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Ajwain a potential source of phytochemical for better health

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Abstract
Ajwain seeds have enormous health benefits. The therapeutic, medicinal and pharmaceutical potential of ajwain seeds is attributed to its phytochemical composition and their bioavailability. The present study was therefore conducted to see the effect of various grinding conditions on the major phytochemicals of ajwain seeds. The three Ajwain varieties showed an increase in the polyphenolics, flavonoids and antioxidant activity in the cryo-ground seeds. The effect of cryo-grinding was found more in AA93 variety than in AA1 and AA2 variety. Among the two solvents i.e Methanol (80%) and Diethyl ether, Methanol proved to be better for extraction of Polyphenols and flavonoids from Ajwain. Whereas, the DPPH scavenging potential was estimated more in the diethyl ether extracts. Total phenolic content (TPC) was maximum (0.033 mg GAE /g) in the methanol extract of cryo-ground sample of AA1, and that in AA-2 and AA93 was 0.021 mg GAE /g and 0.027 mg GAE /g. Total Flavonoid content (TFC) was maximum (0.046 mg QE /g) in the methanol extract of cryo ground sample of AA93 variety, whereas in AA1 and AA2 it was 0.042 mg QE /g and 0.043 mg QE /g. The DPPH scavenging potential was highest in ether extract of cryo-ground sample of AA2 (61%) and that in AA1 and AA93 was 54% and 57% respectively. Since, phytochemicals acts as ingredients for exerting the medicinal values, the present study on the cryogenic grinding proves that cryo-ground Ajwain powder is a potential source for medicinal and health related applications.

Keywords: Phytochemical, ajwain, health, food, pharmaceutical, medicinal

Introduction
Ajawain and Ajwain Seed
Ajwain is an annual herbaceous and aromatic plant belonging to the family Apicacae[1]. It is an erect, minutely pubescent, branched annual herb. The plant grows 1- 2 ft (60-90 cm) in height. The leaves of the plant are feathery and the flowers are red and white in colors [2]. Ajwain (*Trachyspermum ammi* L.) is a well-known spice. It belongs to the family *Apiacaea*. The seeds are small, egg shaped and brownish or greyish in color depending upon the variety (Zachariah, 2008)[2]. The ajwain seeds are hot and dry, strong in flavor and leave a slightly bitter aftertaste. The flavor of ajwain resembles to that of thyme due to the presence of similar flavoring compounds mainly thymol. The seeds of Ajwain inherit a great potential to subside the cramping, flatulence, any abdominal discomfort due to the presence of certain bioactive compounds, which exhibit pharmacological or health benefits (Vitali, *et al*., 2016) [3]. Ajwain has found its wide applications not only in cookery but also in medicine, cosmetic, food and flavor industry. The ajwain seeds are constituted of various important chemical constituents namely- carbohydrates (24.6%), proteins (17.1%), crude fat (21.1%), crude fiber (11.9%), glycosides, tannins, saponins and flavones (Zarshenas, *et al*., 2014) [5]. The other phytochemical constituents of ajwain includes iron, calcium, cobalt, phosphorous, copper, magnesium, iodine, riboflavin, nicotinic acid and thiamine (Qureshi and Kumar, 2010) [4]. The ajwain seeds are also famous for their essential oil. The seeds comprise of 2.5- 5% essential oil. Ajwain essential oil is a major contributor towards its odor and taste. The essential oil is mainly constituted of thymol and carvacrol which are the principle components of its flavor. Other major components of the ajwain essential oil include γ- terpinene, ρ- cymene, β- pinene, myrcene, limonene, and camphene. These aroma compounds

vary with origin, type and parts of the plant used for the extraction of essential oil (Chahal *et al*., 2017) [6].

Fig 1: Ajwain seed

Ajwain volatile and non-volatile oil

The ajwain as whole or its oil are known to possess numerous health benefits and hence, the spice is well known as an ayurvedic herb. It is a part of numerous traditional medicines which have wide medicinal and pharmacological benefits (Zarshenas, *et al*., 2014) [5]. Earlier the spice was used to treat tremor, palsy, paralysis and various other neurological disorders. The drops composed of ajwain extracts were used to treat infected eyes and ears. The ajwain was also proved to be effective against cough, dysphonia, pleurisy and other respiratory problems. Without any doubt the ajwain seeds are still being used for correcting the gastrointestinal disorders such as – vomiting, abdominal cramps, nausea, reflux, and loss of appetite. The herb possesses strong carminative and stimulant properties. Ajwain was also used as antidote against toxic agents present in nature. It was also believed that seeds of ajwain can help melting the stones if consumed with wine. The seeds are considered as an anthelmintic, galactogogue, aphrodisiac and diuretic agent (Tonekhaboni, 2007) [7].

Functional characteristics of ajwain for better health

The ajwain is highly valued as a gastrointestinal medicine and as an antiseptic. The seeds exhibit various properties such as antiflatulence, asthma, polyuria, indigestion, common cold, toothache, bronchitis, cardialgia, antiarthritic, antirheumatic, migraine, aphrodisiac, antispasmodic, carminative, expectorant (Rajeshwari, *et al*., 2011) [8]. Also, improves premature ejaculation and enhances virility. The ajwain seed oil however, exhibit a wide spectrum of antibacterial, antifungal, fungitoxic, insecticidal, antimicrobial, antispoilage, antioxidant, antifilarial, nematicidal, anti-inflammatory and other medicinal properties (Anwar, *et al*., 2016; Chahal, *et al*., 2017) [9, 6]. The ajwain is also being studied for its hypotensive, anti-tussive, bronchodilatory, hepatoprotective, analgesic and anti-nociceptiveactivities (Chahal, *et al*. 2017) [6]. Other uses of ajwain include its wide application in the food and flavor industry. The ajwain is being used a preservative in beverages, pan mixtures, confectionaries. Bakery products such as mathri, ajwain flavored salty pastry and breads uses ajwain in their preparation. Curries in Indian cusuins, pickles and pulses also include ajwain as their indispensible part. The aroma compounds of ajwainhave found their place in the perfumery industry. In the cosmetic industry the ajwain oil is an essential component of ointments and lotions (Malhotra& Vijay, 2004; Malhotra, 2006) [10, 11]. The above literature very well justifies the potential of ajwain. In general almost all spices have such therapeutic and bioactive potential, but these spices tend to lose their potential when they are subjected to the size reduction at high temperature grinding. At ambient grinding when the energy is utilized to break the seeds into smaller particles heat is generated, this heat is detrimental to the flavor and overall quality of the product. Mostly the problem is caused by the fat content of the spices. The spices lose out on a significant fraction of their essential oils or flavor compounds because of the rise in temperature (Singh & Goswami, 1999) [12]. In order to improvise on the quality of spices and retain their flavor cryogenic grinding systems were developed. The extremely low temperature of the cryogenic systems embrittles the spice, thereby solidifying its essential oil content. During grinding the spice crumbles easily without any temperature rise. Hence, preservation of quality as well as flavor is achieved. Development of these cryogenic grinding systems requires a prior and detailed knowledge of the thermal properties of the spice that is to be grinded (Singh & Goswami, 2000) [13].

Objective of study

The present study was envisaged to study the effect of ambient and cryogenic grinding on the antioxidant activity, total phenolic content and total flavonoid content. Antioxidants, phenols and flavonoids are the major bioactive compounds of ajwain exhibiting various health benefiting activities.

Material and Methods

The samples of ajwain were collected from ajwain seed research station and same were used for this study. The collected varieties were studied for their phytochemical retention over ambient and cryogenic grinding conditions.

Sample preparation for study

The matured seeds of ajwain were harvested and after drying those seeds were cleaned and stored at spice production and research station and same seeds were collected.For the analysis the ajwain seeds were grinded under the cryogenic and ambient conditions.

Nutritional composition of ajwain

The nutritional profile of the ajwain seeds were analyzed with the help of standard methods given in AOAC and other literature.

Extract preparation

The extracts were prepared from the grounded powder using different solvents by Cold Extraction method. 10gm of ajwain powder of all varieties were taken and soaked for 24hrs in 100ml of each solvent. The solutions were then stirred magnetically for 6 hours and centrifuged for 15 minutes at 200g. The collected supernatant was directly used for the analysis within two days.

DPPH scavenging activity

The extracts were determined for DPPH scavenging activity using the method as mentioned by Baba, *et al*., (2018) [15] with slight modifications. 1 ml of extract was treated with 1 ml of 0.1M methanolic DPPH solution. 3ml of methanol was added to the tube. The solution was mixed homogenously with the help of vortex. The solutions were then incubated at room temperature for 30 minutes and then absorbance was measured using spectrophotometer at 517 nm. Results were expressed as percentage DDPH scavenging activity.

Total Phenolic Content

The total phenolic content was estimated using the method as

reported by Gani, *et al*., (2016) [16] with slight modifications. 0.1 ml extract was mixed with 0.5ml FC (FolinCiocalteau) reagent. The mixture was incubated for 5 minutes at ambient temperature and then 1.5ml of 20% Sodium carbonate was added. Later 7.9 ml of distilled water was added to make the volume and the mixture was mixed homogenously using vortex. The mixture so prepared was incubated at 37 ^{0}C for 1 hour and then absorbance was taken at 765 nm. The results were expressed as mg GAE/ g sample using calibration curve (r^2= 0.989).

Total Flavonoid Content

The total flavonoid content was estimated using the method as reported by Shafi, *et al*., (2017) [17] with certain modifications. 0.1 ml of extract was mixed with 0.4 ml aluminum chloride with concentration of 25 g/l. To the mixture 0.5 ml of sodium acetate was added with 100 g/l concentration. Volume was made up using 4ml of distilled water. For homogenous solution the mixture was mixed using vortex. The solution was then incubated at room temperature for 30 minutes and absorbance was taken at 430nm. The flavonoids were expressed as mg Quercetin equivalent/g of sample using the quercetin calibration curve (r^2= 0.9697).

Results and Discussions

Nutritional composition of ajwain

Ajwain is a good source of various nutrition needed for human body, specifically it is rich source of phytochemicals which were determined using AOAC methods of analysis (2012) [14]. Nutritional composition of ajwain determined with the help of proximate analysis is shown in Table 1. It is found that the ajwain is very good source of fat, carbohydrates, protein, fibers and minerals and different values of individual nutrientgs can be found in Table 1.

Table 1: Nutritional contents of the ajwain

Nutrient	Contents
Moisture (%)	8.03±0.23
Carbohydrates (%)	52.49±0.03
Proteins (%)	15.43± 0.01
Crude fat (%)	16.35±0.25
Crude Fiber (%)	18.78± 1.1
Mineral content (%)	7.70± 0.03

Antioxidant Assay- DPPH scavenging activity

The DPPH assay was used to determine the antioxidant potential of the extract. Fig. 2 shows the effect of various grinding and extraction conditions on the DPPH scavenging activity. Through the graph it is clear that the scavenging activity is found to be more in samples which are cryogenically grinded than the ambiently grinded. Moreover, the diethyl ether shows better retention of the antioxidant activity than methanol. Similar results were also analyzed by Kenny, *et al*., 2013[18], where they found more DPPH scavenging activity with less polar solvents. AA2 variety is estimated to have highest DPPH scavenging activity i.e. highest antioxidants followed by AA93 and AA2.

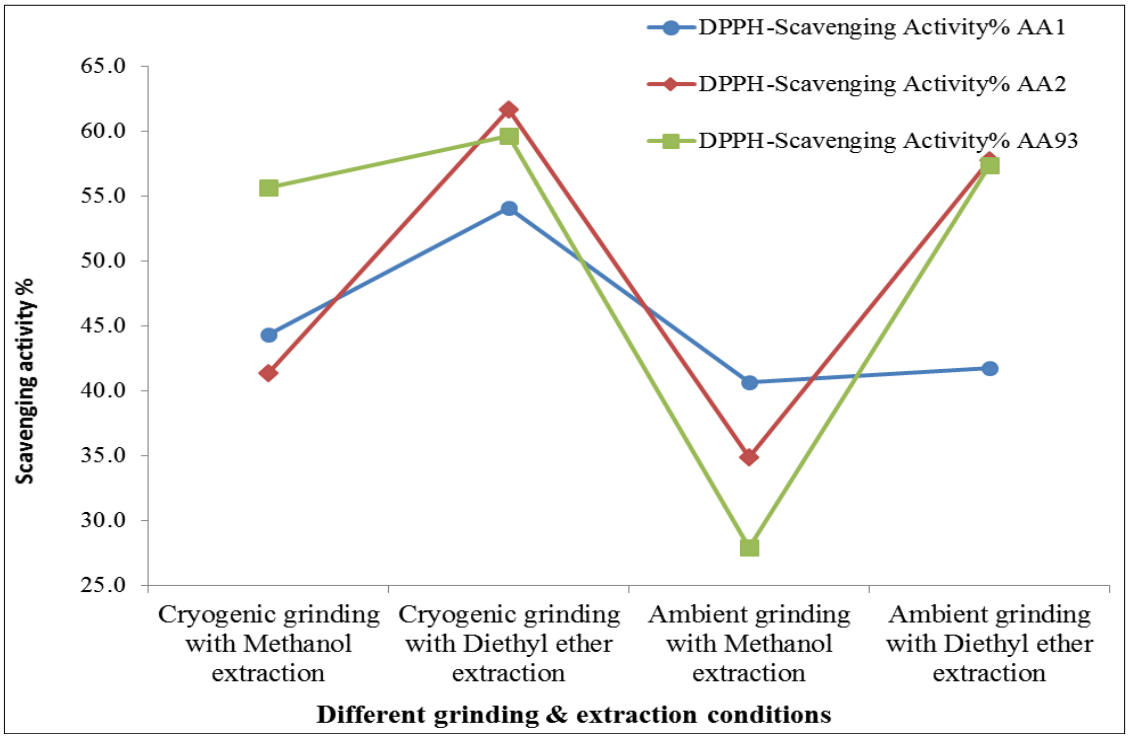

Fig 2: The variations of scavenging activities with different grinding and extraction conditions

Total phenolic content

The phenolic compounds possess redox properties which enable them to exhibit antioxidant activities. Therefore, the phenols act as hydrogen donors, reducing agents and quenches the singlet oxygen (Baba, *et al*., 2018) [15]. Fig. 3 shows the effect of different grinding conditions and solvents on the extraction of total phenolic content. The total phenolic content of ajwain varied greatly with the grinding conditions and various solvents. Under both the grinding conditions the total extractable phenolic content can be seen higher with methanolic extraction. Also, the retention of total phenolic content is seen higher in case of cryogenic grinding than in the ambient grinding. The total phenolic content was found to be highest in AA93 variety under cryogenic grinding with methanolic extraction, followed by AA2 and AA1. Diethyl ether extraction of polyphenols was found to be almost negligible in both cryogenic and ambient grinding.

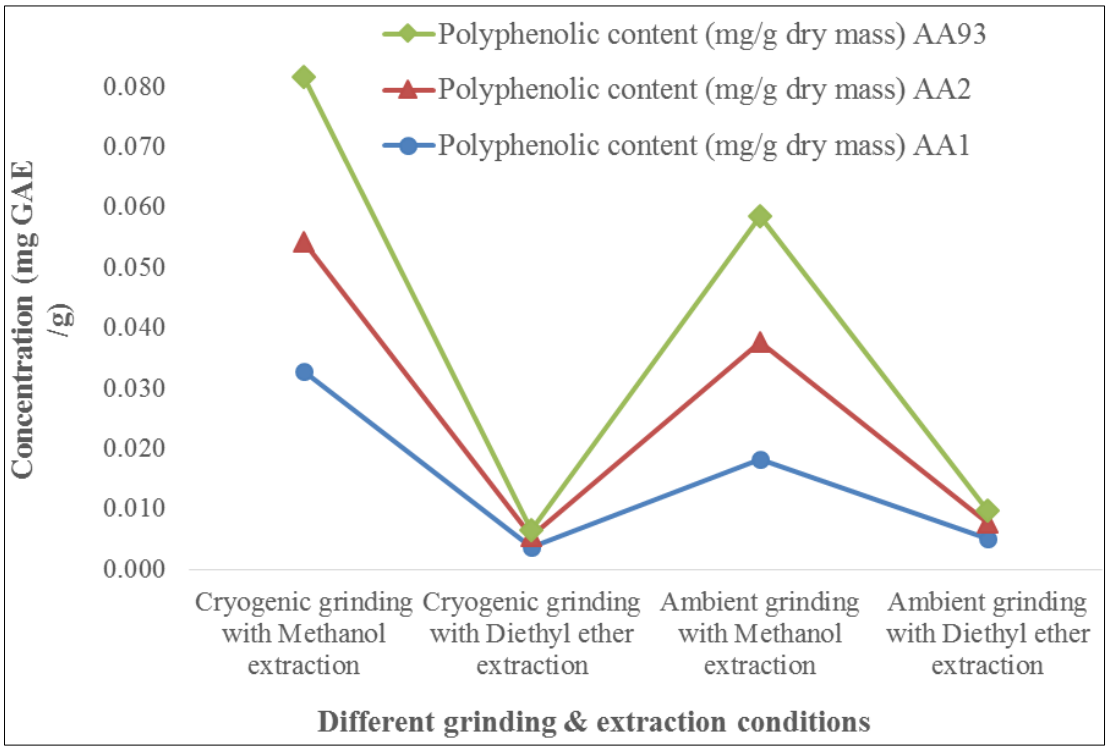

Fig 3: The variations of total phenolic content with different grinding and extraction conditions

Total flavonoid content

Flavonoids are one of the most important bioactive compounds which have numerous pharmacological benefits and can bring about many biochemical changes. Some of the most important health benefits of flavonoids include antioxidant activity, anti-inflammatory, antimicrobial, antitumor, anti-atherosclerotic and also exhibits anti-allergic effects(Baba, *et al*., 2018) [15]. The flavonoids are also known for their proton donating nature which enhances their radical scavenging activity. Fig.4 shows variations in the flavonoid content grinded under different conditions. From the figure it can be concluded that methanolic extraction of flavonoids yields better results than the diethyl ether extracts. This may be due to the higher polarity of methanol, which is able to dissolve maximum flavonoids from the sample. Also, the total extractable flavonoids are higher in case of cryogenic grinding than in the ambient grinding condition.

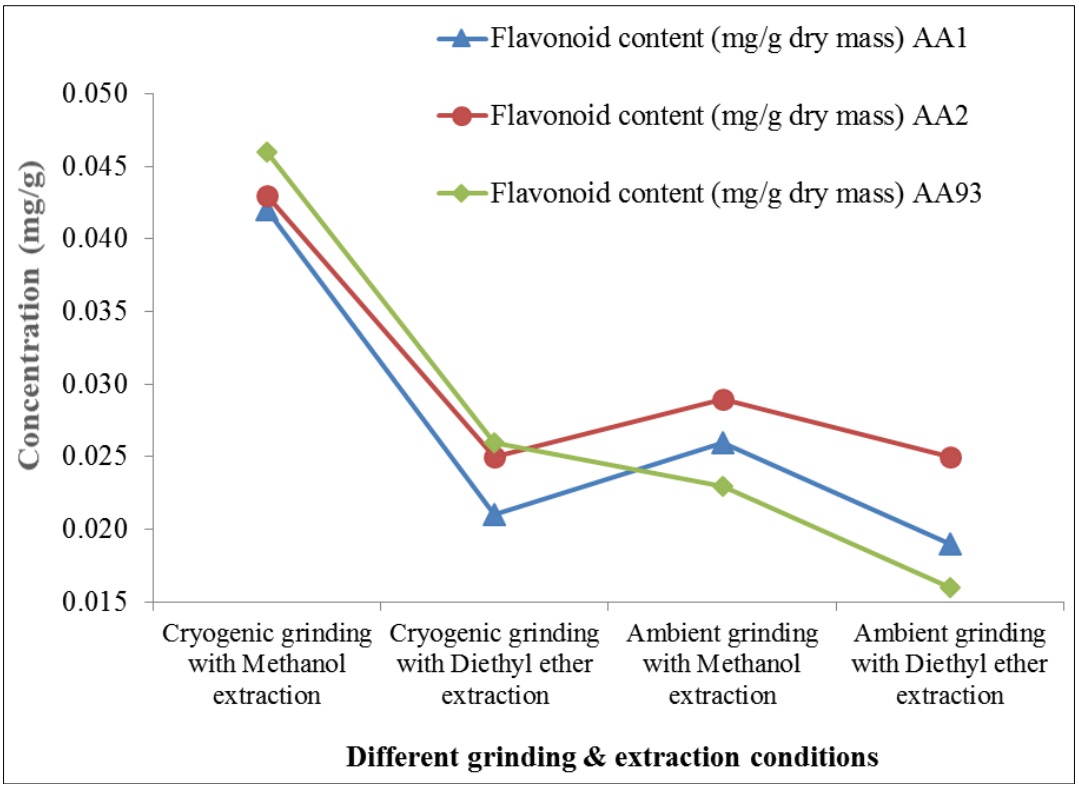

Fig 4: The variations of scavenging activities with Different grinding and extraction conditions

Conclusions

It can be concluded that ajwain has a great therapeutic and pharmacological potential contributed by its antioxidant, antirheumatic, carminative, antiflatulant, diuretic, hepatoprotective, antimicrobial, antifungal, antibacterial and many more similar properties. These properties make ajwain a potential source as natural food additive. The high content of antioxidants mainly polyphenols and flavonoids in ajwain makes it a probable source for developing nutraceuticals. Moreover, as it was seen through the results, methanol is found to be a better solvent for the extraction of polyphenols and flavonoids. This may be attributed to the higher polarity of methanol. Whereas, the total antioxidant activity was observed more with diethyl ether as solvent. So, further studies need to be conducted to know about the best solvent that may recover the most and all different kinds of antioxidants.

Conflicts of Interests

There are no conflicts of interest.

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