negative bacteria only. The GC-MS analysis showed the presence of antimicrobial compound phytol besides other major compounds which could be responsible for antibacterial activity against *E.coli* and *P.aeruginosa* as this compound inactivates the protein and enzymes present in the microorganisms. Phytol which is a diterpene, is a member of the group of branched-chain unsaturated alcohols. It is the product of chlorophyll metabolism in plants; hence, phytol is abundantly available in nature. It also blocks the teratogenic effects of retinol. Along with the free radical scavenging activity, these plants also have high reducing power which provides the potentiality or ability to reduce and scavenge free radicals. The importance of the study is due to the biological activity of some of these compounds. GC-MS analysis is the first step towards understanding the nature of the bioactive compounds and this type of study will be helpful for further detailed study. Isolation of individual phytochemical constituent and subjecting it to biological activity will definitely give fruitful results. The presence of these active compounds suggest that their contribution on the pharmacological activity should be evaluated.

Although, *A. odorum* L. is abundantly available in Manipur, it is either very rare or not available in the mainland markets of the country. This could be the reasons that it has not yet been enlisted in the list of 52 spices published by Spices Board of India, under the Ministry of Commerce and Industries, Govt.of India. Furthur studies can be carried out to identify the bioactive components responsible for higher antioxidant activity and exploitation for largescale production for used in pharmaceutical industries. The present study provides a baseline data for future studies geared towards the therapeutic benefits of *A.odorum* L.

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