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Bioactive constituents, sorption behaviour, antioxidant and antimicrobial activity of solar dried ajwain (*Trachyspermum copticum* L.) leaves

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The stability of active compounds, sorption studies, and antioxidant and antimicrobial activity of solar-dried ajwain (*Trachyspermum copticum* L.) leaves were studied. Fresh ajwain leaf (FAL) on solar drying yielded solar-dried ajwain leaf (SDAL) and was packed in polyethylene pouches for further studies. SDAL was rich in protein, fibre and mineral matter. Active compounds such as β-carotene (17 and 89 mg/100 g) and total chlorophyll (112 and 82 mg/100 g) contents were found in FAL and SDAL respectively. Similarly, higher ascorbic acid and polyphenols content were found in SDAL. Moisture sorption isotherm studies indicated that the SDAL was found to be non-hygroscopic in nature and stable at room temperature. Antioxidant activity as assayed by methanolic extract indicated SDAL had better activity than FAL. The ethanol extract of FAL and SDAL was evaluated for antimicrobial activity by disc diffusion method, which showed that the extract was sensitive at 20 µg/mL concentration on Gram-negative and positive bacteria. The study encourages the isolation and characterization of active ingredients from FAL and SDAL and their application in foods as nutraceuticals.

Keywords: Antimicrobial activity, Antioxidant activity, Bioactive components, Solar dried ajwain leaves, Sorption studies

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Introduction

Ajwain (*Trachyspermum copticum* L.) is an annual aromatic herb, which belongs to the family Apiaceae. It is native to Egypt and widely cultivated all over Iraq, Iran, Afghanistan, Pakistan and India. Ajwain has been well-known as an Ayurvedic spice since ancient times. The most utilized part of the herb is the small caraway-like fruit, which is especially popular in Indian savoury recipes. It is a small shrub with soft leafy branches, feather-like leaves growing up to 90 cm in height. The herb has a very pungent smell and emits a strong aroma when rubbed, similar to the smell of thyme. In India, the major Ajwain producing states are Rajasthan and Gujarat, where Rajasthan produces about 90% of India's total production. Ajwain possesses some pharmaceutical effects such as antiviral, anti-inflammatory, antifungal, antipyretic, anti-filarial, analgesic, antinociceptive, and antioxidant activity[1-8]. Essential oil of ajwain seed (2.5 to 5%) constitutes 35-60% of thymol. Other compounds such as α-pinene, p-cymene, limonene and γ-terpinene are also found in the seed oil. Seed extracts have been reported to have antioxidant, antiviral, insecticidal and anti-tussive activity[9]. Active compounds present in ajwain seed is used as a preservative in food[10].

In India, ajwain seeds are used as a digestive relief for abdominal pain and indigestion[11]. Essential oils from *ajwain* seed reported to have antifungal activities such as inhibition of *Aspergillus parasiticus* growth, aflatoxin production and also food preservative effects by protecting from fungal infection[12]. GC-MS analysis of essential oil from ajwain seeds showed thymol (54.5%), γ-terpinene (26.1%) and *p*-cymene (22.1%) as the major terpenoid compounds[13]. The total lipid of ajwain seed extracted using a mixture of chloroform: methanol (2:1, v/v) at room temperature, reported to be rich in monounsaturated fatty acids (68.3%) and polyunsaturated fatty acids (26.3%) and low quantities of saturated fatty acids (5.4%)[14].

Drying is a simple and economical method for the preservation of food materials and various drying techniques have been developed over the years for food materials. It has advantages like reducing volume and bulk, easy transportation and adding value in terms of nutritional benefits and economic advantage. Solar energy is an important alternative source of energy. It is relatively preferred to other

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sources because it is free, abundant, inexhaustible and non-pollutant in nature compared with higher prices and shortage of fossil fuels. It is a renewable and environmentally friendly technology that is also economically viable in most developing countries. Various studies have reported the application of solar drying for fruits, vegetables, grains, seeds, beans, herbs, spices and medicinal plants. The effect of solar drying at different temperatures and the quality parameters in terms of colour (L* a* b*), total phenols and radical scavenging activity in olive leaves were reported[15]. Green leafy vegetables occupy an important place among food crops as these are rich sources of β-carotene, ascorbic acid, riboflavin, folic acid and minerals like calcium, iron and phosphorous[16].

Literature on the composition of vegetative parts such as the stem and leaf of ajwain is limited. The present study was carried out to prepare solar-dried ajwain leaf with a specific objective to analyse its physicochemical composition, stability of active compounds, sorption studies, and antioxidant and antimicrobial activity.

Materials and Methods

Collection, authentication and taxonomy of ajwain

Ajwain seeds (Lamb Variety) were procured from the local market in Hyderabad and cultivated at CSIR-CFTRI Resource Centre Hyderabad Campus during the month of October 2019. Ajwain seed sample was identified by Dr M. Venkata Ramana, Assistant Professor, Botany Department, Osmania University, Hyderabad. The Taxonomy of Ajwain shows that it belongs to the family of Apiaceae, genus *Trachyspermum* and species *copticum.* Ajwain (*Trachyspermum copticum* L.) leaves were collected during the month of December before flowering from the garden.

Chemicals used for the study

Chemicals and solvents used in the study were of analytical grade and purchased from SD Fine Chem Ltd (Mumbai, India). Standard chemicals 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2-azino-bis (3-ethyl benzothiazoline 6- sulphonic acid (ABTS), 2,6 Dichlorophenol indophenols (DCPIP) were procured from Sigma-Aldrich.

Solar drying of fresh ajwain leaf (FAL)

Freshly collected *T. copticum* leaves were washed under running water to remove dust and dirt. The cleaned leaves were spread in stainless steel (550 × 260 × 20 mm; solar photovoltaic panel 60 W) trays and dried in a solar drier (Model SDM-8, SEED, Hyderabad). The solar-dried leaves were ground in a laboratory mixer (M/s. Preethi, Mumbai, India), passed through 60 BSS mesh (240 μ) for obtaining uniform dry powder, which was packed in polyethylene (PE) pouch and stored at 28±2ºC room temperature (RT) for further studies.

Physico-chemical composition

The fresh ajwain leaf (FAL) and solar-dried ajwain leaf (SDAL) were evaluated for Moisture, total ash, fat, protein, and crude fibre using reported standard methods[17].

Colour measurement

The colour of FAL and SDAL was measured using Hunter Colorimeter (Hunter Associates Laboratory, USA). Among the three colour coordinates, ‘‘L*’’ represents the lightness index, ‘‘a*’’ represents red-green, and ‘‘b*’’ represents yellow-blue colour components. The measurement of L*, a*, and b* values of colour was carried out in triplicate and the average values were reported.

Antioxidant activity

Determination of ABTS assay

The ABTS (2, 2-Azinobis {3-ethyl-benzothiazoline-6-sulfhonic acid) assay of the FAL and SDAL was measured according to the method reported in literature[18]. FAL and SDAL samples were extracted with methanol for 2 h using a magnetic stirrer. The extract was filtered using a Whatman No. 1 filter paper. Varying concentrations of FAL and SDAL extracts were taken in different test tubes and were dissolved in 1 mL of water and 3.0 mL of diluted ABTS solution, and incubated at RT in the dark for 10 min and OD was recorded at 734 nm. The per cent ABTS inhibition was calculated based on the optical density of the reagent control and sample and compared with the butylated hydroxytoluene (BHT) equivalent and the percentage inhibition was calculated using the following expression

$$\text{ABTS assay \%} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Estimation of total polyphenol content

The total polyphenol content in FAL and SDAL was determined using Folin-Ciocalteau reagent and expressed as mg of gallic acid equivalent/100 g

sample[19]. One gram of the sample was ground and extracted in 50 mL of 85% ethanol at room temperature (RT) using a magnetic stirrer. An aliquot of the extract (0.5 mL) was mixed with 0.5 mL Folin-Ciocalteau reagent and 5 mL water. The contents were vortexed for 2 min and allowed to settle at RT for 5 min. A saturated solution of sodium carbonate (1 mL) was added to the contents, and the volume was made up to 10 mL with distilled water. All the contents were mixed thoroughly by vortexing for 2 min. The contents were allowed to stand at RT for 60 min. The colour developed was measured at 675 nm and total polyphenol content was calculated from a standard gallic acid calibration curve (19-76 µg/mL) and expressed as mg per 100g sample.

Estimation of active compounds

Estimation of active compounds such as β-carotene, lycopene and chlorophyll in FAL and SDAL was carried out by extracting 1 g sample in a 100 mL solvent mixture, (acetone:hexane, 4:6) using a magnetic stirrer at room temperature for 30 min[20]. The contents were filtered and passed through anhydrous sodium sulphate and the absorbance of the filtrate containing the pigment components was measured using a UV-Visible spectrophotometer (UV-1800, Shimadzu) at 452, 505, 645, and 663 nm. The quantification of active compounds was done using the following expressions and reported as mg per 100 g sample.

β-carotene (mg /100 mL) = $0.216 \times A_{663} - 1.220 \times A_{645} - 0.304\ A_{505} + 0.452 \times A_{453}$

Lycopene (mg /100 mL) = $- 0.0458 \times A_{663} + 0.204 \times A_{645} - 0.304\ A_{505} + 0.452 \times A_{452}$

Chlorophyll (mg /100 mL) a = $0.999 \times A_{663} - 0.0989 \times A_{645}$

b = $0.328 \times A_{663} + 1.77 \times A_{645}$

Estimation of ascorbic acid

Ascorbic acid content in FAL and SDAL was determined as per standard method reported in literature[17]. Sample of 0.5 gm was dispersed in 100 mL metaphosphoric acid (3%) and the contents were mixed for 25 min using magnetic stirrer. The contents were titrated with DCPIP dye (50 mg DCPIP (2,6 Dichlorophenolindophenol) mixed with 42 mg $NaHCO_3$ and dispersed in 200 mL distilled water, incubated at 4°C overnight) with an end point of pink colour. Ascorbic acid content was calculated from a dye factor using standard ascorbic acid and expressed as mg/100 g sample.

Antimicrobial activity

Extraction

Ground leaf (50 g) was soaked in ethanol for 24 h at room temperature with shaking using a mechanical shaker followed by decanting and filtration. The fresh solvent was replaced and agitated for 10 min, decanted and filtered. The extracts were pooled and concentrated in a rotary vacuum evaporator and allowed to air dry and finally kept in the refrigerator for further analysis.

Bacterial culture

A total of six standard pathogenic bacterial strains were used which were maintained as glycerol stocks at CSIR-CFTRI, RC-Hyderabad. They were Gram-positive bacteria such as *Staphylococcus aureus (ATCC-25923), Streptococcus pneumoniae (MTCC-655)* and Gram-negative bacteria namely *E. Coli (ATCC-25922), Pseudomonas aeruginosa (ATCC-27853), Klebsiella pneumoniae (MTCC-4031)* and *Salmonella typhi (MTCC-733)* respectively.

Antimicrobial susceptibility test

The antimicrobial activity was determined by the disc diffusion method using the Kirby-Bauer method[21]. The discs of 6 mm diameter were prepared with Whatman No 1 filter paper. The ethanol extract reconstituted with Dimethylsulfoxide (DMSO) at a concentration of 20 µg/mL was applied to the discs. Inoculum was prepared with the fresh cultures of bacterial strains, which were grown in nutrient broth for 18 h at 37±1ºC (3×10^6 cells/mL). Inoculum density was compared with Mac-Farlands standard solution. The Muller Hington agar was inoculated with the culture and incubated at room temperature for 5 min. The discs were arranged on the surface of the inoculated agar plates and pressed gently to adhere to the surface of the agar. The plates were incubated for 24 h at 35-37ºC. After incubation, the diameter of the zone of inhibition was measured.

Equilibrium moisture content-relative humidity studies

Equilibrium moisture content-relative humidity (EMC-RH) studies of SDAL powder were carried out using the reported method in literature[17]. Equilibrium Relative Humidity (ERH) studies of SDAL powder were carried out by exposing it to different relative humidity conditions such as 10, 30, 50, 70, 90, and 100% using sulfuric acid solutions. The powder was exposed to the above conditions by keeping 5 g of sample in different petri plates and moisture absorbed by the sample was determined at regular intervals

until they attained constant weight. The samples were observed critically for adverse changes like lump formation, discolouration, and mould growth during the study. Moisture sorption isotherms of samples were plotted by drawing graphs of relative humidity (RH) versus equilibrium moisture content (EMC).

Results and Discussion

Physico-chemical composition

The unit operations involved in the production of solar-dried ajwain leaves (SDAL) are presented in Fig. 1 along with the photograph of fresh ajwain leaf (FAL). The nutritional composition of FAL and SDAL is presented in Table 1. Fresh ajwain leaf (FAL) after solar drying yielded 11.68% SDAL. Moisture content in fresh ajwain leaf was 88.31% and after solar drying reached 4.15%. Vegetables such as tomato (95.8 to 2.2%), spinach (95 to 2.4%), carrot (93.4 to 2.6%) and mint (89.7 to 2.0%) reached the safe moisture level within 2 to 4 days by exposure to solar dryer[22]. SDAL possessed good quantities of protein (22.99%), fibre (13.28%) and minerals (19.65%). Acidity as citric acid was around 0.188 and 1.49% for FAL and SDAL respectively. Titratable acidity of 16.62, 6.91, 2.07, and 1.47% were reported in *Hibiscus, Rumex, Basella*, and *Alternanthera*[23]. Fresh spinach leaves yielded 4.57% spinach powder possessing 28.23% protein, 19.21% mineral matter and 10.24% fiber[24]. Physico-chemical and mineral composition of leafy vegetable species namely *Vernonia*, *Solanum*, *Amaranthus*, and *Telfaria* were evaluated[25]. It was reported that they possessed crude protein (31.7–34.6 g/100 g) and crude fibre (7.4–9.8 g/100 g) on a dry matter basis. Colour values of FAL and SDAL showed greenness as indicated by a negative value for a* (- 3.82 and - 4.05) and L (56.7) value was recorded for SDAL. The Hunter colour units of dried spinach leaf were L* (56.33), a* (-1.26) and b* (3.51). Higher L* values observed can be attributed to the presence of bound carotenoids[24].

Fresh ajwain leaf (2840 g)

↓ Manual cleaning and water wash

Drying at 50±5°C for 8-9 h in a Solar dryer

↓

Solar dried ajwain leaf (SDAL)

↓ Grinding & sieving [60 BSS mesh (240 µ)]

SDAL powder (314 g)

↓

Packing in PE pouch

↓

Storing at Room Temperature (28±2°C) for further studies

Fig. 1 — Flow chart for the preparation of solar dried ajwain leaf (SDAL).

Stability of active compounds

The stability of active compounds such as β-carotene, lycopene, chlorophyll, polyphenols and ascorbic acid of FAL and SDAL are presented in Table 1. The results indicated that in comparison to FAL, the SDAL was rich in β-carotene (89 mg/100g), total chlorophyll (273 mg/100g), polyphenols (1034 mg/100g) and ascorbic acid (20.71 mg/100g). This may be due to the decrease in moisture content in the controlled temperature of the solar dryer. A similar trend was reported in solar-dried mint leaf[26], they concluded that up to 25% of total chlorophyll was retained in the dried sample. Active compounds such as β-carotene (111 mg) total chlorophyll (338 mg) and ascorbic acid (108 mg/100g) were reported in dried *rumex* leaf[23].

Status of ABTS radical activity

The antioxidant capacities were determined by measuring the decrease in blue colour intensity, which

Table 1 — Physico-chemical composition, colour units, and active compounds of FAL and SDAL

Parameters	FAL	SDAL
Moisture	88.31	4.15
Total ash	2.17	19.65
Acidity as citric acid	0.19	1.49
Fat	-	1.03
Protein	-	22.99
Crude fibre	-	13.28
Carbohydrates by difference	-	38.90
Energy kcal/100g	-	256.83
Hunter colour		
L*	34.22	56.77
a*	-3.82	-4.05
b*	9.31	16.10
Active compounds mg/100 g		
β - Carotene	17	93
Lycopene	37	87
Chlorophyll a	80	191
Chlorophyll b	32	82
Total chlorophyll	112	273
Total polyphenol content	149	861
Ascorbic acid content	9	21

Values are the mean of duplicate analyses
FAL (Fresh ajwain leaf) and SDAL (Solar dried ajwain leaf)

results from the reaction between the ABTS+ radical and the antioxidant compounds in the sample. ABTS assay indicated 50% inhibition at a concentration of 5.5 and 0.60 mg/mL for FAL and SDAL respectively (Fig. 2). Solar-dried ajwain leaf showed higher antioxidant activity than fresh ajwain leaf due to the increase in ascorbic acid and total phenolics after solar drying, as antioxidant activity depends upon active compounds. Though there was a decrease in ascorbic acid content after drying, the overall antioxidant activity increased. A similar trend was observed in mango, banana and papaya solar tunnel dried samples[22]. The highest antioxidant radical scavenging activity was also reported in solar-dried olive leaves at 60°C[15]. The dried spinach leaf powder exhibited 69.55% inhibition of ABTS at a concentration of 1.8 mg/mL[24]. ABTS activity of *Momordica* with an IC_{50} value at a concentration of 13 µg/mL was reported[27]. In a similar ABTS assay for *Hibiscus* and *Basella* dry leaf, IC_{50} value of 0.4 mg/mL was observed[23]. The ABTS activity is directly proportional to the concentration of ajwain leaf extract. The capture of more free radicals formed by ABTS by leaf extract resulted in an increase in activity and a decrease in absorbance. The activity also depends on the phenolic concentration in the extract. Polyphenols are the main antioxidants that can influence oxidative processes by chelating metals[28]. The antioxidant activity is significantly related to the total phenolic content in hydroethanolic leaf extract of *E. tectorius*[29]. The ABTS antioxidant activity shown in the ajwain leaf extract is highly responsible for polyphenol and ascorbic acid concentration.

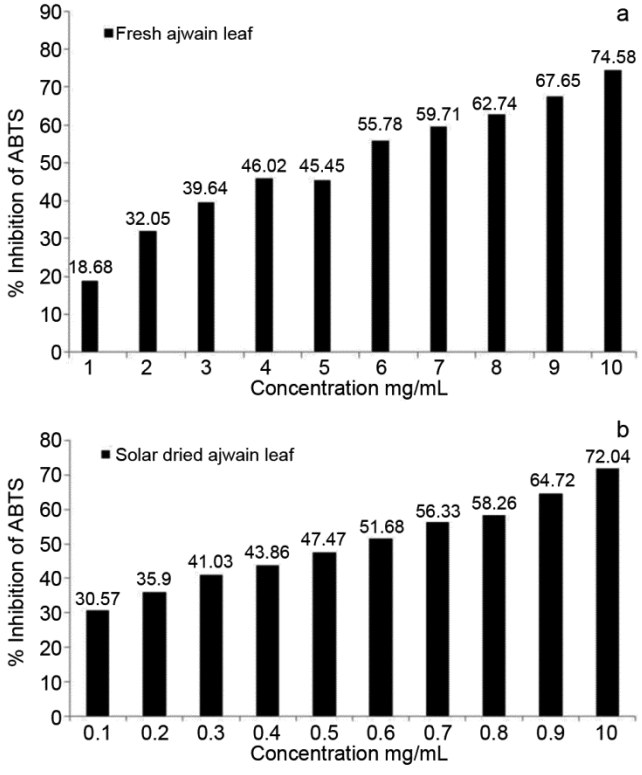

Fig. 2 — Antioxidant activity (ABTS assay) of a) FAL; and b) SDAL.

Experimental sorption isotherm

Moisture sorption isotherm of SDAL is presented in Fig. 3. The results of moisture sorption isotherm studies of SDAL indicated that the critical moisture content of 13.57%, equilibrated at 68% RH. Hence, the SDAL was non-hygroscopic in nature and stable at 63% RH in polyethylene pouches for storage at room temperature. Similar sorption isotherm studies of tray-dried curry leaves and drumstick leaves showed stability in HDPE (high-density polyethylene) pouches at 60% RH. The critical point in terms of moisture level for curry leaves (12.01%) and drumstick (11.21%), were observed when initial moisture content was 2.5% and 5.50% respectively[30].

Antimicrobial activity

The photographs of the antimicrobial activity of ethanolic extract of ajwain leaves are presented in Fig. 4. The results of antimicrobial activity revealed that the extract was sensitive at 20 µg/mL concentration on Gram-negative bacteria *Pseudomonas aeruginosa* (ATCC-27853). Gentamicin (17 mm) and Tobramycin (18 mm) were used as positive controls and DMSO as negative control. Similar trend was also observed in *Indigofera oblongifolia* leaf extract at a concentration of 25 µg/mL for gram positive and negative bacteria[31]. The highest zone of inhibition (14.6 mm) was observed in ethyl acetate extract of

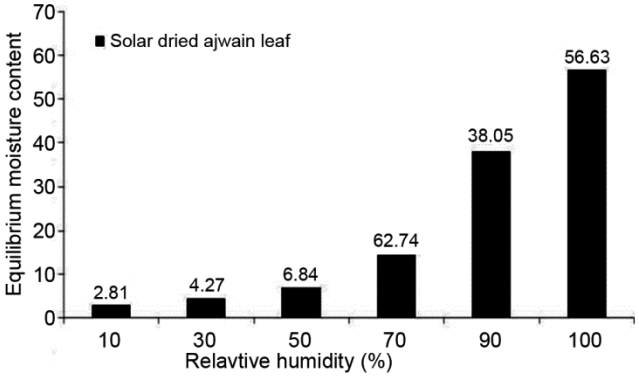

Fig. 3 — Sorption isotherm of SDAL

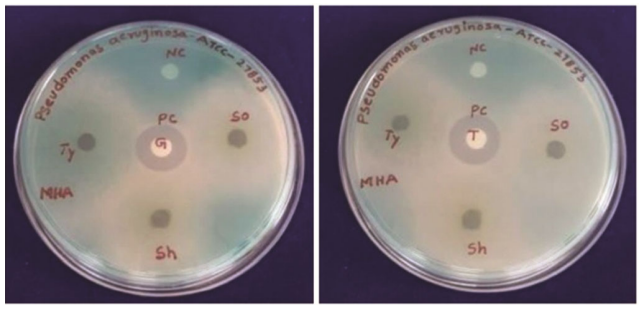

Fig. 4 — Antimicrobial activity of SDAL extracts.

Salvia hispanica flower[32] against the bacterium *Pseudomonas fluorescens* at 50 µg/100 mL. Different species of *mushroom* were reported to possesses similar trend of antimicrobial and antioxidant activity[33-34]. Acetone extract of ajwain seeds showed more antibacterial activity compared to the aqueous extract by agar diffusion assay reported in literature[35]. Similarly, ethanolic extract of Ajwain leaf possessed antibacterial activity at a concentration of 20 µg/mL with the diameter inhibition zone measuring around 7-15mm[36,37]. The bactericidal activity depends on the concentration of active compounds present in ajwain extract, which was reported as large quantities of thymol/carvacrol in its total essential oil and phenolic compounds[38].

Conclusion

In conclusion, this study provides information regarding the valuable nutrients of ajwain leaves. The SDAL is a good source of protein (22.99%), mineral matter (19.65%), and fibre (13.28%). Antioxidant properties of leafy vegetables have great potential because of their use as natural antioxidants in place of chemical or synthetic additives. The experimental results of SDAL showed that it is a rich source of active compounds like β-carotene (93 mg/100g), and polyphenols (861 mg/100g) and retained 27% of ascorbic acid even after the dehydration process. The values of the results are also reflected in the antioxidant activity and antimicrobial activity. The microbiological analysis of ajwain leaf, solvent extract has also revealed that it contains biologically active nutrients. The quantity of active compounds resulting from the herb could serve as valuable sources for isolating and characterizing the lead molecules with specific functions. This approach assists in identifying the compounds that show bioactivity. It has been suggested that the compounds that possess antioxidant activity, can inhibit the occurrence of diseases because they can scavenge the free radicals. Thus, the present study of microbiological and active constituents data suggests that ajwain solvent extracts can be used as a good source of natural antioxidants for health benefits. Fresh leaves can be further explored for preservation in the form of dehydrated or intermediate moisture foods for use in various food products. Further study could be taken up to incorporate the SDAL powder in bakery products as a source of protein, minerals, and fibre. The present study could further encourage in isolating and identifying bioactive compounds to establish their food and pharmaceutical applications.

Conflict of interest

There is no conflict of interest regarding the publication of this manuscript.

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