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Comparative Analysis of Grinding Methods for Health Promoting Phenolic Content, Flavonoids and Antioxidant Potential of Oil-Bearing Cumin Seeds

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ABSTRACT

Cumin is the major seed spice crop of India mainly grown in Rajasthan and Gujarat states. This is a low volume high value crop with very rich medicinal properties. Cumin is rarely exploited crop for advanced scientific research and for its medicinal potential. Antioxidant potential of cumin powder with low temperature grinding and machine grinding has been compared in this study. It is analyzed that cumin powder grinded in liquid nitrogen (low temperature) is having high flavonoid content (0.032 mg/g), high phenolic content (1.02 mg/g), more antioxidant potential (1.12 mg/g), higher of DPPH (97.07%), reduction in the reducing power (0.091), as compared to the machine grinded powder. Results indicated that the low temperature grinding is better technology to maintain the antioxidant potential of the cumin seeds in powder form.

Keywords: Antioxidant, *Cuminum cyminum*, DPPH, Essential oil, Flavinoids

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INTRODUCTION

Cumin (*Cuminum cyminum* L.) is an important socioeconomic low volume high value crop cultivated by farmers in India's arid and semi-arid regions. The crop is one among the important members of Apiaceae family and is herbaceous, dicotyledonous annual plant, diploid. Cumin is a Mediterranean native that is currently grown in a variety of nations, including India, China, Pakistan, Iran, Iraq, Turkey, Syria, and Morocco. In India it is mainly cultivated in arid and semi-arid parts of Rajasthan and Gujarat. The total area under cumin is around ~12.4 lakh hectare with the production of ~8.5 lakh tons. Cumin is a dollar earning crop and India is earning foreign exchange of ~Rs 4200 crore from cumin export during 2020-21. Cumin has been used as food and medicine since humanity (Nadeem and Riaz, 2012). Cumin seeds are very commonly used to give flavor and texture to the food. In addition, it provides nutritional, and health benefits, and is a natural food preservative (Esmaeili, 2015). Volatile oils present in the cumin seeds are responsible for its typical aroma which tastes like warm and spicy with the slight citrus flavor dominated by the principal constituent of which is cuminaldehyde (Sowbhagya, 2013). Normally 3–4% essential oil present in cumin seed and the most significant chemical component of cumin volatile oil is cuminaldehyde, which accounts around 45–50%. Cumin seeds contain an aromatic alcohol (animol) which is responsible for its typical pleasant aroma and spicy taste. Besides the taste and aroma, cumin is equally important for its medicinal and pharmaceuticals properties (Abbdellaoui *et al.* 2019). It is one of the important spices used in India's kitchen. Its use also mentioned in the Roman cuisines in ancient times. The food sector is particularly interested in the antioxidant activity of extract from a number of oil-bearing plants. Cumin utilized to manipulate secondary agriculture technologies to switch out synthetic preservatives with natural ones due to their future application in natural additives. Cumin seed as a whole, its value added and secondary products are employed in food, pharmaceutical, and perfumery industries and products, like cumin oil and oleoresin are exported to many countries. Raw and roasted cumin powder is used in spice blending and curry powders (Jirarattanarangsrin and Muangrat, 2022).

Cumin seeds having several medicinal properties and have been using in the treatment of nervous and immune system, reproductive, gastrointestinal, antioxidant, antimicrobial and chemoprotective activity. The presence of cuminaldehyd, β-pinene, γ-terpinene etc in the cumin essential oil (EO) responsible for the medicinal properties (Paul and Bhattacharjee, 2018). Essential oil constituents and antioxidant potential of crop extracts also studied by several researchers in different oil-bearing crops (Choudhary *et al.* 2017). Antioxidants are the 'free radical scavengers' that inhibit the action and incapacitate and prohibit the free radicals from causing harm. For optimum metabolic activity, an appropriate balance of antioxidants and oxidative stress is indispensable. lack of balance can lead to oxidative stress. Such balances can be accomplished by using the various forms of drugs and additional additives that can sometimes cause adverse effects and other medical conditions. For such antioxidants, natural sources are the safest option for a balanced life. This can be accomplished by conventional cooking methods and the use of some widely accessible spices

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Table 1: Total phenolic content (TPC) and flavonoids content (TFC) in aqueous extract of *Cuminum cyminum*

Method of grinding	Total phenolic content (TPC) mg/g	Total flavonoids content (TFC) mg/g
Liquid nitrogen	1.023	0.032
Mixer-grinder	0.968	0.029

such as cumin, ajwain, coriander, fenugreek, turmeric etc. Cumin seeds are a very excellent source of antioxidant potential and possess various antioxidant, carminative and anti-flatulent phytochemicals (Norman, 1990). The seeds of cumin possess phenolic flavonoid antioxidants such as carotenes, lutein and zea-xanthin are important source of dietary fiber. Cumin is used in Ayurveda for its medicinal properties like stimulant, tonic, arthritis and antispasmodic (Jayaprakasha *et al.* 2001).

In India traditional and old aged method which still has some uniqueness that is the use of *sil batta* (mortar and pestle) or say the old age method of crushing/grinding spices and other medicinal preparation. In this method water is used frequently to reduce the heat generated in the grinding process so in this method all the essence of spices retains as temperature was not rises. Now this technology is replacing by the machine operated grinding technology. Where temperature increases in the grinding process and causes loss of several antioxidants' properties of spices. So, a new technology has been developed in which liquid nitrogen is used to reduce the heat generated by grinding process. Nonetheless, several researchers have done studies on grinding temperature in seed spices, but there have been very few reports accessible comparing the antioxidants properties and their ability. Cumin is a key edible Indian seed spice and grinding temperature impact despite having great pharmaceutical and antioxidant activities. This study used a number of test methodologies to evaluate and quantify the phytochemical and antioxidant properties of methanolic cumin extracts. The current study shows that antioxidants in cumin have a beneficial impact due to their active metabolite and or occurrence of free radical scavenging activity and phytochemicals viz., nitric oxide assay, superoxide, DPPH activity and H_2O_2 tests.

MATERIALS AND METHODS

Sample preparation

Cumin seeds were ground to a fine powder in two batches: one in liquid nitrogen (100 mg each), the other in a mixer-grinder, and a 100mg sample from each was extracted with methanol. The supernatant was collected and kept at -20°C for future use. The experiment was performed in triplicate.

Chemicals and reagents

Aluminium nitrate, ammonium molybdate, 2,2-diphenyl-1-picrylhydrazyl (DPPH), ethanol, ferric chloride, ferrozine and $FeCl_2$, solin-Ciocalteu's reagent, methanol, η-butanol, phosphate, potassium ferricyanide, sodium carbonate, sodium nitrite, sulphuric acid, sodium Trichloroacetic acid (purchased from VWR chemicals, USA).

Estimation of phenolic and flavonoid content

The Jayaprakasha *et al.* (2001) approach was used to determine the total phenolic content of cumin methanolic extracts, with some minor alterations. A 100 µl aliquot of methanol extract was mixed with 500 µl of diluted Folin-Ciocalteu reagent and 400 µl of 7.5 percent (w/v) sodium carbonate. At 760 nm, the absorbance of the reaction mixture was measured after it had been incubated for 30 minutes at room temperature (25°C). The phenolic content was then measured as tannic acid equivalents expressed as mg/g.

Using a modified spectrophotometric approach, the amount of flavonoids was determined (Basu *et al.* 2012). A fine powdered 500 mg portion of each cumin sample was extracted three times with 10ml of n-butanol. All of the extracts were combined and vacuum-condensed at 60 °C. The obtained precipitate was re-suspended in 60% (v/v) of 5ml ethanol and the suspension was washed with 5ml of 30% (v/v) ethanol, and filtered twice. With 30% (v/v) ethanol, the filtrate was diluted to 25mL. A 100 µl aliquot of the extract was blended with 30% (v/v) ethanol (900 µl) and 5% (w/v) sodium nitrite (60 µl), followed by aluminium nitrate of the volume 60 µl of 10% (w/v). After 6 minutes, the reaction was stopped by adding 2 mL of 1 M NaOH. At 510 nm, the combination mixture's absorbance was promptly measured, and the flavonoid concentration was calculated and shown as usual counterparts.

Total antioxidant capacity and DPPH radical scavenging activity

The most used method for assessing the antioxidant capacity of methanolic extract is 2,2-diphenly-2-picrylhydrazyl (DPPH) radical scavenging. The bleaching of a purple-colored methanolic solution of DPPH was used to calculate the samples' radical scavenging properties (Abbdellaoui *et al.* 2019). A 100 µl methanol extract was added to a 1ml DPPH methanol solution of 0.004 percent (v/v) incubated in dark condition for 30 minutes. A 100 µl methanol extract was added to a 1ml DPPH methanol solution of 0.004 percent (v/v) incubated for 30 min in the dark. After 30 minutes of incubation, the absorbance was measured at 517 nm. The non-methanolic extract reaction combination is referred to as the control in this situation.

$[(A_{Control}-A_{Sample})/A_{Control}] \times 100$,

where, A indicates the absorbance.

Estimation of reducing power

Methanol extract (100 µl) was diluted with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 1 percent (v/v) potassium ferricyanide and the reaction mixture was incubated at 50°C for 20 minutes' duration. After incubation, the reaction mixture was centrifuged for five minutes at 8000 rpm with 2.5 ml of 10% trichloroacetic acid (TCA). The aqueous layer of the solution (2.5 ml) was blended with 2.5 ml of distilled water and 0.5 ml of ferric chloride 0.1% (w/v) before being evaluated for absorbance at 700 nm. The greater the absorbance of particular methanolic extracts, the higher the power loss.

Measurement of Fe (II) chelating activity

The Carter system (Carter, 1971) was being used to measure the Fe (II) chelating operation. Methanolic extract (50 µl) was added in to 100 µl of 2 mM FeCl2, 200 µl of 5 mM ferrozine, and thoroughly mixed. The mixed reaction mixture was maintained at 25°C for 10 minutes and the absorbance was noted at 562 nm. The percentage of chelating activity Fe(II) was measured as using the below formula.

$[(A_{Control}-A_{Sample})/A_{Control}] \times 100$

where A represents the absorption and the chelating activity

was expressed as µg of EDTA equivalents g^{-1} of defeated content using a standard curve drawn with EDTA. The non-methanol extract reaction mixture was represented as control.

Estimation of the conjugated diene formation inhibition in linoleic acid suspension

A 100 µl volume of methanol extract was added to a 500 µl linoleic acid emulsion (10mM) with a pH 6.6 and the reaction mixture was incubated at 37°C for 15 hours while shaking. After complete incubation, 100 µl of the solution was added to 1.75 ml of 80 percent (v /v) methanol, and the conjugated diene production was calculated with help of formula (Basu *et al.* 2012) and it was expressed in per cent :

$[(A_{Control}-A_{Sample})/A_{Control}] \times 100$.

Where, #the reaction mixture without the methanolic extract serves as the control, and A stands for absorbance.

Measurement of H_2O_2 scavenging activity

Methanol extract (100µl) was applied to 0.1 M phosphate buffer of pH 7.4 at 750µl in combination with 43mM H_2O_2 solution (prepared in phosphate buffer) at 150µl for H_2O_2 scavenging activity testing. The absorbance value was reported twice after 10min and 40 min incubation and a standard curve was used to calculate the concentration of H_2O_2 in the assay mixture. For each time point for context subtraction a separate blank (devoid of H_2O_2) has been used.

STATISTICAL ANALYSIS

All the analyses were performed in triplicates and the findings of the experiment were represented as means ± SE (n=3). The significance of the statistics was tested using the Microsoft Excel program using the two-sided Student's t-test at P≤0.05.

RESULTS AND DISCUSSION

Total phenolic and flavonoids content

Polyphenolic compounds are efficient hydroxyl and peroxyl radical scavengers, and can also stabilize lipid peroxidation of the membrane. The results of table 1 show that cumin extracts are enriched source of phenols and flavonois. Total phenolic content was higher in cumin powder (1.02 mg/g) grinded in liquid nitrogen and 0.96 mg/g in machine grounded cumin powder. Cumin when grounded in liquid nitrogen showed higher flavonoid content (0.032 mg/g) which was reduced to 0.029 mg/g when grounded in machine (Table 1). Previously, flavonoids, polyphenols and anthocyanins have been shown to contribute significantly to the antioxidant properties due to the existence of conjugated electron systems (Basu *et al.* 2012). In another report, the total phenolic concentration was assessed with the GCMS in cumin variety RZ-209 (101.58 mg GAE g^{-1}) and while GC-4 (109.09 mg GAE/g) seed (Khan *et al.* 2017) estimated the total phenolic content 25.52-40.0 mg GAE/g seed in cumin which was lower as related to results reported in our experiment. The result indicated that crop variety, stage of harvesting and grinding methods played important role in the phenolic and flavonoids content in the cumin crop.

Total antioxidant capacity and DPPH radical scavenging activity

Cumin seed extract's antioxidant content and antioxidant activity were assessed *in vitro* by testing how well it could scavenge DPPH free radicals. The results shown in figure 1 show that both the extracts had a strong antioxidant capacity and the cumin seeds grinded at low temperature (liquid nitrogen) showed more antioxidant potential (1.12 mg/g) whereas low (0.48 mg/g) potential was recorded in machine grounded cumin seeds (Fig.1A). The cumin powder macerated with the aid of liquid nitrogen has shown higher of DPPH radical scavenging activity (97.07%) which is 2.5 fold higher than machine grounded powder (37.8%) (Fig. 1B).

In recent years, many methods have been developed to test antioxidant activity to assess the effectiveness of naturally occurring antioxidants as pure composites or plant extracts. Among those methods of study, the DPPH is a neutral free radical that is most commonly used to test antioxidant potential of plant extracts. Further this compound will transition to a neutral diamagnetic fragment when acquiescent an electron or radical hydrogen. The results are comparable with earlier studies by Abbdellaoui *et al.* (2019) wherein a high antioxidant activity was found in cumin, based on the tests of DPPH (IC50 = 3.32 mg/mL) scavenging ability and compounds like -Terpinene and cuminaldehyde are main reason for the antioxidant potential of the cumin seed. Further, both the DPPH and BHT assays revealed moderate antioxidant activity for cumin seed essential oils (Karik *et al.* 2021). In our studies higher level of total antioxidant capacity and DPPH radical scavenging activity was recorded in the seed grinded in the liquid nitrogen which confers by the finding of the other researchers. A higher scavenging activity depicted by the extract collected from liquid nitrogen is would be due to lower thermal degradation of the metabolites in the

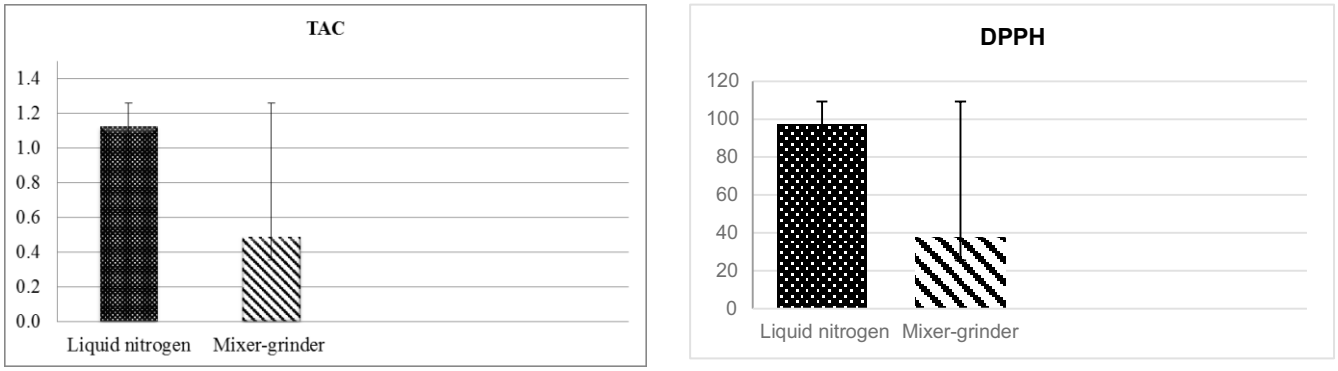
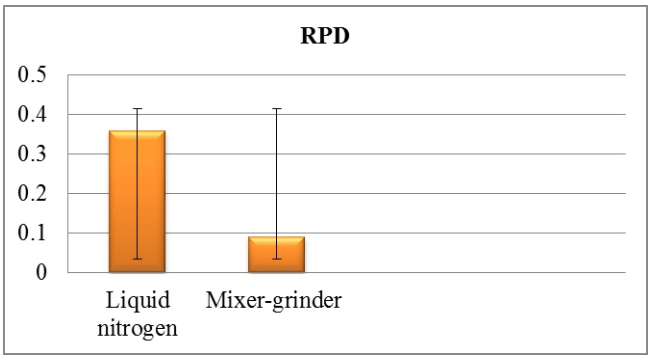
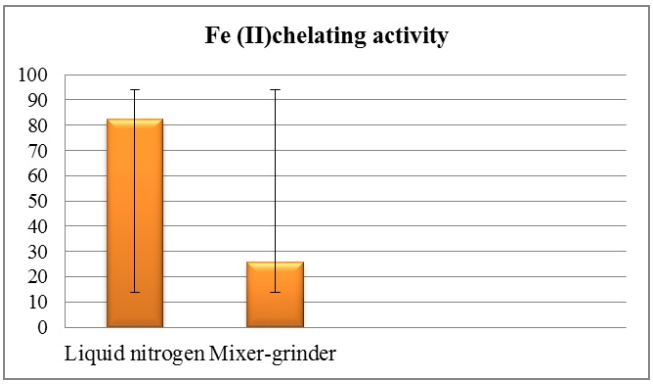


Fig. 1: Total Antioxidant content and DPPH free radical scavenging activity in cumin



(A) Reducing Power Scavenging Assay in cumin



(B) Fe (II) chelation Scavenging Assay in cumin

Fig. 2: Reducing Power Scavenging Assay and Fe (II) chelation Scavenging Assay

seeds of cumin.

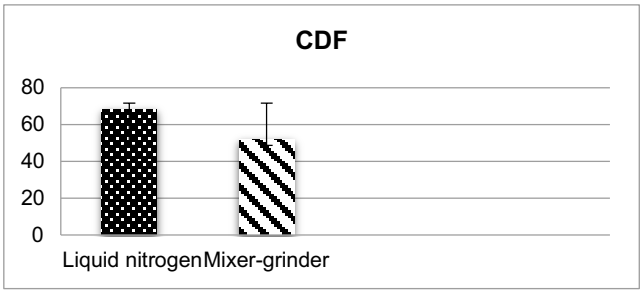
Reducing power and Fe (II) chelation scavenging assay

Reducing power is associated with antioxidant activity and is a crucial measure of antioxidant activity. The extract generated by grinding cumin seeds in a liquid (0.36) has a considerable reduction in reducing power when compared to mixer grinder (0.091) (four times) (Fig. 2A). the Neutralizing and the reducing the level of Fe (II) may be useful in eliminating the oxidative mutilation caused by ROS and enhancing the degree of antioxidant action by inhibiting the catalyzed reaction in metal. Hence the degree of ferrous sulphate chelation by the cumin seeds methanolic extract was analyzed. Fe (II) chelating activity in the cryo-ground cumin was 82.3% which was significantly reduced in machine ground samples to 25.6% (Fig. 2B). In addition to acting as main and secondary antioxidants, reducing substances demonstrate that they are electron donors by reducing the oxidation intermediates of the lipid peroxidation process. The results of reducing power of plant extracts are ascribed due to the presence of reductones that exhibits antioxidant activity by cleaving free radical chain by contributing the of hydrogen atom. In a study by Hajlaoui *et al.* (2010) has reported that the cimun essential oil has significant potential as a reducing agent and they have reported reducing power of EC50: 11 µg/ml essential oil. The antioxidant qualities (DPPH radical-scavenging activity, reducing power of seed extract or essential oil) are extremely important for the food business in order to uncover potential alternatives to synthetic preservatives (namely BHT). Cumin extract has produced intriguing results in this context, being one of the most promising extracts in terms of ability to neutralise free radicals and prevent unsaturated fatty acid oxidation.

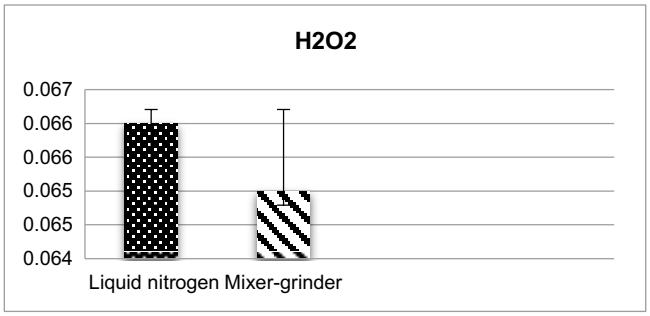
Cells are damaged by lipid peroxidation caused by the oxidative stress produced by superoxide anions, which also produces singlet oxygen and powerful, harmful hydroxyl radicals (Basu *et al.* 2012). The extract had very strong nitric oxide-scavenging activity (IC50= 309 14 g/ml), and antioxidant activity was revealed to be dose-dependent, indicating that activity increased as the concentration elevated. The extract chelated iron ions extremely poorly (IC50 = 1.3 0.07 mg/ml) yet very well in hydrogen peroxide scavenging (Ebrahimzadeh *et al.* 2009). Spices have long been used as food preservatives and health remedies, but they have recently gained prominence as bio nutrients, both as functional food additives and nutritional supplements. The health-promoting activity of spices will improve people's lifestyles and well-being.

Conjugated diene inhibition and H_2O_2 radical scavenging activities

Conjugated dienes are often used to evaluate primary oxidation products, and the capacity of herbal extracts to limit the synthesis of conjugated dienes determines their antioxidant potential. An indication for prooxidant formation may be increased inhibition of conjugated diene formation. The present experiments on cumin seeds ground in liquid nitrogen and mixer grinder showed H_2O_2 scavenging potential of 0.066% and 0.065% respectively (Fig 3B). Same results were obtained for inhibition of conjugated dienes with 68.2 and 52.03 values. Oxidative stress is primarily caused by the ROS accumulation (Fig. 3A). In the present study we used



(A) Inhibition of conjugated dienes in Cumin



(B) H2O2 radical scavenging in Cumin

Fig. 3: Inhibition of conjugated dienes and H2O2 radical scavenging activity in Cumin

cumin extract to determine the ability to inhibit formation of conjugated dienes. The most prevalent kind of ROS produced by mitochondria is cytotoxic H_2O_2 which can alter the structure or function of proteins. As a result, the issue of radical scavenging peroxide is crucial. Improved phenolic content can be due to the ability to scavenge peroxide, which can donate electrons and thereby neutralize it to water (Ebrahimzadeh *et al*., 2009). Cumin extracts yielded intriguing results in this context, being one of the most promising extracts in terms of ability to neutralize free radicals and prevent oxidative stress. The findings provided here may also contribute to our understanding of antioxidative and antiproliferative properties.

CONCLUSIONS

The higher proclivity for antioxidants could be attributed to material buildup, which includes vitamins, flavonoids, terpenoids, carotenoids, phytoestrogens, minerals, and so on. In this work, a linear link between the phenolic compound production and the antioxidant flavonoids in a cumin crop, a commercially significant spice and medicinal plant was observed. We discovered that when cumin was ground at a low temperature, it had the maximum phenolic and flavonoid concentration as compared to machine grinding, which caused antioxidant loss in the cumin. It can well be inferred, in the light of the current observation, that antioxidant properties associated with cumin may be a rich source of therapeutic purposes when grinded in low temperature then machine grinding. Overall, our findings indicate the spice's appealing potential as an excellent source of naturally occurring bioactive metabolites, as well as its critical role in preventing oxidative damage.

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Conflicts of Interest: The authors state that they have no conflicts of interest.

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