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ECDYSTERONE, PHOSPHOLIPIDS AND PHENOLICS IN THE SEEDS OF *AMARANTHUS HYPOCHONDRIACUS* LINN

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ABSTRACT

The grain amaranth available in India, *Amaranthus hypochondriacus* Linn, is subjected to chemical analysis, wherein the seeds were found to contain 20-β-hydroxyecdysterone, upto 110 µg per gm, in addition to β- sitosterol, campesterol and stigmasterol. The oil content of the seeds was 5.49% consisting of glycerides of linoleic acid (40%), oleic acid (27%), palmiticacid (27%) and stearic acid (3%). Phospholipids and galactolipids amounted to 1.266%. The total phenolic was 1.4 mg/gm in terms of gallotannins. The flavonoid located was quercetin (in traces) and phenolic acids present were vanillic and syringic acids. The total antioxidant potential of the seeds was estimated to be IC_{50} = 43.75.

KEYWORDS: *Amaranthus hypochondriacus,* grain amaranth, ecdysterone, Linoleic acid, phenolic acids, Antioxidant potential.

INTRODUCTION

The seeds of *A. hypochondriacus* Linn., popularly known as 'grain Amaranth' (Rajgiro in Hindi and Gujarati) is a food preferred over fasting seasons and festivals in Western India. The small convex seeds have a translucent centre and pinkish marginal ring. The dehulled seeds are translucent white with a central endosperm and a curved embryo coiling around it. The seeds are toasted and popped, boiled for gruel or stews or ground into flour. In Bolivia, the toasted seeds are used as foods for children and invalids. The children are advised to consume this food for better health. In China the seeds are used for making candy.

Amaranth grain was the staple food for Aztecs and was in cultivation since 6,000 B.C. [1] Edible grains are obtained from three species of the genus *Amaranthus such as A. hypochondriacus, A. cruentus* and *A. caudatus.* Though grain Amaranth is a crop of the Americas, some time ago (probably since Columbus) *Amaranthus hypochondriacus* underwent a remarkable migration to Asia. There, during the last century, it became increasingly popular among hill tribes in India, Pakistan [2].

It is now being rediscovered in USA where it is sold as a health food. The seeds contain 67% carbohydrates, 14% fat, 4-10% oil and are rich in vitamins of B group and in minerals. According to ECHO, the protein is of high quality, rich in lysine and free of gluten. The fat consists of esters of linoleic acid (46- 50%), oleic acid (22-26%), palmitic acid (19-20%), stearic acid (3%) and squalene (5-6%). These seeds are reported to be one of the rich sources of phytosterols such as β- sitosterol, campesterol and stigmasterol [3]. The grain and oil are found to reduce non-HDL chole sterol and raised HDL cholesterol, as also lowers very low density lipoprotein cholesterol in hamsters [4, 5]. Most of the chemical data of grain amaranth are based on those of *Amaranthus cruentus* and *A. caudatus*. Therefore the seeds of *A. hypochondriacus* were analysed for their individual and total phenolics, oil components, phospholipids and total antioxidant potential.

MATERIALS AND METHODS

The seeds were obtained from Gujarat State Seeds Corporation, Vadodara, India. The powdered grain was extracted with petroleum ether and the total ether solubles quantified. The GC-MS analysis of the oil was done at DMAPR, Anand, India.

The instrumental conditions were the following: The Equipment was Focus-PolQ GC/MS (Thermo); Column: ZB-5 capillary column (30 m×0.25 mm×0.25 mm); Oven temperature: 80°C for 5 min, then increased 3°C/min to 220°C and held for 5.0min.; Injector Temperature: 230°C, Carrier gas: Helium (1mL/min). The injection volume was 0.5ul and EI-MS: 70 eV in the range *m/z* 30-400. Individual compounds were identified as methyl ester by comparing their mass spectra with library (NIST) and literature [6]. Steroids were extracted in methanol from defatted seeds and were analysed using Shimadzu LC20AT having photodiode array detector.

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The column used for separating ecdysterone was GraceSmart RP C18 column (250x4.6mm, 5µ) using a mobile phase of acetonitrile: water (20:80, v/v), flow rate was 1 ml/min, an injection volume of 20 uL and the peak was detected at 242 nm. Standard steroids including ecdysterone, provided by Prof.A.Banerji (Amruta Institute of Biotechnology, India), were used for identification of these compounds.The phospholipids and phenolics were extracted from defatted material using methanol in a Soxhlet's extractor.

The methanol extract was concentrated and on addition of acetone to this concentrated extract, phospholipids precipitated which were filtered and quantified. The acetone solubles remaining after the separation of phospholipids contained phenolics including flavonoids. Phospholipids were analyzed by TLC (Silica gel) using the solvent systems acetone: benzene: water (91:30:8,v/v/v) and chloroform: methanol: acetic acid: water (170:30:20:7,v/v/v/v). Lecithins were visualized by spraying the developed chromatograms by Dragendorff's reagent (to locate choline-containing lipids) and the cephaelins were located by Ninhydrin reagent. Total phenols were estimated by Folin-Ciocaulteu method [7]. Flavonoids were analyzed by standard methods prescribed by Mabry and co-workers [8] and by co-chromatography with standard compounds. The identification of phenolic acids was done following Ibrahim & Towers [9]. The Total antioxidant activity was measured using the well-known DPPH method [10].

RESULTS

The steroidal fraction on analysis was found to contain 20-β-hydroxyecdysterone, in addition to the three steroids reported earlier (*i.e.*, β- sitosterol, campesterol and stigmasterol). The amount of this anabolic steroid amounted to 110 µg per gm. The total phenolic was 1.4 mg/gm in terms of gallotannins. The flavonoid located was quercetin in traces. The seeds yielded two phenolic acids vanillic and syringic acids. The total antioxidant potential was estimated to be IC_{50} = 43.75. The oil content of the seeds was 5.49% consisting of glycerides of linoleic acid (40%), oleic acid (27%), palmiticacid (27%) and stearic acid (3%). Phospholipids and galactolipids amounted to 1.266%. There were four cephaelins while lecithins were two in number. Galactolipids were three in number.

DISCUSSION

The presence of 20- β-hydroxyecdysterone in grain amaranth, that too in appreciable quantities, elevates the status of grain amaranth as a health food considerably.This is the first time ecdysterone, an anabolic steroid is reported in a staple food material.It is proved that ecdysterone supplementation leads to increase in lean body mass, increased endurance, stimulates metabolism, improves nerve function and enhances erythropoiesis (the development of mature red blood cells) [11, 12, 13]. This compound is known to increase protein synthesis and glycogen content in muscles, stimulates incorporation of glycogen into proteinaceous tissue such as liver and muscle and thus increases growth anabolically in vertebrates including humans [14]. Russian researchers equal ecdysterone directly against Dianabol, one of the most powerful anabolic steroid developed [15]. The other health benefits are cholesterol-lowering effect, Suppression of hypoglycemia as well as hyperglycemia, hepatoprotective action, anti-inflammatory properties

[16, 17, 18]. The other phytosterols obtained from Rajgiro, β- sitosterol, campesterol and stigmasterol exhibit cholesterol lowering properties and many clinical studies have confirmed the efficacy of beta sitosterol in lowering plasma LDL- concentrations. The reduction in non-HDL cholesterol and elevation of HDL cholesterol levels as also lowering of very low density lipoprotein cholesterol in hamsters by the grain and oil [4, 5] can be attributed to the ecdysterone and other phytosterols.

Grain amaranth is rich in oil and this adds to nutritive potential. The fairly high amount of oil (6%), rich in linoleic acid (40%) which is an omega 6-fatty acid, is highly significant because this acid is easily converted to n-6 eicosanoids, n-6 prostaglandin and n-6 leucotriene hormones. This provides targets for drug development in arthrosclerosis, asthma, arthritis, immunity development etc. Linoleic acid is also very popular in beauty products as helping in moisture retention, acne reduction, and anti-inflammatory. Lack of linoleic acid causes dry hair, hair loss, and wound healing. Therefore, the consumption this millet containing oil will yield the same advantages to the consumer. Oleic acid, which is an omega-9 fatty acid, also is equally important having all the health benefits of linoleic acid. In cases of reduced availability of omega-6-fatty acids, omega-9-fatty acids are converted to omega-6-fatty acids.

Though many medical authorities, such as the World Health Organization, say dietary intake of saturated fats such as palmitic acid increases the risk of cardiovascular diseases. In moderation, palmitic acid does display mild antioxidant and anti-atherosclerotic properties, at least in animal studies. Palmitic acid has been thought for many years to raise cholesterol levels if consumed, although a Canadian study in 2002 published in the "Asian Pacific Journal of Clinical Nutrition" examined the effects of high consumption of palmitic acid in healthy volunteers and concluded it does not raise cholesterol if it is combined with linoleic acid. This is significant because in Rajgiro, palmitic acid is present along with linoleic acid. The high amount of phospholipids consisting both lecithins and cephalins, also offer many advantages.

They are a group of best antioxidants. Phospholipids of other cereals like rice, wheat, corn, etc. are not available to the consumer because they are removed dissolved in oils. These compounds are having great role in general metabolism, being concentrated in brain are useful in brain function, behavioral disorders and stress. They help in regeneration of membranes and protect liver, lungs, kidneys, and gastrointestinal tract. These compounds are known to enhance the bioavailability of other nutrients and medicines. Glycolipids present in this seed play an important role in the intercellular signalling that controls the growth and development of the nervous system. In particular, defects in that carbohydrate may result in the uninhibited cell growth that characterizes the genetic disease neurofibromatosis and certain types of cancer. The results have just been published in the well-reputed journal *PNAS*. The phenolics acids identified also have distinct pharmacological properties. Vanillicacid is anthelmintic, anti-fatigue, anti-inflammatory, antilaeukemic, antiseptic and anti-sickling whereas syringic acid is antioxidant, anti-peroxidant and anti-radicular [19].

CONCLUSIONS

The discovery of the anabolic steroid ecdysterone, in seeds of *A. hypochondriacus* elevates the nutritional status of this grain to an important health food as well as a nutraceutical. The constitution of oil rich in linoleic acid, also is in tune with those of other grain amaranths. The phospholipids and glycolipids identified provide additional health benefits to the consumer. The antioxidant potential of the grain is mainly due to the phenolic acids present.

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