



## EXTRACTION OF ESSENTIAL OIL FROM AJWAIN SEED BY USING SOLVENT EXTRACTION METHOD AND ITS UTILIZATION IN VALUE ADDED BAKERY PRODUCT

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### ABSTRACT

In the present study, nutritional composition of ajwain seed, bioactive components of different solvent extracted ajwain essential oil and nutritional composition, textural attributes and sensory properties of ajwain essential oil fortified cookies were investigated. Ajwain seed has shown to have higher amount of carbohydrate (55.38%), fat (10.08%) and protein (15.73%) and also significant ash content (7%) and crude fibre content (18.98%). Furthermore, it contains potential functional qualities, including TFC (2.0608 mg quercetin/gm), TPC (126.021 mg GAE/100 gm), and antioxidant content of 36.36 g/ml. Essential oil extracted by using solvent extraction method with the application of different solvents and its combination was analysed in terms of yield and characterization of bioactive constituents using GC-MS technique. Maximum yield was found in combination of n-hexane and petroleum ether (3.2%). Thymol was found as dominant bioactive constituents both by using petroleum ether (75.831%) and combination of n-hexane and petroleum ether (71.372%) whereas p-cymene showed the least by using combination of combination of n-hexane and petroleum ether (1.602%). Nutritional constituents in terms of carbohydrate (51.89%), fat (31.004%), protein (7.14%), crude fibre (3.14%), Ash (2.59%) and moisture content (4.23%) of 1 ml essential oil fortified ajwainjeera cookies were found as satisfactory. Moreover, fortified cookie was shown best sensory acceptability using 9-point hedonic rating test. Texture profile analysis of essential oil fortified cookie results satisfactory values. Experimental investigation revealed that antimicrobial potential of the essential oil and essential oil fortified cookie against *Staphylococcus aureus*, *Bacillus cereus*, and *E. coli* were highly satisfactory. (If we are reducing word count then some of part missed in abstract.)

## 1. Introduction

One of the herb and spice plants in this family is *Trachyspermum ammi* L. which is generally known as ajwain. Ajwain is an annual herbaceous plant that is widely distributed and harvested in Afghanistan, Egypt, India, and European countries. Ajwain seeds are oval in form, yellowish-brown to greyish in color, and wrinkled (Gaba et al., 2019). Ajwain seed is bitter and pungent, and it also contains

anthelmintic, carminative, digestive, and anti-ulcer effects (Bairwa et al., 2012). One of the most active and beneficial components of medicinal plants that may be isolated from various parts is essential oil. The herbal cure ajwain is often used to treat a wide range of illnesses in both people and animals. chemicals that are antibacterial and antifungal. Moreover, it contains a significant quantity of fat, protein, ash, and carbohydrates. Many phytochemicals,

including alkaloids, chalcones, coumarins, flavonoids, glycosides, saponins, steroids, and tannins, have been shown to be present in ajwain (Shahrajabian et al., 2021). Due to its distinctive odor and bitter flavor, ajwain is used as a seasoning in curries. Its seeds are used as food flavorings and preservatives (Awais Hanif et al., 2021). Ajwain is a well-liked spice that may also be used in medical practices to treat febrile illnesses, dyspepsia, stomach issues, amoebic ailments, and inflammatory disorders (Chahal et al., 2017). One of the most powerful and potent elements of medicinal plants that may be isolated from various sections is essential oil. These mostly oil-soluble compounds come from major components including leaves, seeds, and bark (Zarshenas et al., 2014). EOs are very complex mixtures that may include hundreds of distinct aromatic elements. Even though they are not technically oils, essential oils can have a poor water solubility comparable to oils (Mahian & Sani, 2016). Many biological activities, such as antimutagenic, antibacterial, antimicrobial and antioxidant are present in ajwain essential oils (Chahal et al., 2017).

## 2. Materials and methods

### 2.1. Materials source and preparation

Fresh Ajwain seeds (*Trachyspermum ammi* L.) were collected from a local farm in Timbi village, Bhavnagar, Gujarat. For further examination, the seeds were air dried for 8 days before being placed in a plastic bag with an airtight seal.

### 2.2. Determination of Proximate Composition

The sample's Ash, fat, crude fiber, and Moisture content have been evaluated using (AOAC 2000). For the determination of ash content, we take a 1 gm sample and put it in a muffle furnace. This was done by ashing at 550<sup>o</sup> C for 3 hours. The crude protein content has been identified using the Kjeldahl technique. A standard (AOAC 978.10) approach was used to determine the amount of crude fiber. Model FES04E crude fiber analysis equipment was

used to find crude fiber. The Soxhlet extraction technique was used to evaluate the crude fat content. The difference between the total of all the proximate components and 100% was used to calculate the amount of total soluble carbohydrates.

### 2.3. Determination of TPC, TFC and Antioxidant activity

The total phenolic content in the ajwain seed was determined by using the Folin-Ciocalteu reagent method, as described by (Wanyo et al., 2014). Ajwain powder was dissolved in methanol at a concentration of 1 mg/ml (w/v) by adding 0.010 g of the powder to 10 ml of methanol. Add 9.6 ml of distilled water to 0.4 ml of an ajwain extract solution in a test tube. 2.5 ml of Folin-Ciocalteu's reagent (10%) was then added. 2 ml of Na<sub>2</sub>CO<sub>3</sub> (7.5% w/v) was added after the reaction had been going for 5 minutes. Three duplicates of the test samples were created. After that, it was left to develop for 30 minutes without light. Finally, a UV-VIS spectrophotometer was used to assess the absorbance of test samples at 730 nm.

Using the aluminium chloride method, the total flavonoid content of the Ajwain seed extracts was determined. (Khatiwora et al., 2010). 6 ml of several methanolic extracts were poured into the various test tubes to determine the total flavonoid concentration. 0.2 mL of correctly mixed 10% AlCl<sub>3</sub> should then be added. A further addition of 0.2 ml of 5% Na-K tartrate solution was made. The last addition was 5.6 mL of distilled water. After 30 minutes of incubation, the solution was well mixed, and the absorbance at 415 nm was measured using a UV-V spectrophotometer. Using the quercetin calibration curve, the quantities of quercetin in the test samples were determined and expressed as mg of quercetin equivalent per g of sample. The method provided was used to determine the antioxidant activities. (Saikia et al., 2016). The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical's ability to scavenge free radicals was used to assess the antioxidant activity of ajwain extracts. First, 4 mg of DPPH were dissolved in 100 ml

of methanol to create a DPPH solution. Test tubes were filled with a 0.4-ml solution of the methanolic extract. The mixture included 5.6 ml of DPPH solution. 30 minutes were given for the mixture to remain at room temperature after it had been mixed and kept in the dark. Using a spectrophotometer, the solution's absorbance at 515 nm was determined.

#### 2.4. Evaluation of Antimicrobial Activity

Antibacterial properties in the isolates are allowed to permeate into the medium and interact in a plate freshly spread with the test organisms. If the test organisms are sensitive, it will result in the production of circular zones of inhibition (Murugan et al., 2018). The (MTCC 7190), *Escherichia coli* (MTCC 443) and *staphylococcus aureus* (MTCC 7190) strains were then serially diluted to  $10^{-2}$ , and 0.2ml *E. Coli*, *bacillus cereus* and *S. Aureus* were dispersed separately on nutrient agar (himedia) plates. Then, drill a well and pour ajwain essential oil and cookies methanolic extract into it and using paraffin, seal the agar plates and place them in the incubator for 24-48 hours at 37°C.

#### 2.5. Essential oil extraction of Ajwain seed

For extracting essential oil from ajwain seed, we are using the Soxhlet extraction method. In this, we take an ajwain powered sample in a thimble and put it into a solvent chamber, and then we set the temperature according to the solvent boiling point for 5–6 hours. After extracting a sufficient amount of oil from the solvent, we can go for distillation to remove the solvent by using a rotary evaporator.

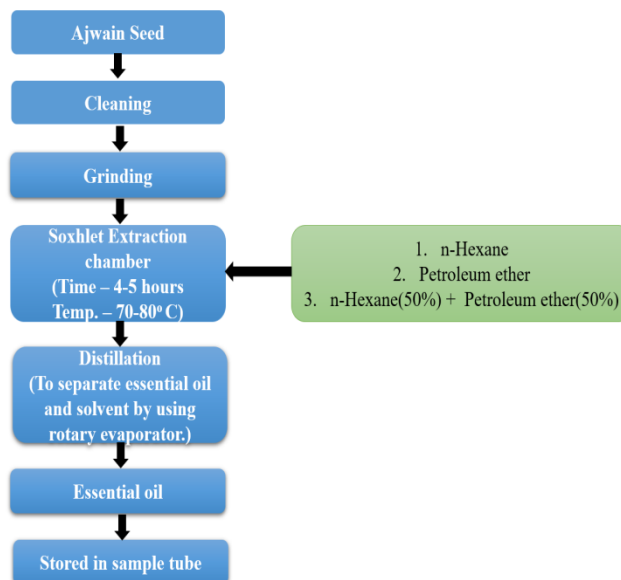


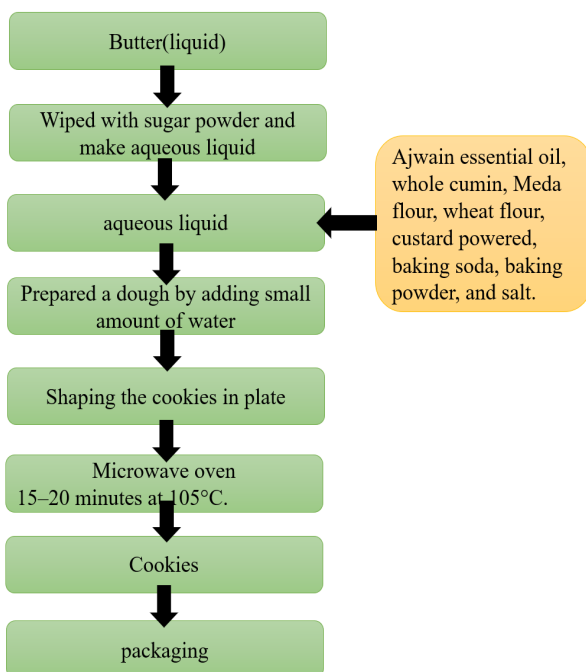
Figure 1. Essential extraction process.

#### 2.6. Characterization of bioactive constitute of ajwain essential by using GC-MS

The identification of different bioactive constituents of Ajwain essential oil that were extracted by different solvents and their combinations, like n-hexane, petroleum ether, and mixtures of both solvents, was carried out using GC (model Clarus 680 GC), equipped with a HP-5 capillary column (60 meter long, 250  $\mu$ m diameter), and a mass spectrometer (model Clarus 600 MS). The analysis was conducted with a helium carrier gas flow rate of 2 ml/min and an injector temp. of 280°C. The column temperature was initially 60°C for 1 min, then continuously increase to 200°C at 7°C/min, and finally increased to 300°C at 10°C/min. For GC-MS detection, an electron ionization system was used with an ionization energy of 50 to 600 Da.

#### 2.7. Development of ajwain essential oil fortified cookies.

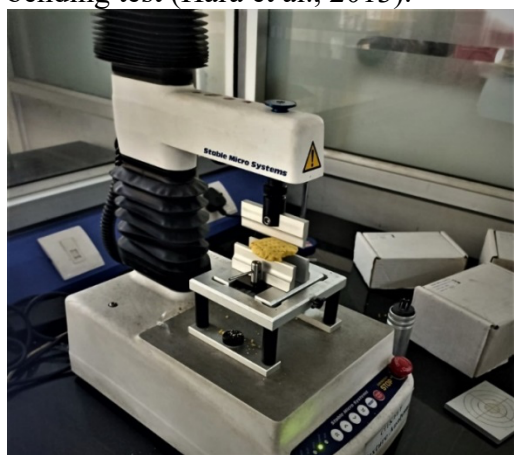
By applying essential oils to cookies in different quantities, ajwain essential oil fortified cookies were developed.



**Figure 2.** Preparation of development of ajwain essential oil fortified cookies.

### 2.7.1. Texture profile analysis and three-point bending test.

In order to determine the various textural attributes, such as hardness, chewiness, cohesiveness, gumminess, resilience, and springiness, a texture profile analysis (TPA) was used (Kek et al., 2013), and for fracturability measurement, we performed a three-point bending test (Hara et al., 2013).



**Figure 3.** Model TA.XT plus texture analyser

## 2.8. Sensory Evaluation

According to (Singh et al., 2018), the nine-point hedonic rating test was used to evaluate the sensory attributes of ajwain essential oil fortified cookies.

## 3. Results and discussions

### 3.1. Proximate composition of ajwain seed

The results of the proximate composition of Ajwain seed are shown on Table 1. Result showed that Ajwain seed is rich in carbohydrate (55.38%), fat (10.08%) and protein (15.73%). Carbohydrates are listed first, followed by protein, and then lipids, with a range of nutritional values from 42.76 to 15.73%. We obtained a greater proportion of fat (10.23%) and a lesser percentage of fat (4.3%), as determined by (Javed et al., 2012). Because of High percentage of fat in Ajwain seed we can easily extract essential oil.

**Table 1.** Proximate Analysis of Ajwain seed

Ajwain constituents	seed	Composition (%)
Carbohydrates		42.76±0.68
Protein		15.7325±0.87
Crude fibre		18.98±0.35
Fat		10.08±0.57
Moisture		5.44±0.40
Ash		7±0.27

All values are expressed as means ± SD. \*Significant ( $P \leq 0.05$ ).

### 3.2. Functional composition of ajwain seed.

Ajwain essential oil has a TPC, TFC, and DPPH radical-scavenging activity range at various concentrations of 14.29% to 56.17%, 6.02 to 16.52 mg GAE/g, and 0.04 to 3.89 mg QE/g, respectively, according to a research study (Chahal et al., 2017). The DPPH radical scavenging activity (total antioxidant activity) of ajwain seed was found as 36.36%. Occurrence of significant total phenolic content (TPC) and total flavonoid content (TFC) establishes its functional importance.

**Table 2.** Total phenolic, flavonoid and antioxidant content

Functional parameters	Result
TPC (Gallic acid equivalent $\mu\text{g/ml}$ )	126.0216 $\pm$ 0.46
TFC (mg Quercetin/ml)	2.0608 $\pm$ 0.32
Antioxidant activity (mg/ml)	36.36 $\pm$ 0.44

All values are expressed as means  $\pm$  SD. \*Significant ( $P \leq 0.05$ ).

### 3.3. Essential oil extraction yield with different solvent

As part of our investigation, we extract essential oils utilizing a variety of solvents and their mixtures. Our investigation guided us to the conclusion that solvents have an impact on the yield of bioactive compounds and extraction yield. The range of ajwain EO yields was within the ranges described in the literature(Chahal et

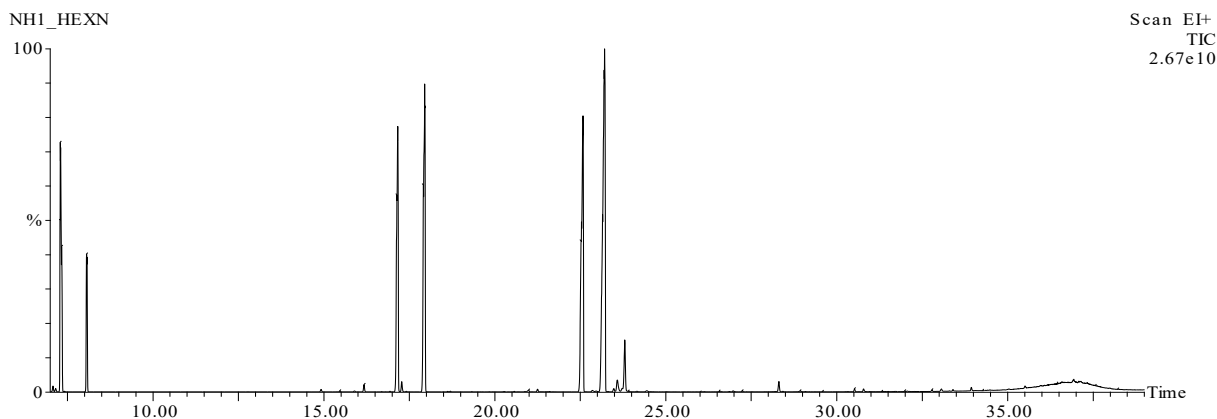
al., 2017), ranging from 1.5 to 3.4% (v/w). In terms of extraction yield, we found that using a combination of solvents provides highest extraction yield 3.2%. Additionally, there is a little variation in the extraction yield between petroleum ether and n-hexane.

**Table 3.** Extraction yield of ajwain E.O.

Solvent	Yield % (v/w).
n-Hexane	2.1 $\pm$ 0.64
Petroleum ether	2.5 $\pm$ 0.76
N-Hexane (50%) + Petroleum ether (50%)	3.2 $\pm$ 0.58

All values are expressed as means  $\pm$  SD. \*Significant ( $P \leq 0.05$ ).

### 3.4. GC-MS results



**Figure 4.** n-Hexane extracted essential oil GC-MS graph

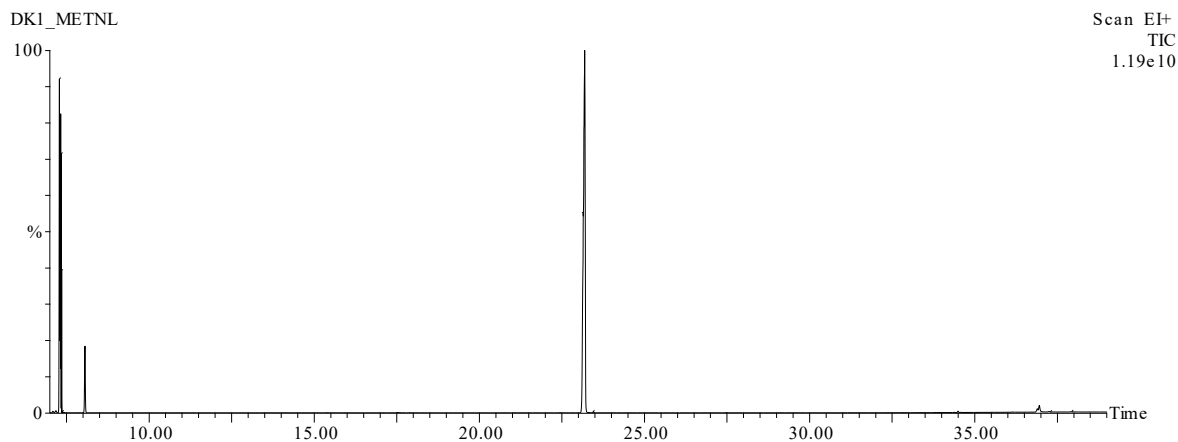


Figure 5. Petroleum ether extracted essential oil GC-MS graph.

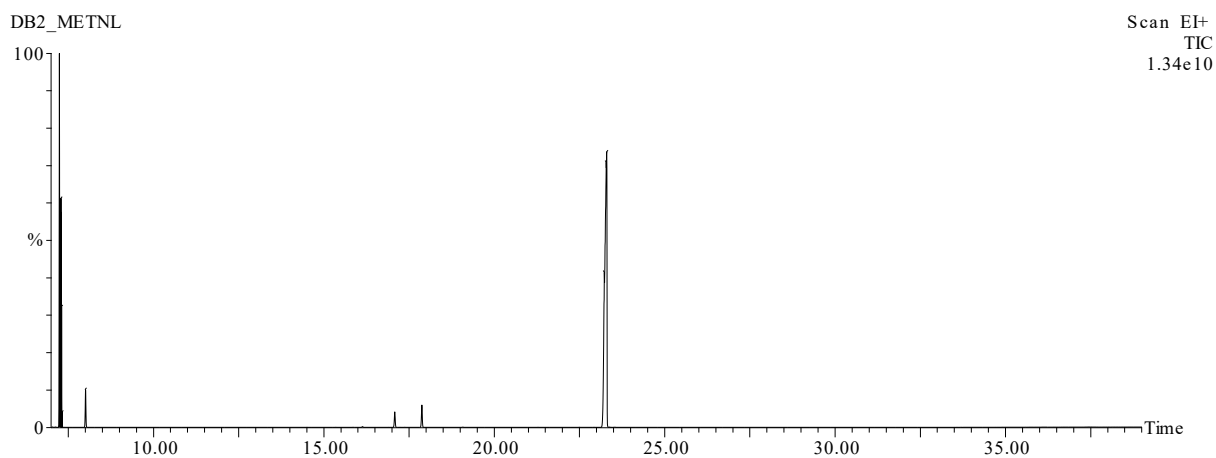


Figure 6. n-Hexane (50%) + Petroleum ether (50%) extracted essential oil GC-MS graph.

Table 4. GC-MS analysis of ajwain seed essential oil

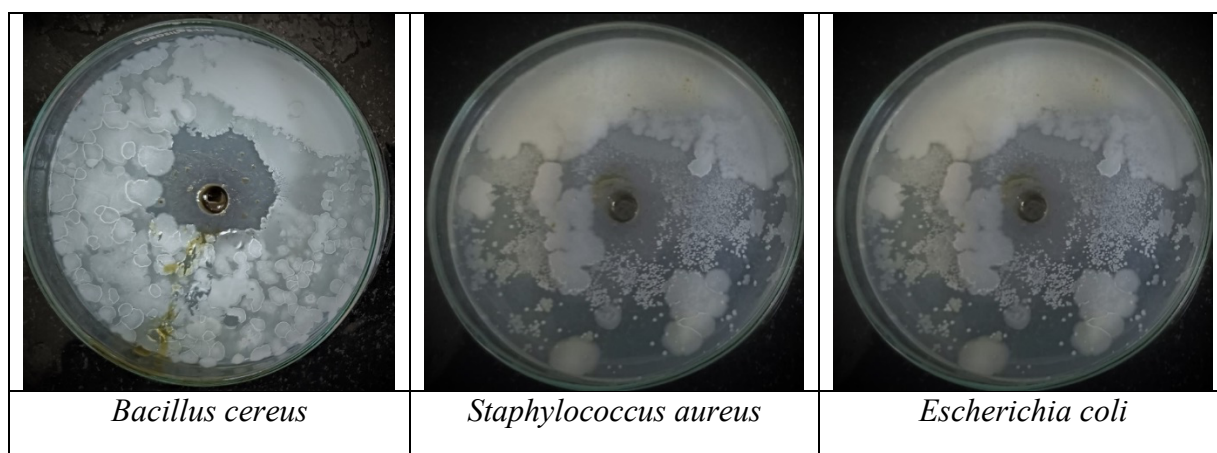
Solvent	N-Hexane	Petroleum ether	N-Hexane (50%) + Petroleum ether (50%)
Terpinene-4-ol	-	10.426%	16.535%
P-cymene	12.029%	-	1.602%
Gama-terpene	14.793%	-	2.389%
Thymol	23.092%	75.831%	71.372%
Cyclopentanone, 2-(1-methylpropyl)-	4.360%	-	4.037%
3-heptene, 2-methyl-, (e)-	-	4.133%	-
Methyl 9-eicosenoate	-	0.955%	-
9,12-hexadecadienoic acid, methyl ester	-	0.420%	-
Methyl 8-methyl-nonanoate	-	0.138%	-
3,4-dimethylbenzyl alcohol	5.363%	-	-

About bioactive substances Petroleum ether is more effective in extracting the major bioactive components thymol than a mixture of solvents. More of the terpinene-4-ol compound is extracted using a mixture of solvents compared to petroleum ether. Additionally, petroleum ether is used to extract several minor chemicals that cannot be extracted using a mixture of solvents, such as 3-Heptene, 2-Methyl-, (E)-, Methyl 9-Eicosenoate, 9,12-Hexadecadienoic Acid, Methyl Ester, and Methyl 8-Methyl-Nonanoate. as well as several bioactive compounds that can only be extracted using a mixture of solvents, such P-Cymene, Gama-Terpene, and Cyclopentanonea 2-(1-Methylpropyl). The major bioactive elements identified by (Chahal et al., 2016) GC-MS study of ajwain essential are p-cymene,  $\gamma$  -terpinene, and thymol, with corresponding percentages of amount of 6.18, 12.31, and 31.4. Using innovative GC-MS techniques, (Gaba et al., 2018) investigated the properties of *Trachyspermumammi* L. essential oil and observed that thymol constituted an even higher percentage, with minor amounts of other bioactive compounds such as  $\rho$ -cymene (14.16%),  $\beta$ -phellandrene (0.27%),  $\gamma$ -terpinene

(9.29%), and  $\alpha$ -terpineol (0.17%). Based on this study, we have concluded that the phytochemicals found in solvent extracts of ajwain essential oil are different based on the solvent used. These phytochemicals include flavonoids, phenols, tannins, and alkaloids.

### 3.4. Antimicrobial activity in ajwain essential oil

Antimicrobial activity of essential oil and essential oil fortified cookie were tested against both gram-positive and gram-negative bacteria e.g. *Bacillus cereus*, *staphylococcus aureus* and *Escherichia coli* and represented in table 13. Maximum zone of inhibition in essential oil was found in *bacillus cereus* (34.2mm). However, maximum zone of inhibition in essential oil fortified cookie was found in *staphylococcus aureus* (33.8mm). Therefore, it was concluded that antimicrobial potential in both these two cases were significant. In our investigation, we observed a significantly larger inhibition zone when using ajwain seed extract as compared to the findings reported by Bhatt et al. in 2018. Specifically, the inhibition zones for *S. aureus* and *E. coli* were measured at 19.02 mm and 16.29 mm, respectively.



**Figure 7.** Antimicrobial zone of ajwain essential oil



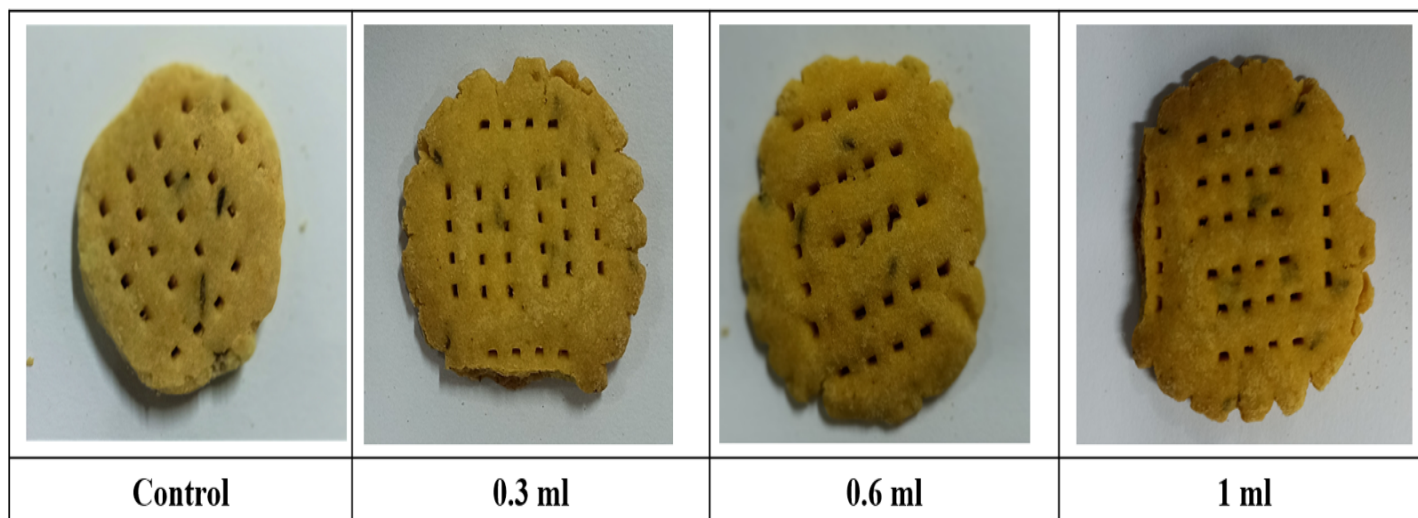
**Table 5.** Zone of inhibition against ajwain essential oil

Bacteria name	zone of Inhibition (mm)
<i>Bacillus cereus</i>	34.2 ±0.26
<i>staphylococcus aureus</i>	21.7 ±0.36
<i>Escherichia coli</i>	18.9 ±0.24

All values are

expressed as means ± SD. \*Significant ( $P \leq 0.05$ ).

### 3.4.Sensory analysis of ajwain essential oil fortified cookies.



**Figure 8.** Pictorial representation of control and ajwain essential oil fortified cookies.

Here we developed 4 sample of ajwainjeera cookies. 10 eligible judges from the Department of Food Engineering and Technology at the Central Institute of Technology, Kokrajhar, participated in the sensory evaluation of ajwainjeera cookies.

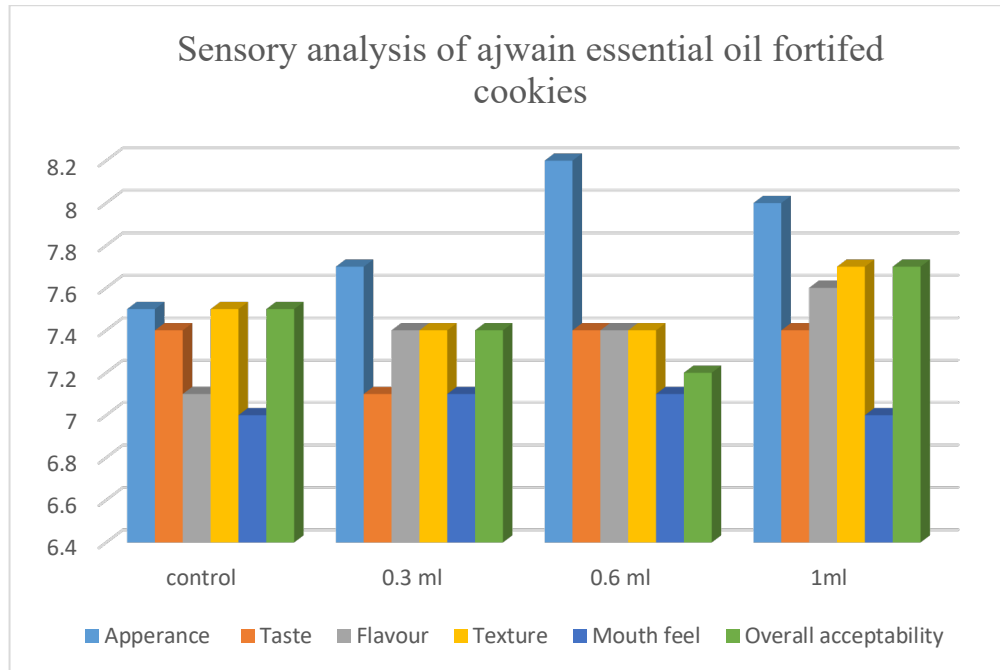
The judges were asked to score for the sensory attributes viz. appearance, taste, flavour, mouthfeel and overall acceptability, on a 9-point Hedonic scale. It may be concluded that ajwain essential oil could be effectively added to cookies, as shown by the fact that overall acceptability of sample 4(1ml essential oil

fortified cookies) was greater than that of other formulations.

The maximum mouth feel was produced by fortifying essential oil, according to a comparison of the control sample and 0.6 ml of fortified essential oil cookies.

Also, for 1 ml of essential oil fortification, we can find the highest flavor, texture, and overall acceptability. This is a novel food product; previously, there was no development on the basis of this concept, so there is not any sensory data for comparison.





**Figure 9.** Sensory graph of a ajwain essential oil fortified cookies.

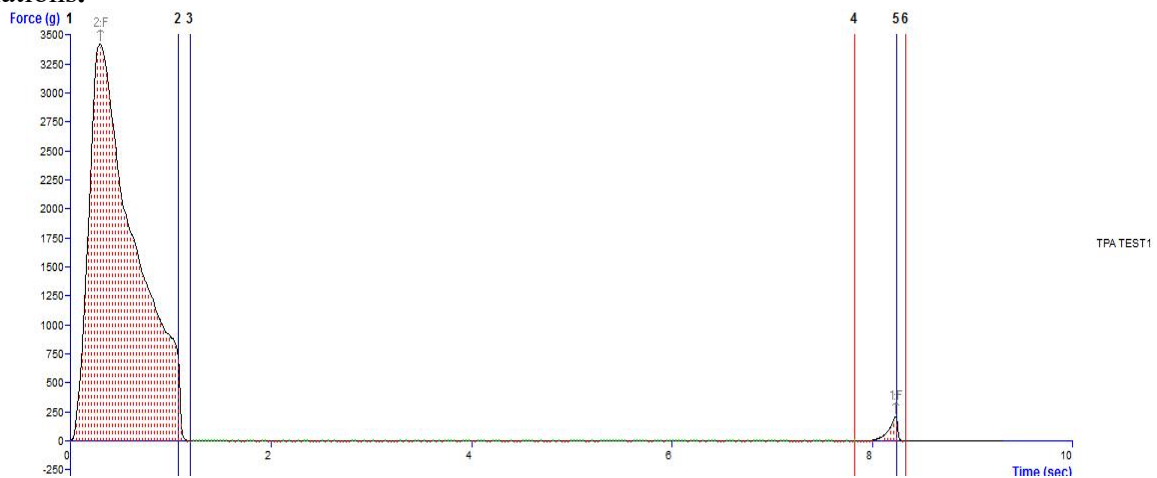
All values are expressed as means  $\pm$  SD. \*Significant ( $P \leq 0.05$ ).

Scores are based on 9- point hedonic scale like extremely, 9; like very much, 8; like moderately, 7; like; slightly, 6; neither like or dislike, 5; dislike slightly, 4; dislike moderately, 3; dislike very much, 2; dislike extremely, 1

### 3.5. Textural attributes of cookies

#### 3.5.1. Texture profile analysis

Textural attribute like hardness, gumminess and chewiness are satisfactory for 1 ml ajwain essential oil fortifies cookies than that of other formulations.



**Figure 10.** Graph of texture profile analysis of ajwain essential oil fortified cookie.

**Table 6.** Evaluation of textural attributes

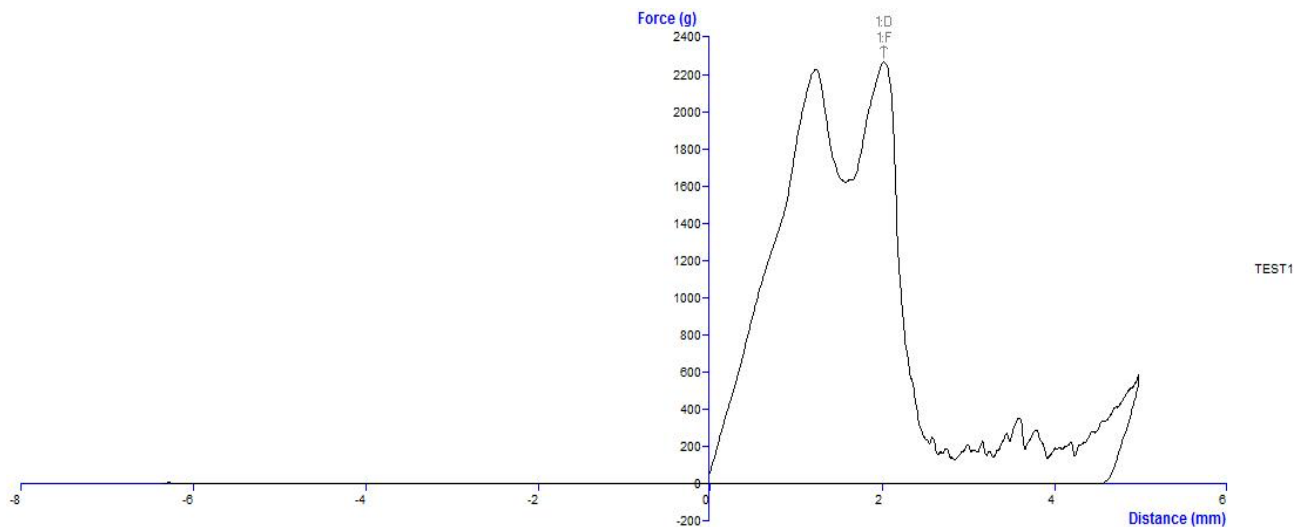
Sample name	Hardness (g)	Adhesiveness (g.sec)	Springiness	Cohesiveness	Gumminess	Chewiness	Resilience
cookie	3104.698 ±0.36	-0.034±0.02	0.223±0.26	0.009±0.06	30.454±0.68	7.009±0.14	0.005±0.03

All values are expressed as means ± SD. \*Significant (P ≤ 0.05).

**3.5.2. Three-point bending test**

Generally three point bending test was performed for the measurement of fracturability of product. Fracturability attribute of ajwain

essential oil fortified cookie was found satisfactory and acceptable to consumers.



**Figure 11.** Graph of three-point bending test of ajwain essential oil fortified cookie.

**Table 7.** Value of Three-point bending properties of fortified cookies

Sample name	Hardness (g)	Fracturability (mm)
cookie	2267.94±0.84	2.04±0.27

All values are expressed as means ± SD. \*Significant (P ≤ 0.05).

While comparing our cookie's texture parameters, we found that the previous study (Bawa et al., 2020) on cookie texture parameters had established an accepted level within the specified range. The hardness, fracturability, and cohesiveness of the cookie texture profile analysis were given in Tables 6 and 7.

For mouthfeel, the Ajwain Jeera cookie sample's three textural qualities are important. The results of the study include cohesiveness,

fracturability (mm), and hardness (g) of 0.009, 2.04, and 3104.698, respectively.

**3.6. Nutritional quality of ajwain essential oil fortified cookies**

Nutritional constituents of essential oil fortified cookies were evaluated and represented in table 8. Table 8 was shown the significant nutritional characteristics in terms of higher carbohydrate and fat content.

**Table 8.** Nutritional analysis of 1 ml essential fortified cookies

Cookies attributes	(%) composition
Ash	2.59±0.34
Carbohydrates	51.8926±0.76
Crude fiber	3.14±0.26
Fat	31.004±0.84
Moisture content	4.23±0.36
Protein	7.14±0.68

All values are expressed as means ± SD.

\*Significant ( $P \leq 0.05$ ).

#### 4. Conclusions

The significant nutritional and functional potentials of Ajwain seed has been established as a suitable and effective nutraceutical. The high level of antioxidants principally polyphenols and flavonoids in Ajwain seed makes it as an effective source for creating nutraceutical. In view of the above facts, essential oils derived from Ajwain seed offer a broad range of biological activities. High level of antioxidant activity of Ajwain essential oil has help to preserve the moist food product and also responsible for the value-added bakery product development. Additionally, we are confirmed that the solvent plays a crucial role in solvent extraction techniques since it affects both the extraction yield and the bioactive substance's functionality. Experimental investigation revealed that a combination of solvents that is petroleum ether and n-hexane result satisfactory yields of essential oil. People are becoming more health conscious every day, which will lead to a growth in the popularity of herbal or nutraceutical products. Therefore, the developed ajwain essential oil fortified herbal cookies give significant health benefits due to availability of potential nutritional, functional and bioactive constituents. Moreover, the satisfactory sensory attributes and textural properties of the ajwain essential oil fortified cookie establish its significant importance in fortified cookie.

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