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Evaluation of antioxidant activity of *Pisum sativum* (pod and grain) and detection of its bioactive compounds by GCMS analysis

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ABSTRACT

Pisum sativum is a common fruit which can be consumed in raw form as well as cooked or frozen form. The health concern of humankind is increasing day by day and this leads to the necessity of discovering novel and healthy methods to keep one disease free. This research shows the various compounds present in pea pod as well as pea grain. The presence of bioactive compounds in pea pod is equal to that present in pea cotyledon which indicates that pea pod can also be consumed. Chromatography technique was carried out in the analysis of Pisum sativum. The results demonstrate that pea pod and pea cotyledon contain almost equal amounts of antioxidants, indicating that pea pods can also be consumed.

Keywords: *Pisum sativum*, antioxidant, phytochemicals, polyphenolics.

INTRODUCTION

Pisum sativum is botanically categorized as a fruit, seeing as they contain seeds developed from the ovary of the flower. It is an annual plant with a life cycle of one year. The immature peas are used for vegetable, fresh, canned or frozen. Matured peas are used as dry peas or split peas. It is starchy, high in fiber, vitamins, minerals, proteins and lutein. The dry weight obtained is approximately one quarter protein and one quarter sugar only. The Pea cotyledon peptide fractions have minimum potential to scavenge free radicals than glutathione, but have superior ability to chelate metals and restrict linoleic acid oxidation [1]. Fiber present in the seed coat and the cell walls of the cotyledon add to gastrointestinal function and health, and diminishes the digestibility of starch in peas. The transitional amylose capacity of pea starch also assists to its lower glycemic index and reduced starch digestibility [2].

Gas Chromatography (GC) is the technique used in analytical chemistry to separate and analyze volatile compounds, vaporizing without decomposition. GC is mainly being used to validate the purity of a chemical compound and to separate different components of a mixture. In a few of the cases, GC may help in compound identification [3]. In preparative chromatography, GC can be utilized to prepare pure compounds from a mixture [4]. Mass Spectrometry (MS) is the analytical technique based on the principle of measurement of mass-to-charge ration of the charged biological samples such as peptides. The mass spectrum contains various peaks which correspond to the m/z value of various types of chemical compounds in the original sample [5] [6]. Gas Chromatography – Mass Spectrometry (GC-MS) can be together used for the identification of chemical compounds in a test sample.

Free radicals are generated in normal as well as pathological cell metabolism due to the action of oxidation, which is indispensable to most living organisms for producing energy required for various biological processes. Oxygen-centered free radicals and Reactive Oxygen Species (ROS) have been related to the onset of many diseases and degenerative actions in ageing. Some ROS species are highly toxic but can be promptly detoxified by discrete cellular enzymatic and non enzymatic mechanisms. While plants have many mechanisms to fight increased ROS levels during abiotic stress environment, in other situations, they appear to resolutely produce ROS as signaling molecules to regulate various processes including pathogen defense, stomatal behavior and programmed cell death [7].

Most organisms are well secluded against free radical damage by oxidative enzymes such as superoxide dismutase and catalase or bioactive compounds such as α-tocopherol, ascorbic acid, polyphenols, carotenoids and glutathione. However, these systems are recurrently inadequate to completely avert the damage, ensuing in diseases and accelerated ageing. Innate products with antioxidant activity can be used to help the human body to diminish oxidative damage. Many fruits, vegetables, herbs, sprouts, cereals, seeds have been explored for their antioxidant activities in the recent years [8].

The word “antioxidant” has recently become popular among current biologists because of its ample health benefits. The dictionary definition of antioxidant is rather forthright, but includes a traditional annotation which says that, an entity that resists oxidation or inhibits reactions advanced by oxygen or peroxides, several of these substances (like tocopherols) are being utilized as preservatives in diverse products, like fats, oils, food products for hindering the progression of rancidity, in petroleum products for obstructing game development and other undesirable and unwanted harmful changes. A more biologically appropriate definition of antioxidants would be: synthetic or natural substances which are mixed with products to stop or delay their degradation due to oxidation. In biochemistry, antioxidants are enzymes or organic substances, like vitamin E or α-carotene, which are proficient in preventing the detrimental effects of oxidation in animal tissues. Effective antioxidants are radical scavengers that break down radical chain reactions. In rubber and plastic industries sterically hindered phenols and amines are also often used as antioxidants. In food science, antioxidants have a wider scope. That they include components that prevent fats in food from becoming rancid as well as dietary antioxidants (which reduces adverse effects of ROS) [9].

Much like the other definitions, this definition does not provide restriction on the mechanism(s) of antioxidant pathway. Therefore, a dietary antioxidant can (sacrificially) scavenge reactive oxygen/nitrogen species (ROS/RNS) to impede radical chain reactions, or it can suppress the reactive oxidants from being created in the first place (preventive). Nutritional antioxidants widely include oxidative enzyme inhibitors, antioxidant enzyme cofactors and metal chelators [10] [11].

While autoxidation of a barren object takes place by radical chain reactions, it is interceded by a range of redox enzymes by oxidation in biological systems. However, despite the contrary, radical chain reaction leading to non-enzymatic lipid autoxidation may keep on occurring which ultimately results in oxidative stress. As a consequent, biological antioxidants include enzymatic antioxidants (e.g., superoxide dismutase or SOD, catalase or CAT, and glutathione peroxidase) and nonenzymatic antioxidants such as oxidative enzyme (e.g., cyclooxygenase) inhibitors, antioxidant enzyme cofactors, transition metal chelators and ROS scavengers [12].

Despite the difference in scope, a radical chain reaction inhibitor is commonly regarded as an antioxidant and also the most extensively studied.

The current research is based on analytical study of *Pisum sativum* grains as well as pods. The chemistry of pea pod and pea grains has been studied and co-related to the health benefits.

MATERIALS AND METHODS

Sample preparation:
Two whole Pea Pods with Pea Seeds were obtained from the local market of VIT University, Vellore. 1 gm of Pea Pod and 1 gm of Pea Seed was weighed for the water and methanol extract.

Chemicals:
Ascorbic acid (1%), Ferric chloride (0.1%), Phosphate buffer (0.2M, pH6.6), Potassium ferricyanide (1% w/v), Trichloroacetic acid (10%), and Phosphate buffer used in the experiment were acquired from Instrumental & Food analysis lab of the School of Biosciences and Technology, VIT University, Vellore. Phosphate buffer was prepared by mixing 62.5ml monobasic sodium phosphate along with dibasic sodium phosphate (37.50ml of 0.2M). The mixture was diluted with water and made to 100ml of final solution. All the chemicals were acquired in the highly purified state.

Preparation of Water and Methanol Extract:
The pod fruit (*Pisum* sativum*)* to be examined is an annual plant, with a life cycle of one year. First the Pea Pods were cut into small pieces and weighed.

Approximately 1gm was taken for both water and methanol extract. Similarly 4-5 Pea Seeds were weighed to approximately 1gm. The dry pea pod and pea seeds were then grounded in mortar pestle separately. For the Water Extract, 10 ml of distilled water was added slowly as the pea pod and pea seeds were smashed. Likewise 10ml of methanol was added during the preparation of Methanol Extract. The 4 homogenized mixtures were poured in 4 dried tarson tubes and labeled. Each mixture was ultrasonicated at 100 rpm, for 20 minutes with pulse speed as 6. Ultrasonication was done for cell disruption and exposing the intracellular products for a better antioxidant assay. After the mixtures were brought down to room temperature, it was centrifuged at 3000rpm for 20 minutes in the centrifugation machine. About 2ml of the supernatant was taken in the study of radical scavenging property. The remaining Methanol extract (supernatant + Pellet) of pea pod and pea seeds were carefully poured in 2 sterilized petri dishes and given for drying in the incubator at 37°C to obtain the powder form and use it for GC-MS analysis.

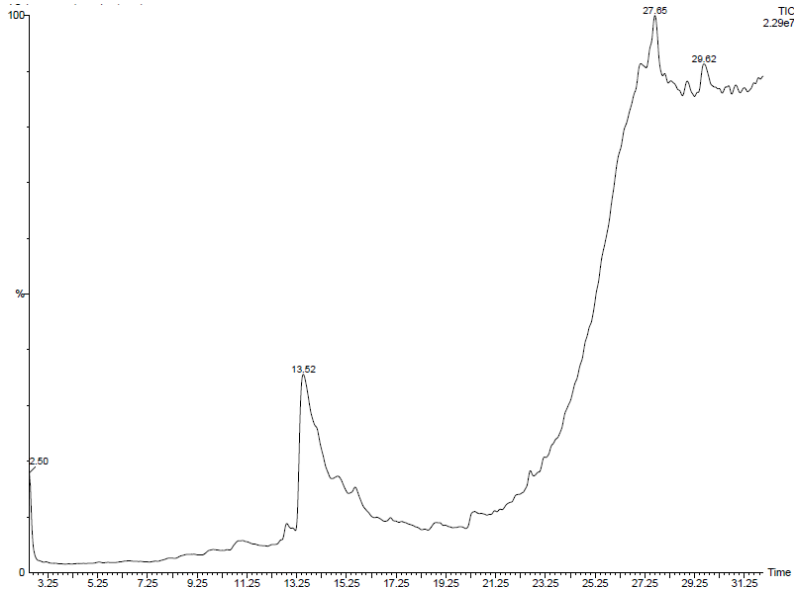
Gas Chromatography – Mass Spectrometry analysis:
The GC-MS used in the present study was Perkin Elmer made, with GC model as Clarus 680 and Mass Spectrometer model as Clarus 680 (EI). The instrumental acquisition parameter was as follows. Oven: initial temperature 60^{o}C for 2 minutes, ramp 10^{o}C to 300^{o}C per minute, hold time 6 minutes, total run time = 32.0 minutes. The carrier gas used was Helium and the column was Elite 5MS. The mass condition (EI) was kept as follows. Solvent delay = 2.00 minutes, Transfer temperature = 230^{o}C, Source temperature = 230^{o}C, Scan = 50 to 600 Da.

Antioxidant property assay:
Antioxidant assay: Reducing power assay
The Reducing power assay has been followed and used for resolving the antioxidant property of the samples under discussion, in respect to Ascorbic acid, which has been chosen as the standard Antioxidant compound. Substances having reduction potential are sensitive to potassium ferricyanide (Fe^{3+}), forming potassium ferrous cyanide (Fe^{2+}), which, when reacted with ferric chloride, produces ferric ferrous complex that has an absorption maximum at a wavelength of 700nm. Phosphate buffer (2.5ml) and potassium ferricyanide (2.5ml) was mixed with the three different concentration of the sample extracts. This mixture was kept at a temperature of 50^{0}C in hot water bath for 20 minutes. The mixture is then cooled to room temperature after which 2.5ml of 10% Trichloroacetic acid was further added and finally centrifuged at 3000rpm for 10 minutes. The pellet is discarded while the supernatant (about 2.0ml) was separated and poured into a clean test tube. It is then mixed with distilled water (2.5ml) and freshly prepared ferric chloride solution (0.5ml). The absorbance was determined at 595nm in the spectrophotometer. Required controls were prepared from before for each sample by just excluding the sample and mixing all other remaining components in the same manner. The antioxidant activity was comparatively studied by measuring the absorbance of the fruit peel extracts with the different concentrations at 595nm.

RESULTS AND DISCUSSION

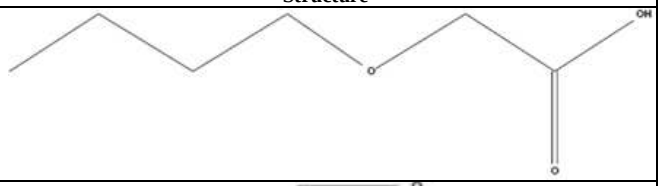
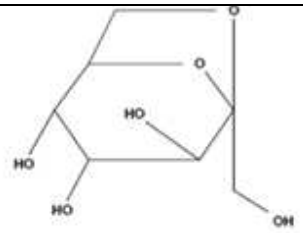
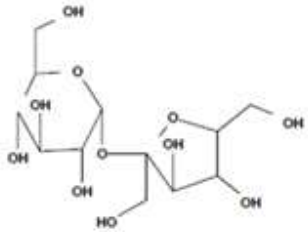
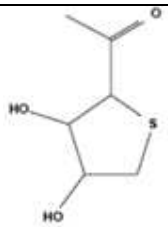
Chromatogram for *Pisum granatum* grains shown in the figure (01)

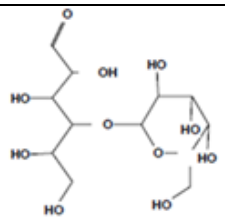
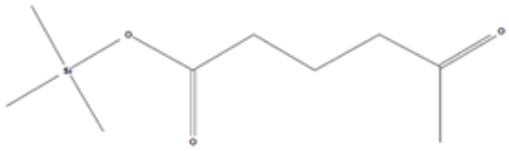
Figure [1]: Chromatogram for *Pisum granatum* grains



Several bioactive compounds were detected in both pea pod and cotyledon as shown in Table [01] and [02].

Table [01]: Bioactive compounds detected in *Pisum sativum* grains

S.no	Compound	Structure
1	Butoxyacetic acid	
2	Sedoheptulosan	
3	Sucrose	
4	Fructose, 1, 3, 6-trideoxy, 3, 6-epitio	

5	Lactose	
6	Hexanoic acid, 5, oxo-trimethyl silyl ester	

Chromatogram of *Pisum sativum* pod shown in the figure (02)

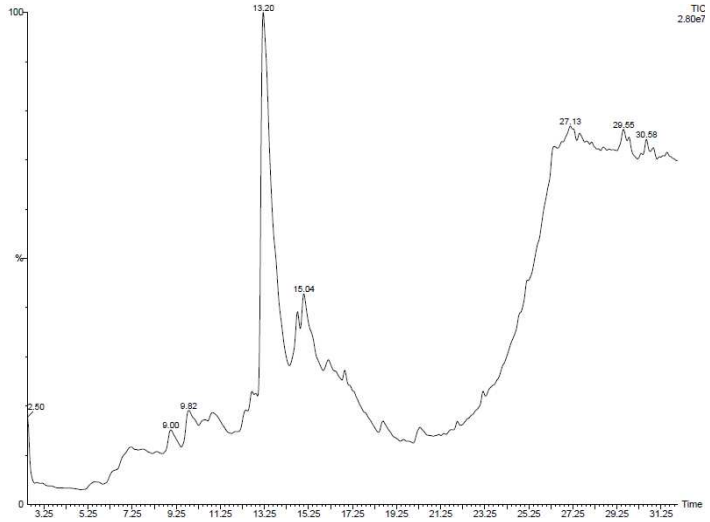
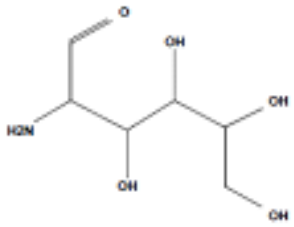
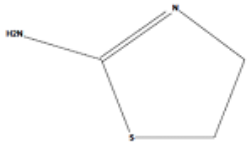
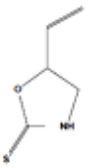
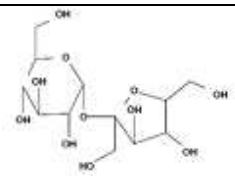
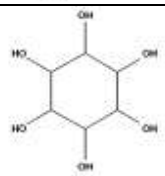
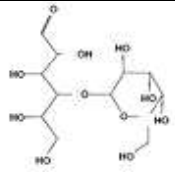
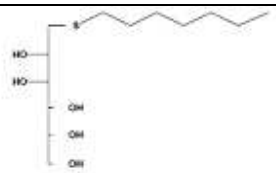
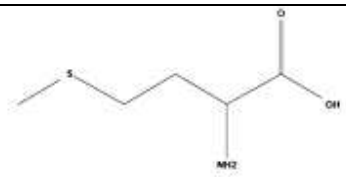


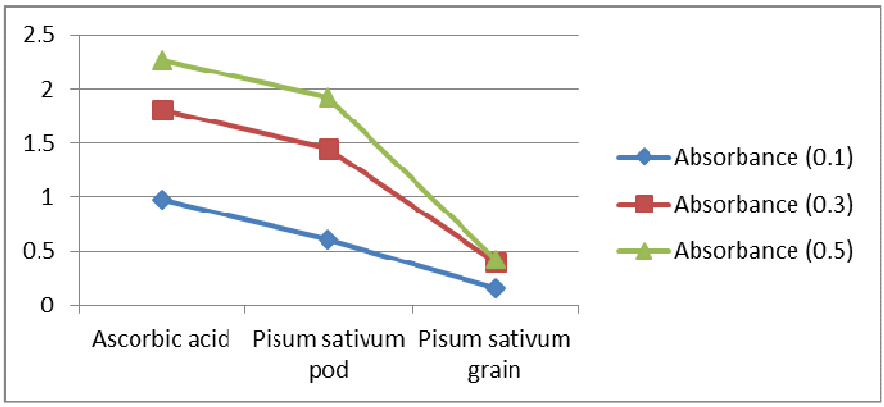
Table [02]: Bioactive compounds detected in *Pisum* sativum pods

S.NO	Compound Name	Structure
1	Mannosamine	
2	2-thiozolamine,4,5-di hydro	
3	Goitrin	

4	Sucrose	
5	Inositol	
5	Lactose	
7	Mannitol 1-thio heptyl -1-deoxy	
8	Methionine	

The activity graph shows that the antioxidant value is directly proportional to the absorbance value detected in the reducing power assay. Table(03) shows the results for antioxidant

Figure [03]: Activity graph *Pisum sativum*



Pharmacokinetic studies indicate that the R-goitrin (epigoitrin) is one of the main constituents accounting for the antiviral activity. Polysaccharide in general is said to be potent antioxidants. Recent studies also state, polysaccharides obtained from bacterial, fungal and plant sources exhibits potent antioxidant activity and therapeutic agent.

Table [03]: Absorbance value detected

S. no	Fruit Peels sample	Concentration (Sample ml/10ml)	Absorbance
1	*Pisum sativum* pod	0.1	0.607
		0.3	1.449
		0.5	1.919
2	*Pisum sativum* grain	0.1	0.160
		0.3	0.394
		0.5	0.421

CONCLUSION

The analytical study shows the presence of various sugar molecules in pea pod as well as pea grains. The sugar molecules include sucrose, fructose and lactose in the majority. Despite the quantity of ascorbic acid in peas being modest, however an approximation of 100 g of peas could possibly add up to around 1/3 of the commended ascorbic acid intake and probably also a substantial part of other various antioxidants. Hence from this point of view selection of pea varieties with high antioxidant content is important. There are various other amino acids and sterically hindered ring compounds, which are responsible for the antioxidant value of pea pods and pea grains. The pea pods or the seed coats are usually discarded and only the pea grains or pea cotyledons are consumed by human beings. The current research supports the consumption of pea pods similar to pea grains. Further investigation must be done to evaluate the harmful chemical compounds in the pea pods. We intend to go for further isolation of bioactive compounds and determine the various medical applications.

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