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## SCREENING OF PHENOLIC CONTENT, ANTIOXIDANT AND *IN VITRO* EYE IRRITATION ACTIVITIES FROM *APIACEAE* FAMILY (DRY SEEDS) FOR POTENTIAL COSMETIC APPLICATIONS

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**ABSTRACT:** Cosmetic applications of spices and herbs are well recognized. Apart from preservative and antimicrobial activities, spices display copious antioxidant activity. Inclusion of these antioxidants in topical supplements has proved to be effective against oxidative aging. However, food and contact allergy to most spices make it mandatory to screen for skin and eye irritation activities before cosmetic applications. In this report, we have aimed to compare ethanolic extracts of five seeds belonging to family *Apiaceae* (*C. carvi*, *C. cyminum*, *C. sativum*, *T. ammi*, *A. graveolens*) concerning their antioxidant activities, phenol and flavonoid content, *in-vitro* cytotoxicity, and eye irritation tests. Our investigation illustrates that *C. carvi* expresses the highest antioxidant activity among these. Further, the polyphenol content and antioxidant activity are positively correlated in all samples. *In-vitro* cytotoxic analysis on chick embryo fibroblasts and *in-vitro* eye irritation assays demonstrate 200µg/ml concentration of these extracts can be safely used for cosmetic application.

**INTRODUCTION:** Medicinal plants have been used for the treatment of various human ailments since long. Various plants have also been used for the isolation of biologically active chemicals used in the development of novel drugs. There has been fervent interest in plant-based products because of their perceived safety and affordability. Moreover, crude extracts from plants are more effective than isolated bioactive due to their synergistic effects<sup>1-4</sup>. Spices are important constituents of cuisines around the world because they impart flavor, serve as preservatives, and have high antioxidant activities<sup>4,5</sup>.

The role of spices in health care and cosmetics is well documented. Some of their therapeutic applications include anti-microbial, anti-carcinogenic, analgesic, anti-inflammatory, and antioxidant properties. Cosmetic applications include skincare, oral care, hair solutions and shampoos, perfumes, and aromatherapy<sup>6-8</sup>.

The key phytochemicals responsible for their therapeutic and cosmetic applications are generally flavonoids and phenolic acids<sup>9</sup>. The antioxidant effects of polyphenols are mainly due to their redox properties, by stabilizing lipid peroxidation or inhibiting various oxidizing enzymes<sup>10</sup>. Evidence suggests that polyphenols protect cell constituents against oxidative damage & tissue deterioration in the body<sup>11, 12</sup>. Antioxidant supplementation to dermis and epidermis has shown to reduce visible signs of aging like wrinkles 13-16 dramatically. An increasing number of skincare products are now incorporating natural or synthetic antioxidant

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preparations<sup>13-18</sup>. Antioxidant supplementation has been used in several clinical conditions like Alzheimer's disease, Atherosclerosis, cardiovascular diseases, Cancer, nutritional deficiency, and diabetes<sup>11, 12</sup>.

Data shows that antioxidant supplementation improves health status and increases longevity, but it certainly does not reverse the effects of these diseases, because they do not arise due to nutritional deficiency<sup>19</sup>.

The *Apiaceae* or *Umbelliferae*, a popular spice family, consists of usually aromatic plants with hollow stems that are annual, biennial or perennial herbs. The defining characteristic of this family is the inflorescence: a simple or a compound umbel. The essential oils or oleoresin present in the plants imparts an aroma typical to this family<sup>20, 21</sup>. A lot of species from this family are used as spices, some of which are *Anethum graveolens* (dill), *Carum carvi* (caraway), *Coriandrum sativum* (coriander), *Cuminum cyminum* (cumin), *Pimpinella anisum* (anise), *Trachyspermum ammi* (ajwain), *Apium sativum* (celery), *Foeniculum vulgare* (fennel), *Ferula assafoetida* (asafoetida). Reports suggest the presence of high amounts of phenolic acids and flavonoids in these species and abundant quantities of antioxidant activity which is associated with these phytochemicals<sup>9</sup>.

We seek to discuss the potential applications of these spices in skin care, particularly as antioxidant supplements. However, a lot of spices are documented as food allergens and have also caused contact dermatitis<sup>22, 23</sup>. Allergies after ingestion of dill, anise, coriander, cumin, and fennel are well described<sup>24, 25</sup>. If spices and herbs are utilized in cosmetics, then every consumer and not just sensitized individual are at risk of contracting irritant contact dermatitis and allergic contact dermatitis. Therefore, assessment of eye and skin irritation is an important part of any comprehensive toxicology program for any chemical to be launched for topical use<sup>26</sup>.

For such reasons, we have assessed not only the antioxidant activity and polyphenol content but also *in-vitro* eye irritation and cytotoxicity evaluations. For our investigation, the following five seeds have been considered - *Anethum graveolens* (AG),

*Carum carvi* (CaC), *Coriandrum sativum* (CS), *Cuminum cyminum* (CuC), *Trachyspermum ammi* (TA).

## METHODS & MATERIALS:

### Materials:

**Plant Material:** All the seeds included in the study were obtained from a local herb store. The specimens were identified and authenticated. A voucher specimen of each has been kept in our laboratory for future reference.

**The Preparation of Extracts:** All the extracts were shade dried at room temperature for 7 days. 30 g of each seed samples were extracted with 300 mL of absolute ethanol using Soxhlet extractor at 60 °C. The extracts were then concentrated under reduced pressure in a rotary evaporator to dryness.

**Reagents and Instruments:** All reagents used were of analytical grade. Ethanol and Methanol were obtained from Merck. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) from Fluka, DMEM, Gallic Acid, Quercetin dihydrate, and Trypsin from Sigma, Fetal Bovine Serum from Biowest. The UV-Visible spectrophotometer used for all the assays was Varian Inc. - Cary 50 and the 96- well plate reader used was MRX Revelation- Thermo Labsystems.

### *In-vitro* Assays:

**DPPH Radical Scavenging Capacity Assay:** The 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) Assay was performed according to the method of Stankovic<sup>27</sup> with slight modifications. The stock solution was prepared by dissolving 24 mg DPPH with 100ml methanol and then stored at -20 °C until needed. The working solution was obtained by mixing the 1ml stock solution with 5 ml methanol. A 1:1 aliquot of this stock with methanol was measured at 517nm using a spectrophotometer such that the reading of control tube corresponded to 2.3 to 2.4 ± 0.02. 0.5ml of seed extract (250-5000 µg/ml) was added to 0.5 ml of DPPH working stock. After 30 min of incubation at room temperature, the absorbance was measured at 517nm using UV - Vis spectrophotometer. Corresponding color blanks were measured. The absorbance of DPPH with methanol measured at 517 nm was used as control. All the readings were taken in triplicates.

Percentage of radical scavenging was calculated as:

The scavenging effect (%) =  $[\text{O.D. of Control} - (\text{O.D. of sample}/\text{O.D. of control})] \times 100$

#### **Determination of Synergistic Action:**

Determination of synergistic effect on radical scavenging action of alcoholic extracts of CuC, CaC, AG, CS, and TA was carried out by DPPH assay. The final concentration of the extracts was maintained at 250, 500, and 1000 $\mu\text{g}/\text{ml}$ .

#### **Ferric Reducing Antioxidant Power (FRAP) Assay:**

Reducing power of the crude extract was determined using the method prescribed by Koksal 28 with slight modifications. 0.5ml of the sample prepared in phosphate buffer (0.2M, pH 6.6) was mixed with 0.5ml of potassium ferricyanide (1%) and incubated for 20 min at 50 °C. The tubes were cooled; 0.5ml of trichloroacetic acid was added and centrifuged at 3000 rpm for 10 min. 0.5ml of the supernatant from this was mixed in a 1:1 ratio with distilled water. 0.1ml of  $\text{FeCl}_3$  (0.1%) was added to each tube, and the absorbance was immediately measured at 700nm using a UV-Visible Spectrophotometer. Ascorbic acid was used as a standard, **Fig. 2**. The assay was performed thrice for statistical analysis.

**Phenol Estimation:** Total phenolic content of all the extracts was evaluated by Folin-Ciocalteu method<sup>2</sup>. 2.5 ml of 10% Folin-Ciocalteu reagent and 2 ml of  $\text{Na}_2\text{CO}_3$  (2% w/v) was added to 0.5 ml of each sample of plant extract solution. The tubes were incubated at 45 °C with intermittent shaking for 15 min. The absorbance of the samples was measured at 765 nm. Quantification was done based on a standard curve using Gallic acid (0-100 $\mu\text{g}/\text{ml}$ ) dissolved in water **Fig. 3**. Results were expressed as micrograms of Gallic acid equivalent (GAE) per mg of extract.

**Flavonoid Estimation:** Aluminum chloride colorimetric method was used for flavonoids determination<sup>2</sup>. 740  $\mu\text{l}$  of a sample of various concentrations diluted with methanol was mixed with 30 $\mu\text{l}$  of 10% Sodium Nitrite, mixed, and incubated at room temperature for 5 min. 30 $\mu\text{l}$  of aluminum chloride was added, and the tubes were again incubated in the dark at room temperature for 5 min. 200 $\mu\text{l}$  of 0.1mM NaOH was added to each tube and incubated again for 5 min. The absorbance

of the reaction mixture was measured at 510 nm with a UV-Visible spectrophotometer. Quantification was done based on a standard curve of Quercetin in methanol (0- 100 $\mu\text{g}/\text{ml}$ ), **Fig. 4**. The concentration of flavonoid was expressed in terms of  $\mu\text{g}$  of Quercetin equivalent (QE) per mg of extract. All the readings were measured in triplicates.

#### **Eye Irritation Assay:**

**Isolation of RBC:** Fresh blood from healthy human volunteers was obtained and immediately mixed with Citrate buffer in 1:10 ratio. The blood was then centrifuged at 1500  $\times$  g for 15 min to remove white blood cells and traces of plasma. The pellet containing the RBCs was then stored in sterile Alsever's solution (NaCl 4.2g/l, Sodium Citrate 8g/l, Citric acid 0.5g/l, D-glucose 20.5g/l, pH 7.4) at 4 °C until required.

**Method:** The assay was performed according to Vinardell and Stankovic 26, 29 with slight modifications. Various test concentrations of the sample were prepared in Eppendorf using PBS (pH 7.4). The volume was adjusted to 975 $\mu\text{l}$ . To each tube, 25 $\mu\text{l}$  of RBC suspension containing about  $8 \times 10^9$  cells /ml was added (such that final concentration of oxyhaemoglobin was 0.125mM,  $E_{576\text{nm}} = 1.59 \times 10^7 \text{ cm}^2/\text{mol}$ ). The tubes were incubated for 10 minutes at room temperature and then centrifuged at 10,000 rpm for 1 min to terminate the reaction. Supernatant from each tube was read at 540nm, 560nm, and 575nm.

RBC suspension with PBS was maintained as a negative control, distilled water as positive control and SDS (final concentration 3.47mM) was used as an internal positive control.

**Hemolysis:** The percentage of hemolysis was determined by comparing the absorbance (560nm) of the supernatants with that of a control sample, which was hemolysed with distilled water. The half-maximal effective concentration, *i.e.* 50% hemolysis (H50/L50) was calculated from the concentration-response curve.

Percentage hemolysis was calculated as-

Hemolysis (%) =  $\text{Absorbance of Sample} \times 100 / \text{The absorbance of Positive control}$

**Protein Denaturation:** Oxyhemoglobin denaturation was quantified at 540 and 575 nm using a UV-Visible spectrophotometer. Denaturation Index (DI) was calculated by the formula-

$$DI (\%) = 100 (R1-Ri)/(R1-R2)$$

Where R1 is the ratio of the absorbance measured at 575nm upon the absorbance measured at 540nm for distilled water, similarly R2 corresponds to the value of SDS and Ri for each sample concentration. The difference between R1 and R2 is defined to be equal to 100% denaturation of oxyhemoglobin.

The ratio of the 50% hemolysis ( $L_{50}$ ) and the protein denaturing potentials (denaturation index, DI) called L/D ratio was used to classify the irritancy of products as given in **Table 7**.

**Cell Cytotoxicity Assay:** Sulphur Rhodamine B Assay was performed to measure the *in-vitro* cytotoxicity using the method prescribed by Houghton<sup>30</sup>. Chick-embryo fibroblast cells were used for this assay. The cell density was adjusted to  $2 \times 10^4$  cells per well-containing 90 $\mu$ l of complete DMEM media in a 96-well plate. The next day, the cells were treated with ethanolic extracts with final concentrations ranging from 25  $\mu$ g/ml to 1000  $\mu$ g/ml. After 24 h of treatment, the cells were fixed

using protein precipitation with 30% trichloroacetic acid (final concentration 10%) for 1 h at 4 °C.

The contents of the plate were then removed by gently inverting the plate, followed by three washes with PBS. The plate was allowed to dry at room temperature. 50 $\mu$ l of 0.4% SRB dye dissolved in 1% acetic acid was added to each well and kept for 30 min. Subsequently, the plate was washed four times with 1% acetic acid to remove the unbound stain. The plate was air-dried, and bound protein stain was solubilized with 100 $\mu$ l 10mM unbuffered Tris base [tris (hydroxymethyl) aminomethane]. The optical density was read at 510nm. Cells treated with sterile PBS served as the negative control.

**Statistical Analysis:** Results were expressed as mean, standard deviation (SD) of three measurements. Statistical analysis was performed using Student's t-test, and  $P < 0.05$  was considered significant.

**RESULTS:** DPPH radical scavenging activity and the FRAP assay widely used to study the antioxidant capacities of extracts. Our samples show a concentration-dependent antioxidant activity ranging from 30-91% inhibition/mg extract, and 28-150  $\mu$ g GAE/mg extract for DPPH and FRAP assay, respectively **Table 1** and **3a**.

**TABLE 1: DPPH RADICAL SCAVENGING ACTIVITY OF C. CYMINUM, C. CARVI, T. AMMI, A. GRAVEOLENS, AND C. SATIVUM TO STUDY ANTIOXIDANT ACTIVITY**

DPPH Assay	Conc. ( $\mu$ g/ml)	CuC	CaC	TA	AG	CS
% inhibition	250	24.9 $\pm$ 1.0	85.7 $\pm$ 1.3	47.4 $\pm$ 1.0	37.9 $\pm$ 0.8	15.1 $\pm$ 1.5
	500	40.5 $\pm$ 3.7	92.4 $\pm$ 0.4	78.9 $\pm$ 4.1	56.9 $\pm$ 3.0	28.4 $\pm$ 4.5
	1000	73.7 $\pm$ 5.3	91.7 $\pm$ 0.7	90.1 $\pm$ 0.4	80.8 $\pm$ 1.5	38.1 $\pm$ 3.1

**TABLE 2: DPPH RADICAL SCAVENGING ACTIVITY TO STUDY EFFECT OF SYNERGISM ON ANTIOXIDANT ACTIVITY**

DPPH Assay	TA+CaC	TA+AG	CaC+AG	CuC+CS	CuC+AG	CS+AG
250 $\mu$ g/ml	91.79 $\pm$ 1.65	63.24 $\pm$ 1.88	93.38 $\pm$ 0.29	39.15 $\pm$ 2.94	35.04 $\pm$ 0.16	25.36 $\pm$ 0.77
500 $\mu$ g/ml	91.51 $\pm$ 5.04	86.44 $\pm$ 1.62	91.60 $\pm$ 4.72	48.88 $\pm$ 2.48	54.74 $\pm$ 1.29	43.12 $\pm$ 2.60
1000 $\mu$ g/ml	89.10 $\pm$ 5.45	74.32 $\pm$ 13.7	91.41 $\pm$ 2.5	74.14 $\pm$ 2.99	73.65 $\pm$ 1.35	66.21 $\pm$ 4.06

**TABLE 3: a) FRAP ASSAY IN TERMS OF ASCORBIC ACID EQUIVALENTS  $\mu$ g/mg, b) ESTIMATION OF TOTAL PHENOL CONTENT IN TERMS OF GALLIC ACID EQUIVALENTS  $\mu$ g/mg, c) ESTIMATION OF FLAVONOID CONTENT IN TERMS OF QUERCETIN EQUIVALENTS  $\mu$ g/mg**

		CuC	CaC	TA	AG	CS
a) Ascorbic Acid Equivalents	$\mu$ g/mg	30 $\pm$ 1.0	150 $\pm$ 1.0	60 $\pm$ 1.0	35 $\pm$ 1.0	28 $\pm$ 1.0
b) Gallic Acid Equivalents	$\mu$ g/mg	50 $\pm$ 1.0	130 $\pm$ 1.0	90 $\pm$ 1.0	65 $\pm$ 1.0	45 $\pm$ 1.0
c) Quercetin Equivalents	$\mu$ g/mg	32 $\pm$ 2.0	140 $\pm$ 1.0	176 $\pm$ 1.0	58 $\pm$ 1.0	30 $\pm$ 1.0

**TABLE 4: CYTOTOXICITY ASSESSMENT USING SRB ASSAY**

SRB Assay		CuC	CaC	TA	AG	CS
% Cell Survival	10µg/ml	84.46	100.61	102.13	87.50	97.26
	25µg/ml	76.78	89.63	98.78	60.98	81.50
	50µg/ml	78.15	66.16	85.37	57.62	77.74
	100µg/ml	74.77	61.34	71.15	53.55	70.02
	250µg/ml	61.94	47.70	46.95	42.07	59.96
	300µg/ml	50.72	44.93	40.85	40.88	55.03
	IC <sub>50</sub> values	300	220	225	130	400

**TABLE 5: DENATURATION INDEX FOR EYE IRRITATION ASSAY**

Eye Irritation Assay	Conc. (µg/ml)	CuC	CaC	TA	AG	CS
Denaturation Index	250	19.99	23.79	7.37	17.63	13.37
	500	25.62	33.70	15.56	29.42	21.06
	1000	34.93	41.60	27.81	36.74	32.36

**TABLE 6: L<sub>50</sub> VALUES AND L/D RATIO FOR EYE IRRITATION ASSAY**

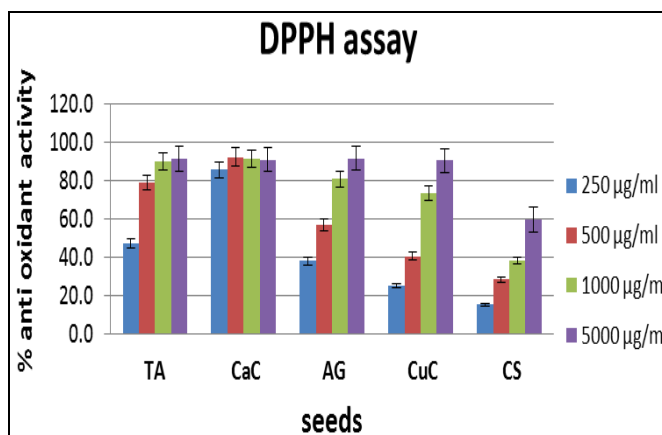
Eye Irritation Assay	Conc. (µg/ml)	CuC	CaC	TA	AG	CS
L/D ratio	250	177.59	78.10	119.80	88.74	104.86
	500	133.54	54.94	73.05	75.59	41.77
	1000	74.71	34.15	37.37	51.68	32.72
L <sub>50</sub> values at 1000 µg/ml		3250	1600	1750	2050	1100

**TABLE 7: CORRELATION BETWEEN THE IN-VITRO L/D RATIOS AND IN-VIVO EYE IRRITATION DATA**

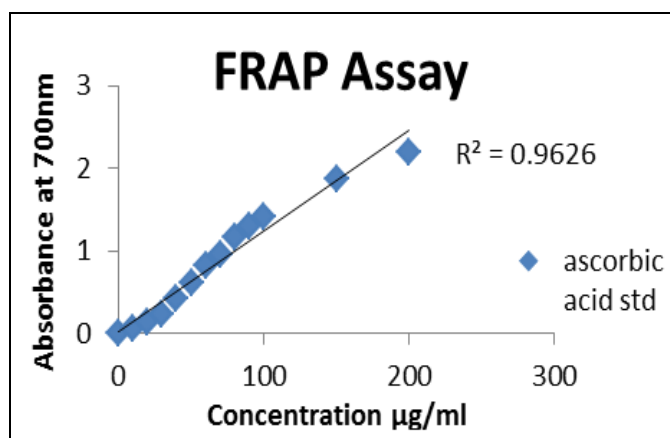
In-vitro L/D Ratio	In-vivo Eye Irritation
> 100	Non-irritant
10-100	Slight irritant
1-10	Moderate irritant
0.1-1	Irritant
< 0.1	Very Irritant

Reference: Order of toxicity and grade for irritancy potential according to Invitox Protocol No. 37<sup>31</sup>

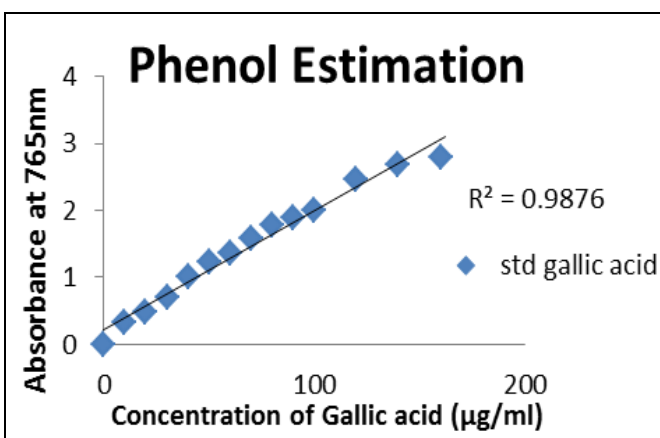
The phenol and flavonoid content was quantitatively estimated and were found to be well correlated with the antioxidant activity. The *in-vitro* eye irritation assay indicates very high L/D ratios for all the extracts validating their safety and non-irritancy. Also, the *in-vitro* cytotoxicity assay prompts an IC<sub>50</sub> value as 200µg/ml for most extracts.



**FIG. 1: DPPH RADICAL SCAVENGING ACTIVITY OF C. CYMINUM, C. CARVI, T. AMMI, A. GRAVEOLENS, AND C. SATIVUM AT CONCENTRATIONS 250, 500, 1000 AND 5000µg/ml**



**FIG. 2: ASCORBIC ACID STANDARD CURVE FOR FRAP ASSAY (Range 0-200µg/ml)**



**FIG. 3: GALLIC ACID STANDARD CURVE FOR PHENOL ESTIMATION (Range 0-200µg/ml)**

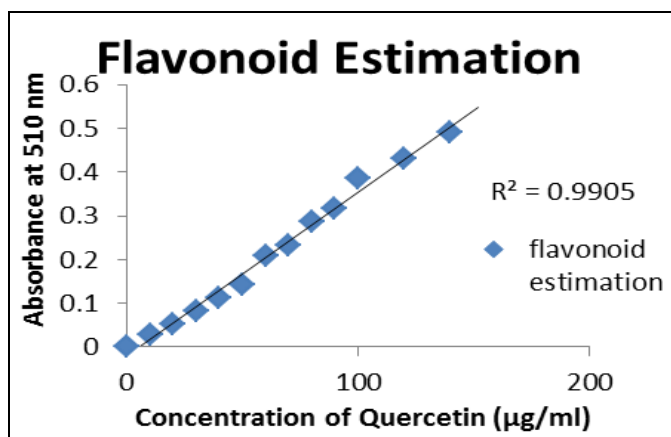


FIG. 4: QUERCETIN STANDARD CURVE FOR FLAVONOID ESTIMATION (Range 0-200µg/ml)

## DISCUSSION:

**DPPH Radical Scavenging Capacity Assay:** The ethanolic extracts from all five seeds displayed a concentration-dependent DPPH scavenging activity, **Table 1** and **Fig. 1**. At 1000µg/ml concentration, all extracts except *C. sativum* showed very potent antioxidant activity. Ethanolic extracts and essential oil from *C. sativum* seeds have previously been investigated for radical-scavenging activity, and it was found that it possessed weak DPPH scavenging activity as compared to the whole plant extracts<sup>32</sup>. This is due to high lipid content observed in seeds, in contrast to *C. sativum* leaves<sup>32,20</sup>.

At 250µg/ml concentration, ethanolic extracts from *C. cyminum* showed around 25% radical scavenging activity. In contrast, Hussain S *et al.*, (2010) observed around 56% and 42% radical scavenging activity from methanolic and hexane extracts respectively at 240µg/ml concentration<sup>33</sup>. Zheng W *et al.* (2001) has reported 10% and 20% antioxidant activity for *C. carvi* and *A. graveolens* respectively by ORAC Assay<sup>34</sup>. However, ORAC assay measures the Oxygen Radical Absorbance Capacity, whereas DPPH relies on proton donating capacity.

Antioxidant activity of plant extracts depends on the type and polarity of the extracting solvent<sup>35</sup>.

Previous studies have shown that the potency of a combination of herbs in a formula does not result from a quantitative addition of the potencies of the individual herbs, but rather from a more complex interaction among herbs with different pharmacological functions<sup>36</sup>. To probe the

synergistic effect of the antioxidant activities of the spice pairs, we randomly selected twin combinations of these five extracts.

Our results show synergistically higher antioxidant activity as compared to the original extracts at the same concentrations, **Table 2**.

**FRAP Assay:** **Table 3a** shows the total antioxidant capacity as measured by the FRAP method for per mg of the extract expressed as Ascorbic Acid Equivalents. Many methods differ in terms of their assay principles, experimental conditions, and particular antioxidants.

These have varying contributions to total antioxidant potential<sup>37</sup>. Concerning FRAP assay, the extracts were divided into three groups according to their reducing ability/antioxidant power –

- (a) Low FRAP (10–50 µg/mg) - *C. cyminum*, *C. sativum*, and *A. graveolens*;
- (b) Good FRAP (50-100 µg/mg) - *T. ammi*;
- (c) High FRAP (100-500 µg/mg)- *C. carvi*<sup>38</sup>.

All the extracts used exhibited capacity in reducing ferric ion ( $Fe^{3+}$ ) to ferrous ion ( $Fe^{2+}$ ) than to scavenging free radicals. *C. carvi*, which exhibited a high DPPH scavenging activity, also exhibited a high ferric ion reducing ability. Annual and geographical climate differences, soil conditions, and pesticide or herbicide use may contribute to variations in antioxidant activity and flavonoid content of plants<sup>39</sup>.

**Phenol Estimation:** The Folin Ciocalteu (FC) method is not an antioxidant test but instead an assay for the quantity of oxidizable substances, like phenolic compounds. Typical polyphenols that possess antioxidant activity are mainly phenolic acids and flavonoids. According to our study, the amount of total phenols in the extracts measured by the FC method ranged from 45 to 130 µg GAE/mg dry weight **Table 3b**.

The highest level of phenols was found in *C. carvi*, while *C. sativum* had the lowest. Some researchers have previously reported that although, spices belonging to this family exhibited very high levels

of phenols but did not exhibit a strong antioxidant effect concerning other spice families<sup>9</sup>.

However, the response order of extracts in our study was found to be significantly correlated with DPPH radical scavenging.

**Flavonoid Estimation:** Total flavonoid content was measured according to a colorimetric assay using Aluminium Chloride. Flavonoids, in our study, were found in the range of 30-176 µg Quercetin equivalents/mg dry weight of extracts **Table 3c**. *T. ammi* presented the maximum flavonoid content while *C. sativum* the least. Apiaceae family spices are a rich source of flavonoids as reported by many studies.

These plants generally accumulate flavonoids mainly in the form of flavones and flavonols<sup>9,40,41</sup>. Our findings are inconsistent with previous data. A positive correlation between the total phenolic compounds and flavonoids has been sighted in previous studies<sup>42, 43</sup>. Our investigation is consistent with these findings. The phenolic and flavonoid content, as well as the phenol content and antioxidant activity, show a positive correlation.

**In-vitro Eye Irritation Assay:** The RBC hemolysis test is a validated and correlated alternative to the *in vivo* Draize eye irritation test for the acute effects of typical surfactant-based formulations and ingredients<sup>26</sup>. **Table 5** represents the Denaturation Index (DI) of the alcoholic extracts. According to the results, even at 1000µg/ml concentration, the extracts resulted in less than 50% protein denaturation. **Table 6** represents the L/D ratio, which decides a compound/ sample's irritancy. Concerning **Table 7**<sup>31</sup>, all the extracts at 1000µg/ml concentration fall under the slightly irritant category. Irritant contact dermatitis and allergic contact dermatitis for many spices in this family have been reviewed before<sup>24,25</sup>.

Although these are rare, a major reason for contact irritation and allergy to these spices, as noted during previous studies is due to cross-reactivity<sup>44, 45</sup>. However, this kind of evaluation for cosmetic applications has not been reviewed previously.

**Cell Cytotoxicity Assay:** **Table 4** represents the *in-vitro* cytotoxicity of alcoholic extracts of five-spice seeds belonging to *Apiaceae* family.

Our results indicate *C. carvi*, *T. ammi*, and *A. graveolens* extracts approached their IC<sub>50</sub> concentrations around 250 µg/ml, while *C. cyminum* and *C. sativum* displayed only 60% inhibition at this concentration on chick embryo fibroblast cells.

Karimi G *et al.* (2002) reported the LD<sub>50</sub> values of aqueous and ethanolic extracts of *A. graveolens* were 3.04g/kg and 6.98g/kg (i.p.) in mice models<sup>46</sup>. *T. ammi* has shown to have antifilarial, anti-insecticidal activities<sup>47, 48</sup>. Shaker S *et al.*, (2010) performed cytotoxic evaluations of ethanolic extract of *T. ammi* against brine shrimps and found LD<sub>50</sub> around 35.48µg/ml<sup>49</sup>. Sayyah *et al.*, reported the LD<sub>50</sub> value for the *C. cyminum* essential oil was 0.59 ml/kg in rats<sup>50</sup>.

**CONCLUSION:** Our investigation demonstrates a comparative analysis of five selected spices (*C. carvi*, *T. ammi*, *C. cyminum*, *C. sativum*, and *A. graveolens*) of the *Apiaceae* family in regards to their antioxidant activity, total phenol content, *in-vitro* eye irritation and cytotoxic analysis. *C. Carvi* and *T. ammi* exhibited very good antioxidant activities and phenol content.

With respect to topical applications, we conclude that a concentration of 200µg/ml, which is non-irritant and is within the cytotoxic limit for all extracts except *A. graveolens*, and can be used safely. The antioxidant activity at this concentration is significantly low when a single extract is used.

However, our report suggests positive synergism on combining two extracts at the same concentrations. We presume a possibility of a further positive synergistic increase in antioxidant activity with the combination of two or more extracts.

Moreover, a comprehensive consideration of cytotoxicity after the combination has to be made. Since several different compounds mediate antioxidant activity, it is suggested that more studies be carried out to isolate the bioactive constituents from these extracts. Efforts are underway to incorporate these extracts in various formulations and additionally assess their effects on anti-aging and skin-whitening.

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**CONFLICT OF INTEREST:** Nil

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