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Phytochemistry, Pharmacognosy and Nutritional Composition of Allium Hookeri: an Ethnic Food Plant Used Among Adi Tribe of Arunachal Pradesh, India

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ABSTARCT

Allium hookeri Roxb. is an ethnic medicinal food plant used among Adi tribes of Arunachal Pradesh, India. *Allium hookeri* has been pharmacognostically discoursed in addition to phytochemical composition and proximate studies. This work also reports fifteen biologically active compounds from this medicinal food plant. Minerals and proximate composition of this plant is reported to be adequate for human consumption.

Keywords: Phytochemistry, Proximate, Minerals, Medicinal plant, Ethnic food.

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INTRODUCTION

Food without dal, wheat, potato, oil and spice are the main characteristic features of the indigenous food system in Arunachal Pradesh, it is an interesting researchable domain. Let food be your medicine, once said Hippocrates (c. 460 – c. 370 BC) over 2500 years ago^{1,2}. Galen- “the father of observational medicine” believed that the fundamentals of good medicine lay in diet; for him “diet saves life- surgery takes it away”. Such medicinal food concepts and belief is still observable in the indigenous food systems practices among indigenous people in various pockets of the world in general and Arunachal Pradesh in particular. In the word of Etkin& Ross³, wild plants that are retained in local food cultures are inseparable from traditional therapeutic systems. Therefore, Pieroni and Price⁴ remarked that it is difficult to draw a line between food and medicine; food may be medicine and medicine may be food. To Guarrera& Savo¹ also, there is no clear dividing line between food and medicinal plants especially in indigenous and local traditions; Food and medicine represent a continuum rather than artificial categories typically imposed in western medicine. Therefore, the interface of diet and medicine under the domain of natural product is the basic concept in the traditional medicinal-food. Therefore, Marriot Bernadette⁵ remarked that links of diet and health are no longer questioned. Historically, wild plants and animals were sole dietary components for hunter–gatherer and forager cultures; still today, it remains very integrated to many agricultural communities⁶ and such practices are prominently observable among the aborigines of Arunachal Pradesh. Domesticated and non-domesticated green leafy vegetables have numerous dietary and health benefits and rich in macro and micronutrients^{7,8}. Wild vegetables are sources of nutrients especially in rural areas where they contributes substantially to proteins, minerals, vitamins, fibers, and other nutrients which are usually in short supply in daily diets⁹. Having said that indigenous folk food is used as food and medicine among indigenous people world around; numerous indigenous medicinal food plants are yet to be studied scientifically in term of proximate, antioxidant, pharmacognosy, phytochemical and other related scientific aspects. In the word of Philipson¹⁰ herbal medicines are, of course, used for their reputed beneficial effects, however, scientific studies for validation are also important and pharmacognosy is one of the basic methods to characterize and validate the drugs of natural origin to give correct and authentic identity. However, despite various modern techniques, identification of plant drug by pharmacognostic study is very reliable¹¹. Therefore, De Pasquale¹² termed “Pharmacognosy” as one of the oldest modern sciences. Pharmacognosy encapsulates medicinal plants and related fields of inquiry with various methods of analysis into drug discovery¹³. Hence, it is pertinent to

characterized crude drugs pharmacognostically. One of the prerequisites for the success of primary health care is the availability and use of suitable drugs¹⁴. Plants have always been a common source of medicaments, either in the form of traditional preparations or as pure active principles¹⁴. In more recent past, the use of plants as medicines has involved the isolation of active compounds, beginning with the isolation of morphine from the opium in the early 19th century¹⁵. The traditional knowledge has been the main clue to lead the search of bioactive compound for phytochemical scientists; for instance, 122 structurally defined compounds from just 94 species of plants have been obtained that are used globally as drugs and out of these compounds 80% are obtained from ethno medicinal plants¹⁶. Butler¹⁷ has pointed out that drug analysis from medicinal plants led to the isolation of important drugs like cocaine, codeine, digitoxin, and quinine. Therefore, folk medicine, which may reveal compound that could be useful for the mankind in the field of health, nutrition and wellbeing, should be a priority area for future research. Therefore, in the present work, *Allium hookeri* (figure 1)- an ethnic medicinal food plants used among aborigines of the East Siang District of Arunachal Pradesh with the vision to reveal boon and gift of nature to mankind under the dimension of ethnobotany, proximate, pharmacognosy and phytochemistry has been attempted to study.

MATERIAL AND METHOD

Ethnobotany

Allium hookeri Thw. (Amaryllidaceae) is an evergreen herb, one nerved almost flat leaves without bulb, base of stem clothed with long narrow membraneous sheaths¹⁸ develop modified stem in form of rhizome and blooming with white flower. This plant is used in stomach pain, cold and cough as well as necklace made of this plant is used to avoid epidemic diseases. As a food, Shoot is used as vegetable, salad and chutney¹⁹. To access utility of *Allium hookeri* 30 villages of East Siang District of Arunachal Pradesh were visited (Boleng Village, Riew Village, Riga Village, Tarak Village, Pangin Village, Kebang Village, Rottung village, Mebo village, Siluk Village, Ayeng Village, Motum Village, Borguli Village, Renging Village, Napit Village, Rani Village, Yagrung village, Miglung Village, Mirem Village, Bilat Village, Ledum Village, Kora Village, Koyu Village, Sile Village, Lingka Village, Rayang Village, Nari Village, Seren Village, BaminVillage, NamsingVillage and Rebo Village). Material for the laboratory analysis was collected from SilukVillage of East Sing district of Arunachal Pradesh. The area under study, the East Siang District of Arunachal Pradesh lies approximately between latitude 27^o 42'' and 29^o 20''

North latitudes and 94° 42'' and 95° 35'' East longitudes with an area of 4005 sq.km. The altitude of this District varies from 130 mtrs to 752 mtrs above sea level²⁰.

Pharmacognostic, Proximate and Minerals study

Characterisation of pharmacognostic parameters were carried out by following methods given in Shah and Seth (2010)²¹, Kakote et al., (2012)²² Wallis (2011)²³. Proximate and mineral study was carried out by following methods given in Iswaran (1980)²⁴ and Thimmaiah (1999)²⁵. Raman (2008)²⁶ was followed in the preliminary phytochemical screenings.

GCMS method

Shade dried sample (500g) was grinded and soaked in ethanol for 48 hours with periodical shaking and filtered in Whatman filter paper No. 1. Filtrate was evaporated to get solvent free slurry extract in rotary evaporator. The ethanol soluble compounds (Allium hookeri extract (90g) was mixed with 100-200 mesh silica gel (2 g) then loaded on column previously packed with 150g of silica gel (Merck 100- 200 mesh) prepared in petroleum ether (60-80 °C). The column was subjected to 100ml each different solvent system, starting with petroleum ether 100% followed by petroleum ether: chloroform graded mixtures (95:5, 90:10, 80:20, 70:30, 60:40 and 50:50) then with chloroform 100% followed by graded mixtures of chloroform: ethyl acetate (95:5, 90:10, 80:20, 70:30, 60:40 and 50:50) then with ethyl acetate 100% followed by graded mixtures of ethyl acetate: methanol ((99:1, 98:2, 97:3, 96:4 and 95:5)²⁷ to get single band on TLC. The elutions were monitored by using TLC. Visualization was done in UV chamber and vanillin sulphuric acid/dragon dorf reagent spraying agent heated 110 °C was used as derivatizing agents. Each time 10 ml elutes were collected and identical elutes were combined (TLC monitored) and kept aside for concentration. At the solvent system Ethylacetate: Methanol (95:5) a single band on TLC was achieved and subjected to GCMS. Elute with multiband on TLC were not studied further. Silica gel 60 F 254 _ pre-coated TLC plates (Merck- Germany) dissected in 10X10 cm were used in this study. Column of 30 X 600 mm (Borosil) with glass stop cork, Silica powder (200-400 mesh) for column chromatography (Merck) was used. All the solvents used were purchased from Bengal chemicals, Calcutta, India. Gas-Chromatography Mass Spectrometry (GC-MS) analyses of the each extract were carried out in Shimadzu GCMS-QP-2010 plus system. RTX-5 Sil MS column (30 m X 0.25 mm id X 0.25 film thickness) was used for the analysis. The operating conditions of the column were as follows: oven temperature program from 80°C to 210°C at 4°C/min with hold time of 2 min and from 210°C to 300°C at 15°C/min with hold time of 5 min, and the final temperature was kept for 20 min. The injector temperature was maintained at 270°C, the volume of injected sample was 0.3µl; pressure 85.4kPa, total flow 76.8mL/min, column flow 1.21 mL/min, linear

velocity 40.5 cm/sec, purge flow 3.0 mL/min, split ratio: 60.0; ion source temperature 230°C; scan mass range of m/z 40-600 and interface line temperature 280°C. The identification of compounds was performed by comparing their mass spectra with data from NIST05 (National Institute of Standards and Technology, US) and WILEY 8.

RESULTS AND DISCUSSION

Pharmacognostic characters

Organoleptically, *A. hookeri* is an evergreen herb bearing white flower, bloom in the month of August to September, and leaf is flat with white scaly leaves just above the ground, without bulb but with reduced rhizome, shoot arise from the lateral rhizome eyes (figure 1 & 2). Very bright white colour roots grow out profusely from the rhizome. The anatomical dissection of rhizome reveals that characteristics leaf traces ramify almost entire tissues; therefore, no particular system of vascular tissue arrangement or proper pith can be distinguishable (figure 5). Root bears thick epiblema and unicellular root hairs. Anatomical discourse reveals that endodermis and pericycle is very conspicuous with six radially arranged xylem and phloem (figure 4); Metaxylem is arranged towards the centre while protoxylem arranged near to pericycle. The central portion of the root is occupied by a cavity.



Figure 1: *A. Hookeri* in field



Figure 2: *A. hookeri* (root & rhizome).

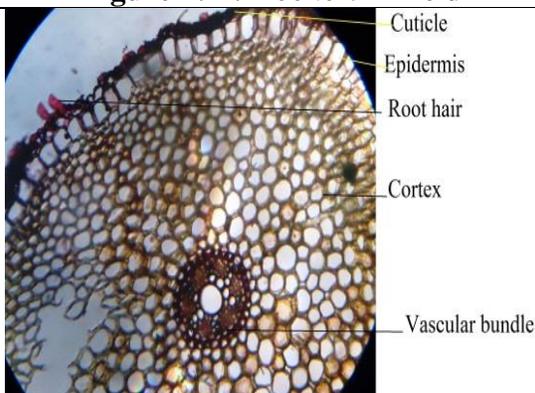


Figure 3: *A. hookeri* root

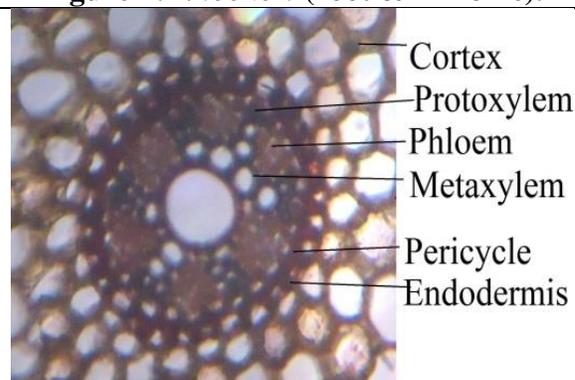
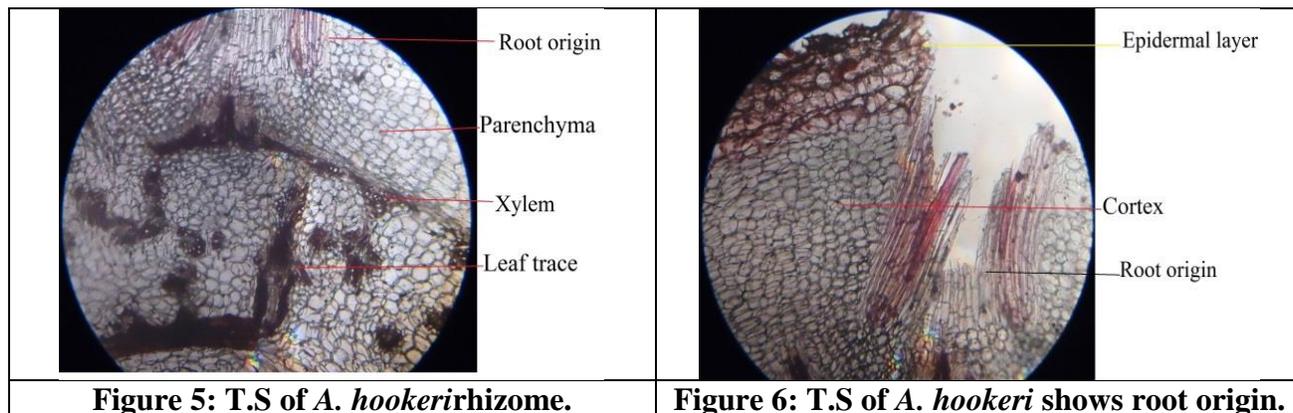


Figure 4: *A. hookeri* V. bundle (root).

Figure 5: T.S of *A. hookeri* rhizome.Figure 6: T.S of *A. hookeri* shows root origin.Table 1: Florescence characters of *A. hookeri* powder

<i>Allium hookeri</i> (whole plant)	Colour	
	Day light	Ultraviolet radiation
Powder as such	Off white	Creamy yellow
Powder + NaOH	Yellow	Pale yellow
Powder + Acetic acid	Yellowish white	Creamy white
Powder + HNO ₃	Orange	Pale yellow
Powder + H ₂ SO ₄	Black	Black
Powder + HCl	Yellowish brown	Creamy brown
Powder + FeCl ₃	Brown orange	Yellowish orange
Powder + water	Off white	Pale yellow

Table 2: Ash profile of *A. hookeri*

Sample	(% = w/w) & n=3		
	Total ash	Acid insoluble ash	Water soluble ash
<i>Allium hookeri</i> (whole plant)	6.60± 0.27	0.66± 0.06	3.33± 0.09

Table 3: Extractive value and corresponding colour of *A. hookeri*.

Sample		(%=w/w) & n=3					
		Methanol	Acetone	Ethyl acetate	Chloroform	Benzene	Petroleum ether
<i>Allium hookeri</i> (whole plant)	Extractive value	20±1.03	8.69±0.94	7.93±	2.43±0.52	1.22±0.01	4.88±0.68
	Extract colour	Urine colour	Bluish yellow	Golden colour	Light golden	Light golden	Light golden

Table 4: Preliminary phytochemical test of *A. hookeri*.

<i>Allium hookeri</i> (whole plant)	Extract reactions				
	Benzene	Chloroform	Ethyl acetate	Acetone	Methanol
Alkaloids					
(a) Mayer's test	-	-	-	-	-
(b) Wagner's test	+	+	+	+	+
(c) Hager's test	+	+	+	+	+
Flavonoids					
(a) Alkaline reagent test	-	-	-	+	+
Phenols					

(a) Ferric chloride test	-	-	+	+	+
(b) Gelatin test	-	-	-	-	-
(c) Lead acetate test	-	-	-	+	+
Detection of volatile oil	+	+	+	+	-
Saponins	-	-	-	+	+
(a) Foam test	-	-	-	+	+
Glycosides	-	-	-	+	-
(a) Borntrager's test	-	-	-	+	-
(b) Legal's test	-	-	-	+	-
Carbohydrates	-	-	-	+	+
(a) Molish's test	-	-	-	+	+
(b) Fehling's test	+	+	+	+	+
(c) Barfoed's test	-	-	-	-	-
(d) Benedict's test	-	-	-	+	+
Detection of proteins and amino acids	-	-	-	+	-
(a) Millon's test	-	-	+	+	-
(b) Biuret test	-	-	-	-	-
(c) Ninhydrin test	-	-	-	-	-
Detection of fixed oils and fats	-	-	-	+	-
(a) Spot test	-	-	-	+	-
(b) Saponification test	-	-	-	+	+

Table 5: Proximate composition of *A.hookeri*.

Sample studied on fresh weight basis; n=3.

Sample name	Moisture (%)	Carbohydrate (%)	Total ash (%)	Crude protein (%)	Crude fibre (%)	Crude fat (%)
<i>Allium hookeri</i> (whole plant)	74.10±0.04	5.30±0.91	6.60± 0.27	0.69±0.09	10.64±1.01	0.32±0.02

Table 6: Mineral concentration of *A.hookeri*.

Sample	Concentration of Element (mg/g)						
	Fe	Mn	Cu	Zn	Mg	Na	K
<i>Allium hookeri</i> (whole plant)	0.485	0.03	0.017	0.0235	1.11	3.2	9

Powder made from underground parts of this plant show yellow to radish florescence characters when observed under day light and UV light(table 1). The total ash of the plant powderis 6.60%, acid insoluble ash is 0.66% and water soluble ash is 3.33% (table 2). From table 3, it can be envisaged that methanol give 20% extractive value with urine colour extract from this plant, Acetone give 8.69% with bluish yellow extract, Ethyl acetate give 7.93 % with golden colour extract, Chloroform give 2.43% with light golden colour extract, Benzene give 1.22% extract with light golden colour extract and Petroleum ether give 4.88% extract with light golden colour respectively. High polar solvent was recorded to extract almost 20% in Methanol (w/w) from this plant whereas low polar solvent gives very low extractive value (1.22%) in Benzene. In

preliminary phytochemical screening; Alkaloids, Flavonoids, Phenols, Volatile oil, Saponin, Glycosides, Carbohydrates, Proteins and Amino acids, fixed oils and fats were found positive from Benzene, Chloroform, Acetone and Methanol extracts (table 4).

Proximate and minerals composition

Allium hookeri contain 74.10% moisture, 5.30% carbohydrate, 6.60% total ash, 0.69% crude protein, 10.64% crude fibre and 0.32% crude fat (table 5). While (table 6) shows to contain 0.485mg/g of Fe, 0.03g of Mn/g, 0.017mg of Cu/g, 0.023mg of Zn/g, 1.11mg of Magnesium/g, 3.25mg of Na/g and 9mg of K per gram minerals.

Phytochemicals composition

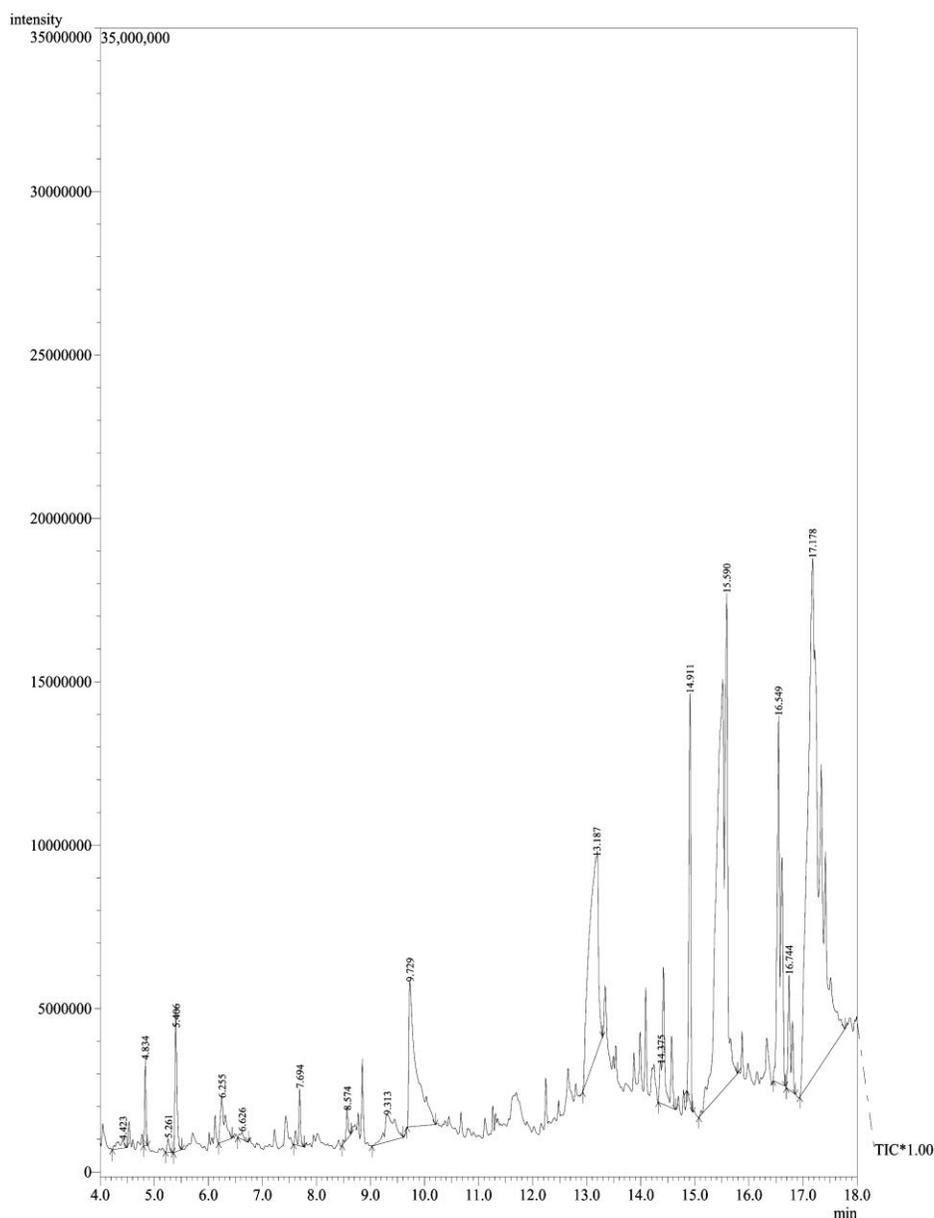


Figure7: GCMS chromatograph of *Allium hookeri*: Ethylacetate: Methanol (95:5)

Peak Report TIC				
Peak#	R.Time	Area	Area%	Name
1	4.423	2098919	0.30	Methyl methanethiolsulfonate
2	4.834	4371268	0.63	1,4-Dioxaspiro[4.4]non-7-ylmethanol
3	5.261	1149986	0.17	Methy methylthiomethyl disulfide
4	5.406	10797691	1.55	Allyl methyl trisulfide
5	6.255	7997412	1.15	Ethyl hydrogen succinate
6	6.626	901342	0.13	2-Methylmercaptoaniline
7	7.694	4359748	0.63	Allitridin
8	8.574	2553208	0.37	Methyl methylsulfinylmethylsulfide
9	9.313	10855196	1.56	Vanillin
10	9.729	42960137	6.16	Benzeneethanol, 4-hydroxy
11	13.187	75413586	10.82	3,4-Dihydroxyacetophenone
12	14.375	20127629	2.89	Pentadecanoic acid
13	14.911	30056606	4.31	Methyl palmitate
14	15.590	171428664	24.60	Ethyl palmitate
15	16.549	52477950	7.53	Methyl linoleate
16	16.744	12793816	1.84	Phytol isomer
17	17.178	246581384	35.38	Ethyl linolate
		696924542	100.00	

Figure 8: GCMS compound table of *Allium hookeri*: Ethylacetate: Methanol (95:5)

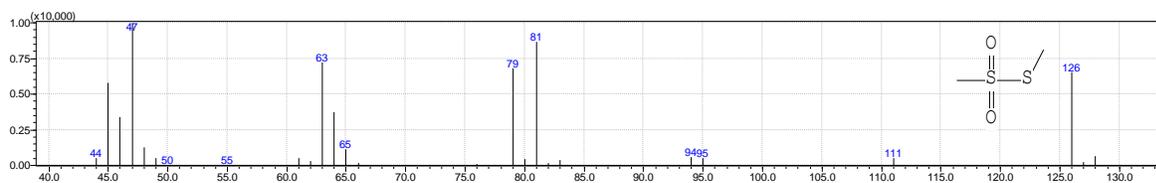


Figure 9: Fragmentation pattern of Methyl methanethiolsulfonate.

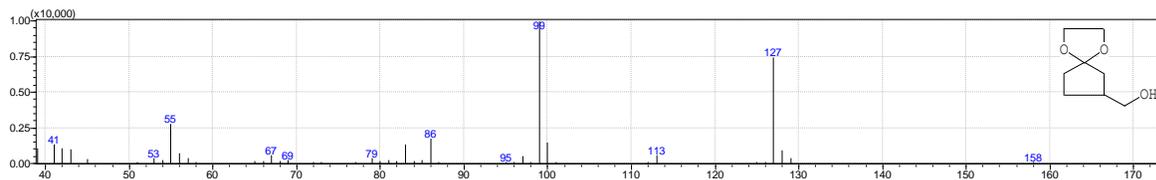


Figure 10: fragmentation pattern of 1,4-Dioxaspiro[4.4]non-7-ylmethanol.

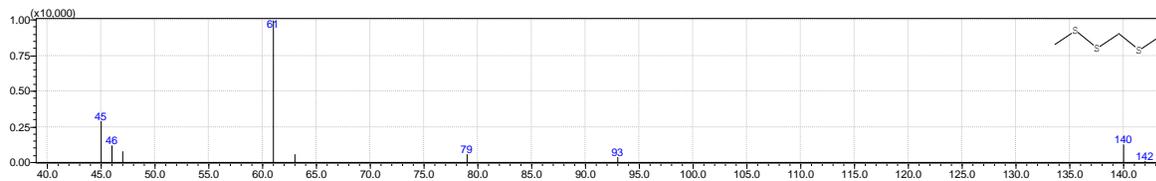


Figure 11: Fragmentation pattern of Methyl methylthiomethyl disulfide.

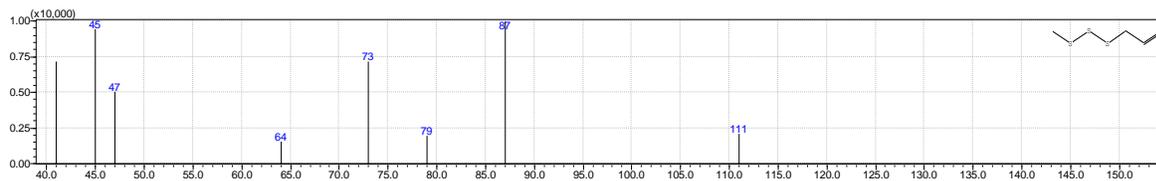


Figure 12: Fragmentation pattern of Allyl methyl trisulfide.

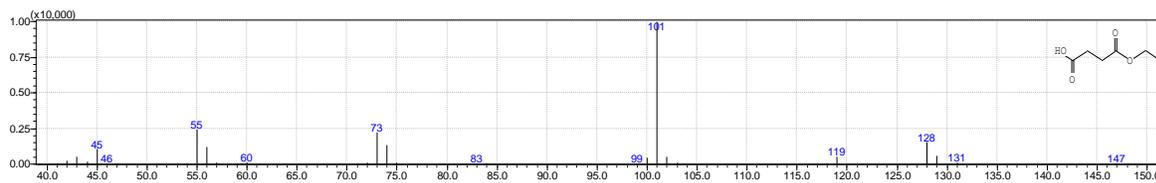


Figure 13: Fragmentation pattern of Ethyl hydrogen succinate

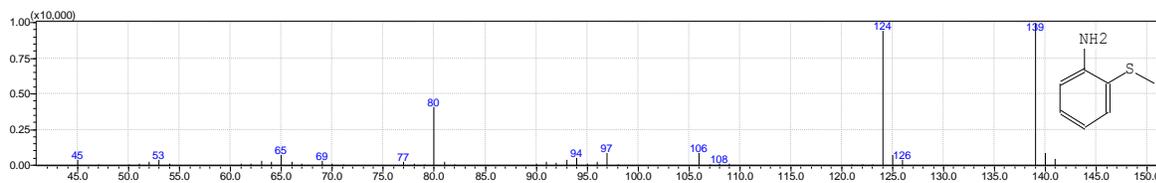


Figure 14: Fragmentation pattern of 2-Methylmercaptoaniline.

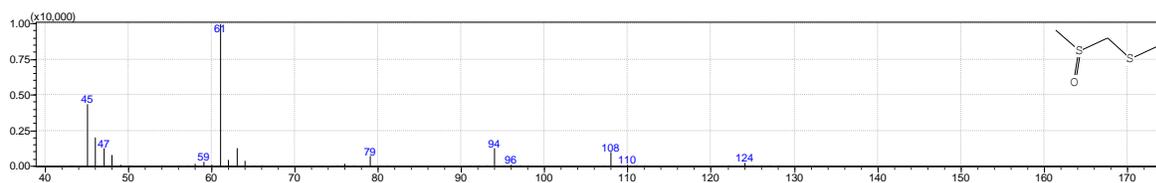


Figure 15: Fragmentation pattern of Methyl methylsulfinylmethylsulfide.

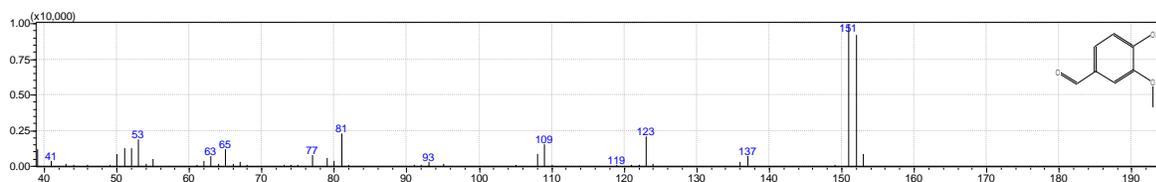


Figure 16: Fragmentation pattern of Vanillin

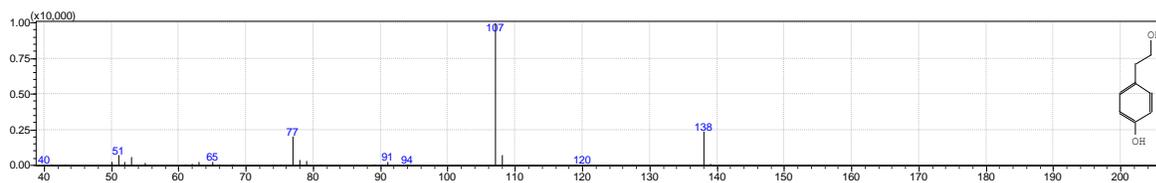


Figure 17: Fragmentation pattern of Benzeneethanol, 4-hydroxy.

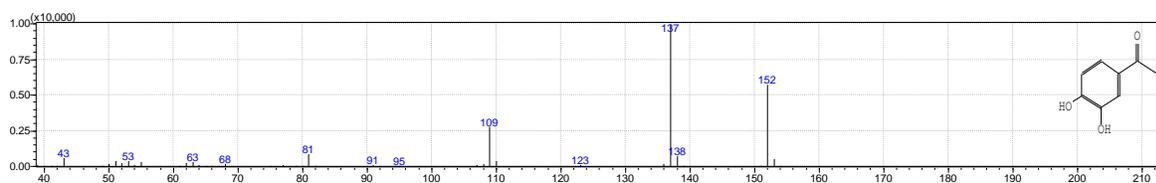


Figure 18: Fragmentation pattern of 3,4-Dihydroxyacetophenone.

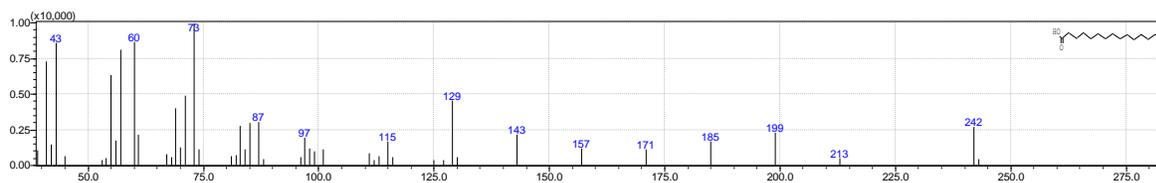


Figure 19: Fragmentation pattern of Pentadecanoic acid.

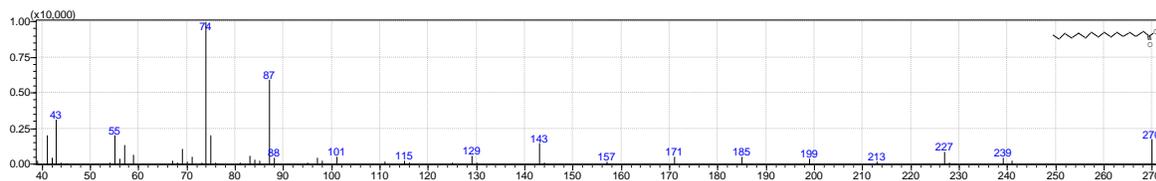


Figure 20: Fragmentation pattern of Methylpalmitate.

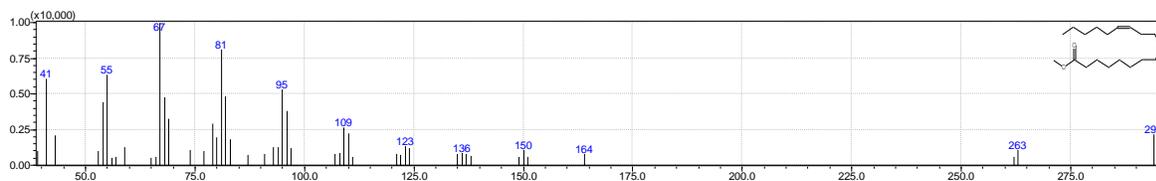


Figure 21: fragmentation pattern of Methyl linoleate.

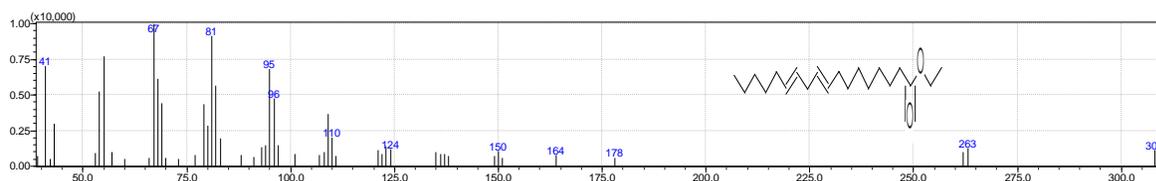


Figure 22: Fragmentation pattern of Ethyl linolate.

The ethanol soluble whole plant extract of *Allium hookeri* that was elicited in column chromatography in the solvent system of Ethyl acetate: Methanol (95:5) gave light green elute weighing 0.742g. In GCMS analysis this extract gave seventeen compounds: Methyl methanethiolsulfonate, 1,4-Dioxaspiro[4.4]non-7-ylmethanol, Methyl methylthiomethyl disulphide, Allyl methyl trisulfide, Ethyl hydrogen succinate, 2-Methylmercaptoaniline, Allitridin, Methyl methylsulfinylmethyl sulphide, Vanillin, Benzene ethanol, 4-hydroxy, 3,4-Dihydroxyacetophenone, Pentadecanoic acid, Methylpalmitate, Ethylpalmitate, Methyl linoleate, Phytol isomer, Ethyl linolate. Vanillin is antianemic, anticancer, antioxidant, antipolio, antitumor, antiyeast, bacteristat, flavor, fungicide, perfumery²⁸; Allitridin act against human cytomegalovirus²⁹; Allyl methyl trisulfide is antiaggregant and anticancer, 3, 4-Dihydroxy acetophenone is antiaggregant and cardiotonic²⁸; Phytol isomer is an antimicrobial, anticancer, cancer preventive diuretic, anti-inflammatory³⁰ Pentadecanoic acid is an antioxidant²⁸.

CONCLUSION

The health benefits on the use of *A. hookeri*, an the ethnic food among the tribal people of the East Siang District of Arunachal Pradesh may be attributed to the identification of above biologically active compounds. The nutraceutical concept of food as medicine and medicine as food is observed and may be partially validated as numbers of biologically active compounds have been reported. Further, adequate proximate and minerals have been reported from this medicinal food

plant. This study conceives the need of further study on this plant in future.

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