

Chemical Investigation of Medicinal Plant *Allium Sativum* L. Family (Amaryllidaceae,) from Sindh Pakistan

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ABSTRACT : The most famous medicinal plants were selected for study; *Allium sativum* L. family (Amaryllidaceae,) Local name in Urdu (Lahsan) English name (Garlic) Habit Bulb Parts used Whole plants The tap water was used for washing the plant material (seeds). Dry under shade for one week at least and grinded by means of grinding machine. Put this powdered form of plant samples in vial. Manage all these chemical tests on plants samples (which are now in powder form) by using SEM/EDS laboratory. The elemental determination of samples takes place in pure and applied Geology, University of Sindh Jamshoro. The elemental analysis determination of the samples. In result we see the elements which are present in *Allium sativum* L are. potassium, calcium, phosphorus, sulfur, magnesium, silicon, aluminum. The material (seeds) were carried in laboratory for fatty acid analysis. . Sulfur 1.48 Magnesium 1.02 Silicon 0.15 , Potassium 0.34 Phosphorus 0.08 , Calcium 0.06, Aluminum , 0.05 .These plants (seeds) were soaked in chloroform + n-hexane (1:1).By rotary evaporator the extract of plants were evaporated for column chromatography and FAME, then the sample were carried for GC-MS isolation of fatty acids from *Allium sativum* L. total 11 compounds were isolated 4 = Saturated, 1 Unsaturated, 6 Hydrocarbon compound. Octadecadienoate (Unsaturated fatty acid) present in the highest amount 39.5% Palmitate (saturated fatty acid) 19.1% Nonadecanoate (Saturated fatty acids) 15.1% , Behenate (saturated fatty acid) 3.55% Arachidate (saturated fatty acids) 2.77% and six different compounds were isolated

INTRODUCTION

Medicinal properties due to the occurrence of ajoene compound which is used as antibacterial, antiviral and antifungal agent. [1]. Garlic have the power to improve the immunity and it also fights against different diseases. [2].In Garlic Sulfur is present in a high amount which motivates the production of various valuable enzymes [3].Garlic and Onions are they oldest cultivated plants. Onion and garlic also used in medicines and as a food. Onion and garlic are rich source of phytonutrient and also used in prevention and the treatment of various diseases [4].Garlic provide the strength. Garlic pungent odor are due to the sulfur compounds [5].

In Pakistan people rely on medicinal plants for treatment some wild plants commonly used eg. Garlic, Artemisia, Ephedra etc [6]. Data show that the Plants are used as a medicine, started from 60,000 years ago in both developed and undeveloped countries. In early civilization, illness was believed the divine punishment [7]. According to WHO estimated that four billion people of world are used herbal medicines which are prepared by plants .Traditional medicinal plants have been used to cure and prevent disease. Many medicinal plants belong to different families which are used traditionally for monitoring and curing variety of dental problem for example clove oil, aloe Vera, turmeric, sesame etc [8]. Various researches reported the importance of biochemical of plants. Plants are rich in fats, carbohydrate and proteins [9 10, 11, 12]. Medicinal plants are the good source of bio-molecules with beneficial values for the treatment of nervousness and depression [13]. Human beings are dealing with different types of stresses due to the modern life style and the habits of smoking also their stresses cause the imbalance between antioxidant –

oxidants species in the body and lead towards the inflammatory conditions. Various herbs which help in it are involved in our daily life e.g ginger, turmeric etc. The herb *Adhatoda vasia* L. is used to neutralize tobacco smoke indeed oxidative stress [14]. STDS and AIDS are very common in our society now a days but the treatment of STDS and AIDS are very costly due to this many people take help from the other systems of medicines for example Chinese, Ayurvedic naturopathy and homeopathy. This is the fact that plants were used as herbal medicines science long time. Plants are also used in the treatment of AIDS and other sexually transmitted diseases. Herbal formulations for vaginal treatment and tropical micro besides from herbal origin [15]. There is no existence of life without plants. Plants are the basis of medicine some very important drugs which are used today derived from the traditional herbs [16].

In the past two decades it is confirmed that fatty acid is very necessary nutrients cure various diseases. Cellular membrane of brain and retina has docosahexaenoic acid (22:6n-3), which is dynamic component. Fatty acids affect on different disease such as inflammatory disease etc [17]. The body of human and animals must requires essential fatty acids (FFAs) for good health but cannot synthesize them. Such as omega-3 and omega-6 fatty acid. These fatty acids are available in the food [18]. In growth and progress EFAs omega-3 fatty acid play a important role [19]. Eicosapentaenoic acids (EPAS) effect on the fetal and non natal brain development. In central nervous system docosahexaenoic acid and arachidonic acid are basic components. During fetal development these fatty acids are come from the placenta, and are collected in the brain and other organs. Research recommended that for growth and development of the fetus fatty acids are important [20]. The tetus derives its EFAs from it mother's store [21]. The mother should take the right amount of fatty acids because infant take these fatty acids from mother feeding [22]. If we want good health we should take proper fatty acids in our meal and take fruits, vegetables, nuts etc in our diet. A substance that can not be decomposed into simple substances by chemical means, and is made up of atoms all with same number of protons. The role of element in human / plant is very important. Every thing around us is composed of chemical element. Elements are the building blocks. In our body different elements perform different functions. Those elements which are present in high amount are called macro elements and if elements which are present in small amount are called micro or trace elements. In human body sixty elements were found and 29 perform important role in human life [23]. About 118 elements were discovered now. Calcium, Phosphorus, potassium, Sulphur, Magnesium, Iron, Copper, Chlorine, Sodium, Silicon, Iodine are knows as trace elements. Different elements present in our body in different percentage. Our body requires calcium to make and maintain strong bones, heart muscles and nerves. Deficiency of calcium cause weak bones. Aluminum deficiency cause different disease in human. Magnesium help in immune system and if magnesium in low amount cause different disease. Iron play essential role in production of hemoglobin and deficiency of iron cause myocardial infection [24]. Iron deficiency also cause short breathiness [25]. Manganese improve memory and act as catalyst for oxidation [26]. Sulfur is very important component of muscle protein. Potassium is one of the most important electrolytes in the human body. Deficiency of Potassium cause hypokalemia. Silicon in important for the growth of skin, hair and nails. About sixty minerals and 15 vitamins daily required for human body. We get all these minerals and vitamins from food. Toxic element even in small quantity can cause harm to body. Important elements are produced by the plants [27]. Ca, P, Fe, Cu, Mn are present in excess amount in seeds [28]. Mg, Ca, Zn, Mn increase the human immunity [29]. Se and Zn cure male infertility [30]. Different elements performs different function in human body such as calcium keep our bones strong, Fluorine make our teeth healthy. If we do not take the proper food so deficiency of elements occurs. The quantity of elements effect the human body so it is necessary to measure the element of medicinal plants. Various problems are observed if the quantity of elements disturbed in human body [31]. Toxic elements are in small quantity can cause great

harm to human body. The low amount of trace element put effect on human [32]. In nature vitamins, protein, lipids and carbohydrates found in different proportion. When we over cook legumes seeds they loose their effectiveness [33]. There is great role of elements in functioning of human body. Alteration in trace elements level cause a verity of disorders e.g. cardiovascular disease [34]. Pulmonary tuberculosis [35]. Protein calorie malnutrition [36]. Minerals, nutrients, elements all put effect on human body and their function [37]. Elements used to cure and prevent the diseases [38]. Magnesium is very essential mineral that need for physiological function. Magnesium is present in the whole human body.it also has the physiological importance. Those human and animal have good health which depend on plant for food. The noble metals are used for the cure of various diseases. Calcium phosphate are very helpful in bone repair. Calcium and Phosphate have a great biological and medicinal significance. Deficiency trace elements like Zinc,Copper ,Magnesium which cause infertility, pregnancy wastage ,congenital disease, still born and low birth weight. Minerals and trace elements also effect on physical activities. 200 million people affected by osteoporosis worldwide. Some are beneficial elements for growth such as Si, Na, Co, Se, Al. These beneficial elements improve nutritional value and the production of crops. Silicon have beneficial effects on growth on crop e.g wheat, barley etc.

RESEARCH METHODOLOGY (FATTYACIDS ISOLATION)

MATERIAL

Material were purchased from local market (different areas). Pakistan (Sindh). Taking information from the survey literature. Plants (material) were recognized botanically and matched with the flora of Pakistan [39]. The medicinal plant types bulb and This medicinal plant belong the family, Amaryllidaceae,

EXTRACTION PROCESS

Water was used to wash the material after that dry the material or samples, at normal room temperature, about twenty days. Now the material or samples those which are in dry form. The dried (seeds) plants were crushed into small pieces and then soaked in hexane + chloroform (1.1) ratio for around thirty days at normal room temperature. After one month plant extract was filtered. By using rotary evaporator, evaporated the extract of plants under reduced pressure 40°C .Result is thick residue.

PROCESS OF COLUMN CHROMATOGRAPHY (CC)

The thick residue which made up of fatty acids. Now we use column for fraction. Chromatography by silica column. The silica which was used made up of particles that size was 70 to 230 mesh. And silica was belong to Merk company. First of all column washed with pure hexane. In first fraction or fraction “A” we take n-hexane and diethyl ether in (85:15) ratio. In second fraction or fraction “B” we take hexane; diethyl ether in (80:20) ratio. In third fraction or fraction “C” we take hexane; diethyl ether in (75:25) ratio. In fourth fraction or fraction “D” we take hexane: diethyl ether in (70:30) ratio. All these fractions were thick and oily.

PREPARATION

(FATTY ACID METHYL ESTERS) OR (FAME)

The plant or seeds oil extract (5ml) was dried under nitrogen. Then put the clean oil in 20ml of 0.5N solution of potassium hydroxide (KOH) in methanol is added. The sample was refluxed at 70c⁰ for 30 minutes then it cooled to normal room temperature. Now extracted it with ten milliliter (10ml) of hexane three times and

than washed with deionized water to remove extra alkali. Hexane phase (upper layer) was collected and was dried over (Na₂SO₄) moved into vial and then by GC-MS analyzed the methylated fatty acid fraction.

ANALYSIS (GC-MS)

GAS CHROMATOGRAPHY- MASS SPECTROMETRY AND FATTY ACID METHYL ESTERS

By using Agilent Technologies we analyzed different fatty acids through FAME (Fatty acid methyl ester). Little Fall, GC-6890 N, USA New York This machine also have sampler (auto) 7683-B injector, MS-5975 inert XL and detector and capillary column. The capillary column (30m) in length and its diameter was (0.25 mm). And (0.25 µm film) in thickness. This machine was used to separate the fatty acid methyl esters. The temperature of oven at being time was 150°C was kept for 2 minutes then increase to 250°C for three minutes, then 250°C for five minutes. 1:50 was the split ratio. Carrier gas (Helium) was used and its flow rate was 0.8 mL per minute. The temperature of detector was 260°C and the temperature of injector was 240°C. The (EI) electron ionization/impact model was used to operated MS (Mass spectrometry). Its volts 70 and the range of scan 50–550 m/z. 1 µl of the extracted sample was injected.

IDENTIFICATION

Results were matched with NIST library and with literature [151].

ELEMENTOLOGY

The model was (JEOL JSM-6490 LV). This model have the important component called energy dispersive X-ray spectrometer or Bruker EDS. For the ideal focusing we used suitable parameters of SEM. The ideal magnifications of the samples is up to x 80. With the help of scanning electron microscope and energy dispersive X-ray spectrometer, elemental determination of the samples takes place. A blank reading was also taken. In the end calculate the percentage concentration of various elements.

RESULTS AND DISCUSSION

Table 1: Elemental analysis of *Allium sativum* L. by E.Ds spectrum: acquisition

Element	Symbol	Norm. C [wt.%]
Aluminum	Al	0.05
Calcium	Ca	0.06
Magnesium	Mg	1.02
Phosphorus	P	0.08
Potassium	K	0.34
Silicon	Si	0.15
Sulfur	S	1.48

Table 2: *Allium sativum* L., (Saturated and unsaturated fatty acids) analyzed as a methyl ester

No.	Name (Systematic)	Name (Common)	Formula (Molecular)	Molecular Wt.	R.R.T	%
	n-Hexadecanoate	Palmitate (saturated FA)	C ₁₇ H ₃₄ O ₂	270	9.66	19.1

2	n-Octadecanoate	Nonadecanoate (Saturated FA)	$C_{19}H_{38}O_2$	298	12.61	15.1
3	n-Eicosenoate	Arachidate (saturated FA)	$C_{21}H_{42}O_2$	326	14.09	2.77
4	n-Docosanoate	Behenate (saturated FA)	$C_{23}H_{46}O_2$	354	16.53	3.55
5	Octadecadienoate	Octadecadienoate (Unsaturated FA)	$C_{19}H_{34}O_2$	294	17.10	39.5
6	5-Octadecene (E)	Octadecene (other compound)	$C_{18}H_{36}$	252	18.53	2.32
7	Trichloroacetic acid, pentadecyl ester	Trichloroacetic acid (other compound)	$C_{17}H_{31}Cl_3O_2$	373	20.80	1.99
8	3-Eicosene, (E)	Icosane (other compound)	$C_{20}H_{40}$	280	21.48	0.54
9	Hepatacosane	Higher alkanes (other compound)	$C_{27}H_{56}$	380	22.05	2.43
	Heneicosane	Other compound	$C_{21}H_{44}$	296	25.51	10.7
	Tetratetracontane	Other compound	$C_{49}H_{90}$	619	26.53	1.81

In *Allium sativum L.* total = 11 compounds were isolated

4 = Saturated, 1 = Unsaturated, 6=Hydrocarbon compound.

DISCUSSION

The fatty acid of *Allium sativum L.* were analyzed by using (GC-MS) Gas Chromatography-Mass spectrometry technique. Total 11 compounds were isolated 4 Saturated, 1 unsaturated and 6 other different hydrocarbon compounds are found. The 4 saturated fatty acids were n- Hexadecanoate, n-octadecanoate, n-Eicosenoate, n-Docosanoate. 1 unsaturated fatty acid was Octadecadienoate. The 6 hydrocarbon compounds were 5-octadecene (E), Trichloroacetic acid pentadecylester, 3-Eicosene, Hepatacosane, Heneicosane, Tetratetracontane. The present study shows the relative percentage of saturated fatty acid in *Allium sativum L.* were n-Hexadecanoate (19.1%), n-octadecanoate (15.1%), n-Eicosenoate (2.77%), n-Docosanoate (3.55%). The relative percentage of unsaturated fatty acid in *Allium sativum L.*, was Octadecadienoate (39.5%). The relative percentage in six hydrocarbon compounds were, 5 octadecene (E) (2.32%), Trichloroacetic acid pentadecylester (1.99%), 3 Eicosene (0.54%), Heptacosane (2.43%), Heneicosane (10.7%), Tetratetracontane (1.81%). The highest relative percentage in *Allium sativum L.* was unsaturated Octadecadienoate (39.5%), and the lowest relative percentage in *Allium sativum L.* was in 3-Eicosene (E), (0.54%), which is in hydrocarbon. Previously reported work in the same plant. This study explained the antioxidant and antigenotoxic activities of garlic. He prepared garlic extracts by using different methods. The result of aged-garlic extract showed that phenolic content present in high amount in it (562.6 ± 1.92 mg/100g). And the result of raw garlic extract or heated garlic extract showed that phenolic

content low in it. Garlic protected the DNA from damaging, determined by GC/MS, in Garlic 70 Fatty acid and determined 14 of that above 0.4% and only 4 above 2.5%. Due to the presence of ajoene compound garlic is used as an antibacterial, antiviral and antifungal agent. Garlic has power to improve the immunity and it also fights against the diseases. Sulfur is present in garlic in high amount which motivates the production of certain enzymes.

examined the plants which were used in diabetes mellitus treatment. Plants like *Allium cepa* L., *Allium sativum* L., *Ficus bengalensis* L., *Gymnema sylvestre* L., *Pterocarpus marsupium* L. etc, act directly on pancreas and motivate insulin level in blood R. Shukla, et al (2000): [38].

reported that garlic has many benefits. Traditionally garlic is used as a medicine. Alliin is an active component of garlic [39]. Worked on garlic and onion. These both plants were the oldest plants, and mostly garlic and onion are used as a food and medicinal purpose. Phytonutrients were present in these plants in high amount which were used in the treatment of various diseases [40]. Describe the importance of garlic. People used garlic as a spice in many cultures. Cancer, Alzheimer's disease, Cardiovascular disease, is cured by garlic. Dermatologic application hypertension etc [41] reported the medicinal value of garlic in daily life. Sulfur is present in high amount in garlic due to this sulfur garlic cures many diseases. Garlic cures against microorganism (bacteria, fungi and viruses) etc [42].

This investigation determined by Atomic Absorption spectroscopy shows that *Allium sativum* L., was maximum K, Fe, Mn, Pb, concentration. In *Allium sativum* L., these elements were detected Sodium (1.636mg/L), Potassium (55.23mg/L), Calcium (35.84mg/L), Magnesium (8.623 mg/L), Copper (0.461 mg/L), Zinc (0.207mg/L), Iron (7.148mg/L), Manganese (0.219 mg/L), Lead (1.029) [43]. Sodium (Na) helps in the production of energy, transport of glucose and amino acids in body. Potassium (K) helpful in maintaining cardiac rhythm in human body. The deficiency and excess of potassium put affect on human body []. Calcium (Ca) helps in maintain the high blood pressure, heart attack and keep the bones strong. Copper (Cu) is important for skeletal health. By AAS analysis find the chemical composition of garlic. These garlic cultivated in . These were detected P (0.013), Ca (0.277), K (2.489), Mg (1.104), Mn (0.0024), Cu (0.0012). These minerals made garlic very valuable [44]. Previously reported work in elemental of *Allium sativum* L. showed that various trace elements are present in *Allium sativum* L., like Ca, Na, Mg, Cu, Zn, Fe, Pb and Mn in different concentration. The elements are responsible for cure different diseases. reported that onion and garlic have effects on human life. In onion and garlic sulphur is present in high amount and sulphur stops the growth of bacteria and it also inhibits the oxidation of fatty acid [45]. Isolated the trace elements in garlic, onion and shallot. The results show that elements such as Fe, Zn and Mn are valuable to our health are abundant, and other elements but Pb, Ni, Cd and Cr are poor [45]. Examine the *Garlic*, *Rheum australe*, *Terminalia chebula* by atomic absorption spectroscopy. Present study shows that each plant sample has different concentration of trace elements due to plant species were used in different treatment of different disease [46]. Examined the eight medicinal plants of Karachi city (Pakistan) and isolated the trace metals in it. Such as Neem, Cloves, Curry Leaves, Ajwain, Fennel, Cabbage, Turnip and Black pepper. [47]; By using Atomic Absorption Spectroscopy and Flame Photometry. The results show that nine heavy metals (Co, Cd, Fe, Pb, Cr, Cu, Zn, Ni, and Mn) and two alkali metals (K and Na) in it. In these plants Ni, Cr, Co, Pb, Cd were the toxic metals. In all the samples sodium and potassium were very high. Sodium was highest in the roots of cabbage and curry leaves. In fennel potassium was 9038 µg/g. Fe is highest in plant samples of ajwain, cabbage, fennel. Lead is present in all the sample. Na, K and Mn and Cu are found very high in turnip roots and Fe is high in leaves [48]. By using atomic absorption spectroscopy analysis. He examined the chemical composition of Garlic which grows in Nigeria. Results

obtained that garlic contain P,Mn ,Cu which show that garlic is source of minerals. Garlic also contain fatty acids (8.4%). The iodine value of garlic is also high (126.9 ± 0.1). This shows that garlic is good for health (49)

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