

***Syzygium aromaticum*: Traditional uses, antioxidant, anti-inflammatory activities and photo-protective properties**

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Abstract:

Syzygium aromaticum commonly called clove belongs to the Myrtaceae family used in traditional Moroccan medicine as diuretics, analgesics, anti-inflammatory drugs and to treat dental problems.

The objective of this study is to evaluate the biological activities of the fixed oils of the buds of *Syzygium aromaticum*.

The UV-Visible spectrophotometer was used to evaluate the photo-protective activity of the fixed oil through the UV absorbance test. We have proven that fixed oil and clove ethyl acetate extract have high UVB absorption capacity.

The study of fixed oil *in vitro* has shown that it has significant antioxidant capacity by inhibiting DPPH free radicals. Note that the maximum value of this activity is 95.13% (IC₅₀ = 0.163 mg / ml.).

The anti-inflammatory activity of the fixed oil of *Syzygium aromaticum* was evaluated in rabbits by the inflammation test induced by the injection of carrageenan. The results show that the fixed oil's maximum inhibitory effect on inflammation was 87.7% achieved by the fifth hour.

Keywords: Antioxidant, Carrageenan, Fixed oil, *Syzygium aromaticum*, Ultraviolet.

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Introduction

Syzygium aromaticum commonly named as “kranfal” and in French (giroflie, clou de girofle) belongs to the family of the Myrtaceae. It's native to the Maluku Islands (or Moluccas) in Indonesia (Batiha et al. 2019). It is a plant always green of ten to twenty centimeters in height with lanceolate leaves and flowers yellowish. The tree is grown in Indonesia, in Tanzania, Madagascar, Sri Lanka, India, Malaysia, Brazil, Jamaica and the Guinea (Ghedira et al. 2010). The essential oil of clove is used for the treatment of several diseases including: anticancer and antimutagenic (Ghedira et al. 2010). Furthermore, the clove bud oil has shown acaricidal activity against *dermatophagoides farinae* and *dermatophagoides pteronyssinus* (Kim et al. 2003). In Morocco, the cloves are used in traditional folk medicine as diuretics, analgesic, Cardiotonic, spices and condiments to carminative properties and simulative as well (Kamatou et al. 2012). On the other hand, the oil of *S. aromaticum* contains higher eugenol content, in addition to acetyleneugenol, chavicol, acetyl salicylate and humulenes (Ghedira et al. 2010). Eugenol which is a phytochemical bioactive component, contributes in photochemistry reactions (Mihara et al. 1982) and has been reported to possess strong potential as antioxidant and (Fankem et al. 2017), insecticidal agents (Singh et al. 2014). Generally, low concentrations of eugenol acts as an antioxidant and anti-inflammatory, while its high concentrations acts as a pro-oxidant resulting from enhanced generation of free radicals (Asha et al. 2001; Chogo et al. 1981). Therefore, the present study was designed to evaluate the cosmetic properties of the lipids of *S. aromaticum* buds.

Recent ethnopharmacological studies have shown that cloves are used to treat a variety of ailments in many parts of Morocco, such as dental problems, inflammation, diabetes, high blood pressure, ailments stomach upset and diarrhea (Table 1).

S. aromaticum (Myrtaceae) is widely used in traditional Moroccan medicine to treat dental problems, rheumatism, against lung diseases, antiseptic is used as an anti-inflammatory (ElHaouari et al. 2018 ; Ziyat et al. 1997). In general, decoctions of seeds, fruits, leaves and flowers can also be used to treat diabetes, heart disease, high blood pressure and toothache (Skalli et al. 2019; Mrabti et al. 2019 ; Eddouks et al. 2002 ; Laadim al. 2017 ; Tahraoui et al. 2007 ; Naceiri Mrabti et al. 2021 ; Khabbach et al. 2012). Clove powder mixed with the leaves of *Lawsonia inermis* is used for hair care (Abouri et al. 2012 ; Elhassan et al. 2020 ; Hseini et al. 2007).

Materials and Methods

Plant Materials

The plant material used for this study is the flower bud of the plant *S. aromaticum*. It was purchased at the herbalist's market in the city of Fez, Morocco. The dry buds of *S. aromaticum* were washed, cleaned, dried and then stored for use.

Plant extract preparation

The buds of *S. aromaticum* were ground to a fine powder. Then the powder was suspended in 20% chloroform. The resulting filtrate was evaporated in an oven at 40 ° C overnight to remove chloroform. The extract is stored at 4°C for different tests.

DPPH assay

The antioxidant power of the fixed oil of *S. aromaticum* was evaluated using the 2,2-diphenyl-2-picryl-hydrazyl radical test (DPPH) and carried out as previously described (Hamdouch et al. 2022). Briefly, the concentrations of fixed clove oil are added to DPPH (0.004%). Optical density was measured at 517 nm after 30 min incubation in the dark at room temperature. The positive control was performed with butyl-hydroxy-toluene (BHT) and ascorbic acid under the same conditions.

The ability to scavenge DPPH radical was calculated by the following equation: DPPH radical scavenging activity (%) = [(Abs control – Abs sample)] / (Abs control) x 100.

Evaluation of the UV absorption

The different concentrations of fixed oils were prepared in chloroform, in order to measure their capacity for absorption of ultraviolet rays. Optical density was measured with a UV spectrophotometer (Cary 3E UV-visible spectrophotometer) and absorption spectra (280-340 nm) were performed by Scanning Kinetics Software.

Anti-inflammatory activity

The method described by Zouhri and by Winter, (Zouhri et al. 2017; Winter et al. 1962) was used for the evaluation of the anti-inflammatory properties of *S. aromaticum*.

Three groups of rabbits (weight 1600 to 2000 g) were formed for the study, each containing three animals, group treated with 0.1 mg / ml of fixed oil of *S. aromaticum*, negative control

group (untreated), control group positive (reference product) treated with 0.5 mg / ml of diclofenac sodium, a non-steroidal anti-inflammatory drug.

The products tested were administered intradermally 30 min before the injection of 1% carrageenan and at a dose of 0.5 ml per paw. Inflammation is measured using the principle of plethysmography.

Rabbit foot volumes were recorded 0, 1, 2, 3, 4 and 5 hours after the injection of carrageenan. For each treated group, the average volumes obtained in these different readings (VT) were compared to that obtained before any treatment (V0). Thus allowing to calculate the percentages of edema (percentage of inflammation), by following the formula: $((V_t - V_0) / V_0) * 100$.

Results

The main aim of this study is to evaluate the properties of the buds of *S. aromaticum*. The fixed oil of *S. aromaticum* has been tested for their photo-absorption, anti-oxidation and anti-inflammatory properties.

Evaluation of the photo- protective properties

The evaluation of the photo-absorption properties is appreciated by determining the absorption spectrum in UVB (280 nm to 315 nm) and UVA (315 nm to 400 nm). The results showed that the fixed oil of *S. aromaticum* has a higher UVB absorption capacity than UVA (Figures 1 and 2).

The absorptions (1.23 in UVA and 1.86 in UVB) and (2.02 in UVA and 1.42 in UVB) were observed at concentrations of 5 mg / ml and 50 mg / ml respectively. Under the same conditions, at a concentration of 5 mg / ml, the ethyl acetate extract of *S. aromaticum* showed absorption of 0.95 in UVA and 1.6 in UVB.

Two positive control sunscreens (sun protection factor 80 (SPF 80) and sun protection factor 30 (SPF 30)) were evaluated for their photo-absorption. The results obtained showed a maximum absorption of (4.16 in UVB and 4.11 in UVA) and (1.27 in UVB and 0.86 in UVA) respectively.

Evaluation of the antioxidant activity

The free radical scavenging activity of the fixed oil of *S. aromaticum* was examined *in vitro* using the DPPH assay. The results obtained were compared with standard compounds (ascorbic acid and butylhydroxytoluene (BHT)).

Figure 3 shows that the antioxidant activity of *S. aromaticum* is dose dependent. Note that for a dose of 2 mg / ml, the maximum value of this activity is 95.13%, with an IC_{50} of 0.163 mg / ml. The calculated IC_{50} of ascorbic acid and BHT is 0.11 mg / ml and 0.07 mg / ml respectively. At the same time, the ethyl acetate extract had a maximum value of 95.06% and an IC_{50} of 0.38 mg / ml.

Evaluation of anti-inflammatory activity

The anti-inflammatory activity of the clove bud part was evaluated. The results of this study indicate that for the negative control group, intramuscular injection of carrageenan will increase inflammation by 30% after one hour, then 20% by the third, and 6.61% by the fifth hour. For the positive control group (reference product) with a dose of 0.5 mg / ml, the percentage of inflammation at the 1st, 3rd, 5th hour was 17.3%, 3.12% and 2.12%, respectively. The percentage inflammation of the oil set at 0.1 mg / ml *S. aromaticum* at the first, third and fifth hours was 13.3%, 4.82% and 0%, respectively (Figure 4). The anti-inflammatory effect of fixed oil of *S. aromaticum* (0.1 mg / ml) was greater than that of diclofenac (0.5 mg / ml) at the 5th hour.

Discussion

In order to prevent and protect the skin from the side effect of UV radiation, several products are available in market nowadays in the form of creams, lotion, and gel having labeled SPF. However, the use of natural products for prevention and treatment of different skin diseases is enjoying great popularity in recent years since they have a high potential due to their antioxidant activity, primarily (Korać and Khambholja 2011), they can also prevent the development of other skin conditions caused by solar radiation, such as aging, wrinkle formation, undesired pigmentation and loss of collagen (Duale et al. 2010; Seite et al. 2000).

The main intention of the present study was to investigate the cosmetics potential of fixed oils of *S. aromaticum* buds. For this end, our work focused on the characterization of the photo-protective, antioxidant and anti-inflammatory properties of this tested plant.

The obtained results showed clearly that the fixed oil of *S. aromaticum* has a UVA and UVB absorption capacity. The absorption in the UVB waveband is greater than in the UVA. Starting from the concentration 5mg/ml we notice significant absorptions with a value of 1.6 and 0.95 respectively. Comparing these results with those of commercial sunscreen lotions with SPF 80 and SPF 30, it is noted the fixed oil of *S. aromaticum* has an average absorption capacity between these two references. These results suggest that the buds forming part of *S. aromaticum* have potent photo-protective properties. The investigation of the antioxidant activity of *S. aromaticum* showed that this later endowed with high and dose-dependent DPPH radical scavenging activity. The maximum value of this activity is 95.13% with an IC_{50} value of 0.163 mg / ml. The latter is comparable to that of BHT obtained under the same conditions. In parallel, the extract with ethyl acetate exhibited a maximum of 95.06% and an IC_{50} value of 0.38 mg / ml.

Several previous studies have shown the antioxidant activity of the essential oil of *S. aromaticum* (Abdel-Wahhab and Aly 2005). According to the bibliographic data, the fixed oil of *Syzygium aromaticum* studied has higher antioxidant properties than the seed oil of *Pistacia lentiscus* reported by Ait mohand et al. (2022), and lower than that of *Brochia cinerea* essential oil reported by Hamdouch et al. (2022). The possibility of contamination of the fixed oil, in our case, by the essential oils is unlikely that the lipid extract of the obtained plant was evaporated in the oven overnight.

The anti-inflammatory activity of fixed plant oil was evaluated using carrageenan-induced paw edema. The maximum inhibition of inflammation was 87.7% for *S. aromaticum* fixed oil and 78.5% for Diclofenac. The evaluation of the percentage of inhibition shown clearly that the fixed plant oil tested has a potent an anti-inflammatory. This observed activity could be due to the various bioactive constituents with health-stimulating properties that *S. aromaticum* contains. According to the bibliographic data, the fixed oil of *S. aromaticum* studied has higher anti-inflammatory properties than the hydro-ethanolic extract of the seeds of *Ammodaucus leucotrichus* reported by Es-safi et al. (2020), and lower than that of *Anacyclus pyrethrum* (L) reported by Jawhari et al. (2020).

Our findings are in agreement with those found by Öztürk, et al. and Bennacer, et al. (Öztürk et al. 2005; Bennacer et al. 2021).

Conclusion

The present study showed that the fixed oil from the buds of *S. aromaticum* has anti-inflammatory, photo-protective and antioxidant properties. The presence of these three properties makes the fixed oil *S. aromaticum* explain their use in traditional Moroccan medicine.

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Table:Table 1: Ethnomedical uses of *S. aromaticum* in Morocco

Place, Morocco	Parts used	preparation methods	modes of administrations	Ethno medical uses	References
Oriental Morocco	Leaves, Fruits	-	-	Haircare, against pulmonary diseases, febrifuge, antiseptic, mouth hygiene	(Ziyyat et al. 1997)
Oriental Morocco	Flowers	Powder	External application	Head problems	(Jamila et al. 2014)
Taza (Northern Morocco)	Flowers	Powder	Oral	Cold, rheumatism, sedative, poison antidote, anti-inflammatory, urinary antiseptic, stimulant, dental problems.	(ElHaouari et al. 2018)
Taza (Northern Morocco)	Fruits	Powder	Local application	Dental analgesic	(Khabbach et al. 2012)
Taza (Northern Morocco)	Leaves, Flowers	Decoction, Powder	Oral	Diabetes	(Mrabti et al. 2019)
Rabat (Western Morocco)	Clove	-	-	Hair care, back pain, toothache, antipyretics	(Hseini et al. 2007)
Rabat (Western Morocco)	Seeds	Ground Powder	Oral	Diabetes	(Skalli et al. 2019)
Sidi Slimane (northwestern Morocco)	Fruits	Infusion	Oral	Diabetes	(Laadim et al. 2017)
Settat (central Morocco)	Clove	Decoction	Oral	period pain, stomach pain and diarrhea	(Tahri et al. 2012)
Beni Mellal	Leaves, Flowers	Decoction Powder, Maceration.	Oral	Diabetes	(Naceiri Mrabti et al. 2021)
Tafilalet (south-east region of Morocco)	Flowers	-	-	Cardiac disease, hypertension	(Eddouks et al. 2002)
Zagora (Southeastern of Morocco)	Clove	Raw, Infusion	External application	Anti-nociceptive	(Boufous et al. 2017)
Errachidia (south-eastern Morocco)	Clove	Decoction, Infusion	Oral	Hypertension	(Tahraoui et al. 2007)
Tata (South-eastern Morocco)	Clove	Powder	Powder mixed with <i>Lawsonia inermis</i>	Hair care,	(Abouri et al. 2012)
Tarfaya (Moroccan Center South)	Clove	Poultice	External application	Hair care	(Idm'Hand et al. 2020)

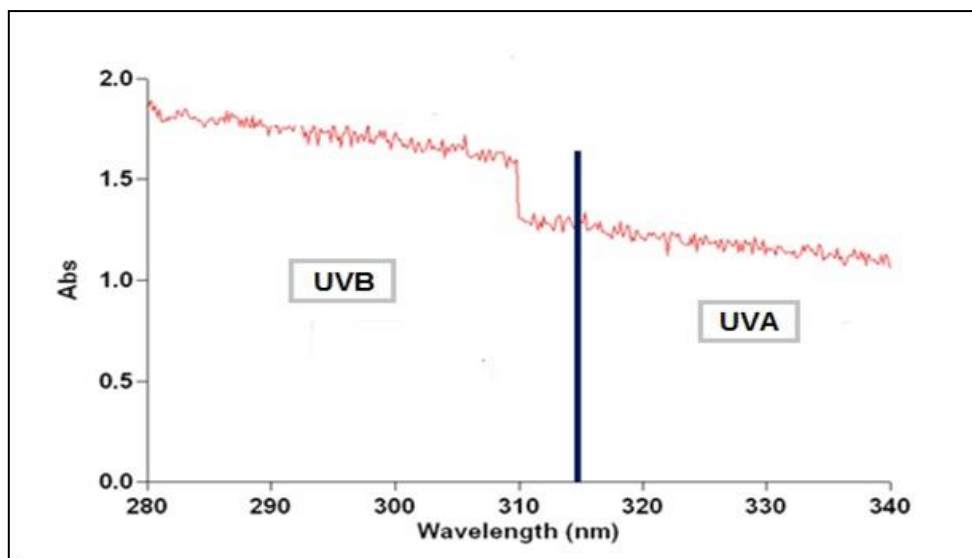
Figure:

Figure 1: Absorption spectrum of the fixed oil of *S. aromaticum* (10 mg/ml).

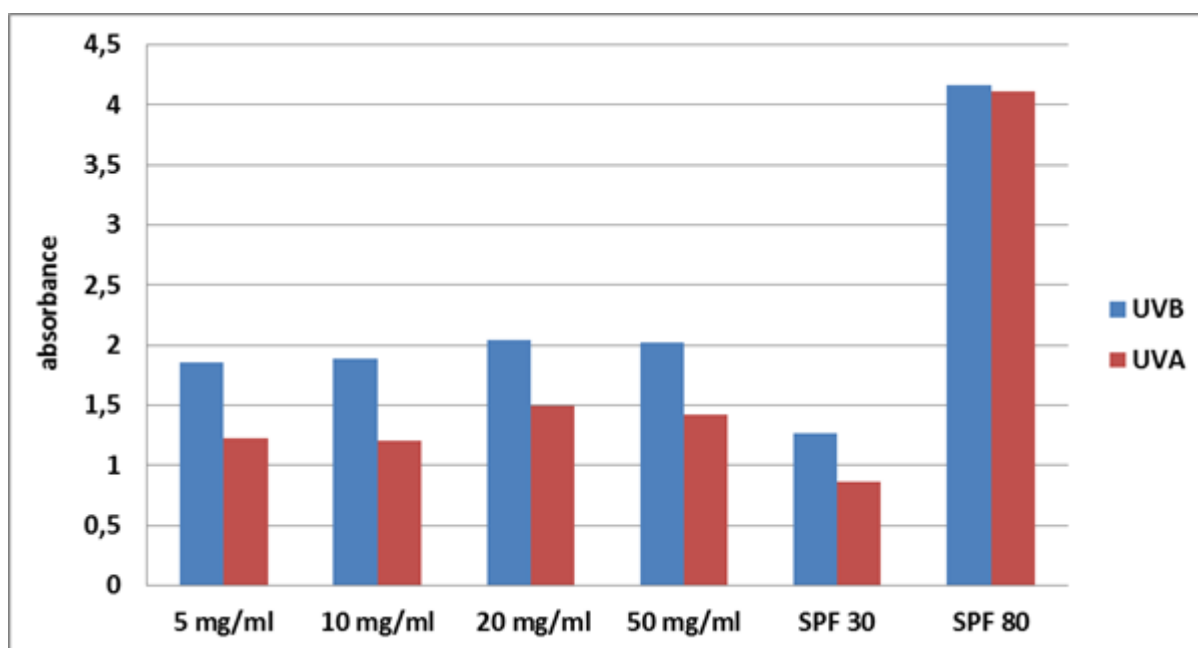


Figure 2: Maxima absorption of the fixed oil of *S. aromaticum* at different concentrations compared to the reference sun creams SPF 80 and 30.

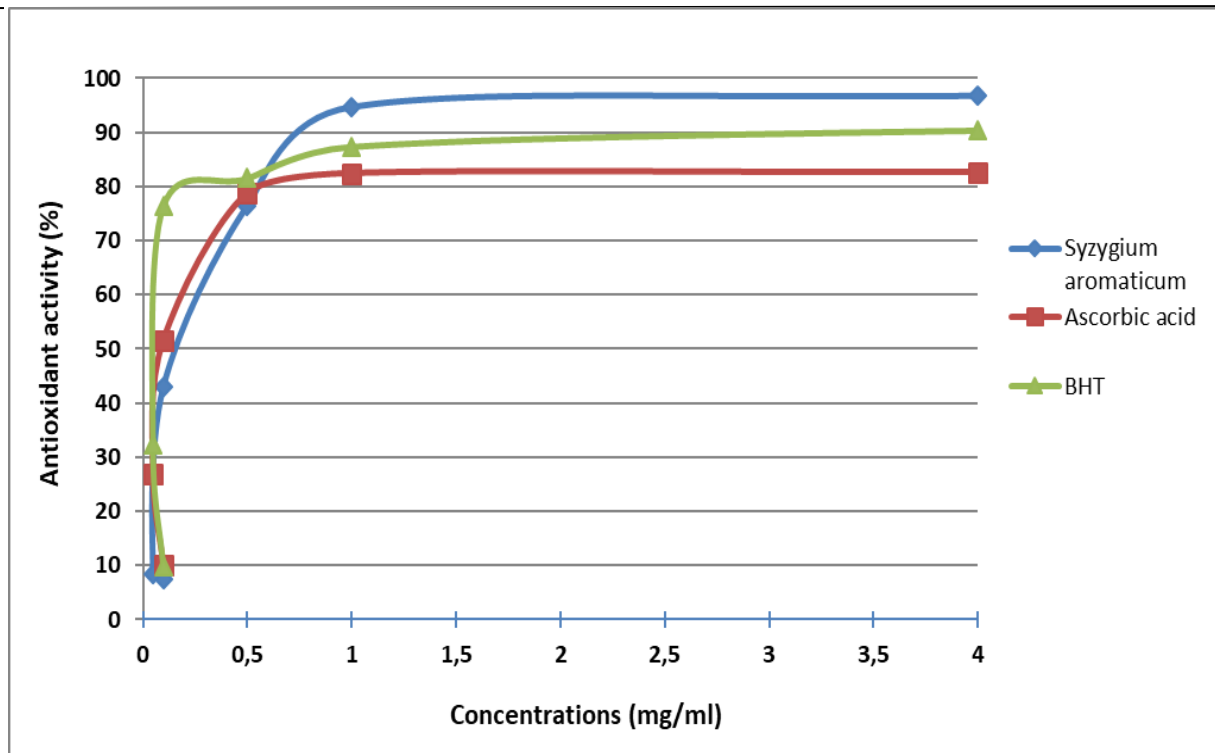


Figure 3: Antioxidant effect of the fixed oil of *S. aromaticum* compared to BHT and ascorbic acid.

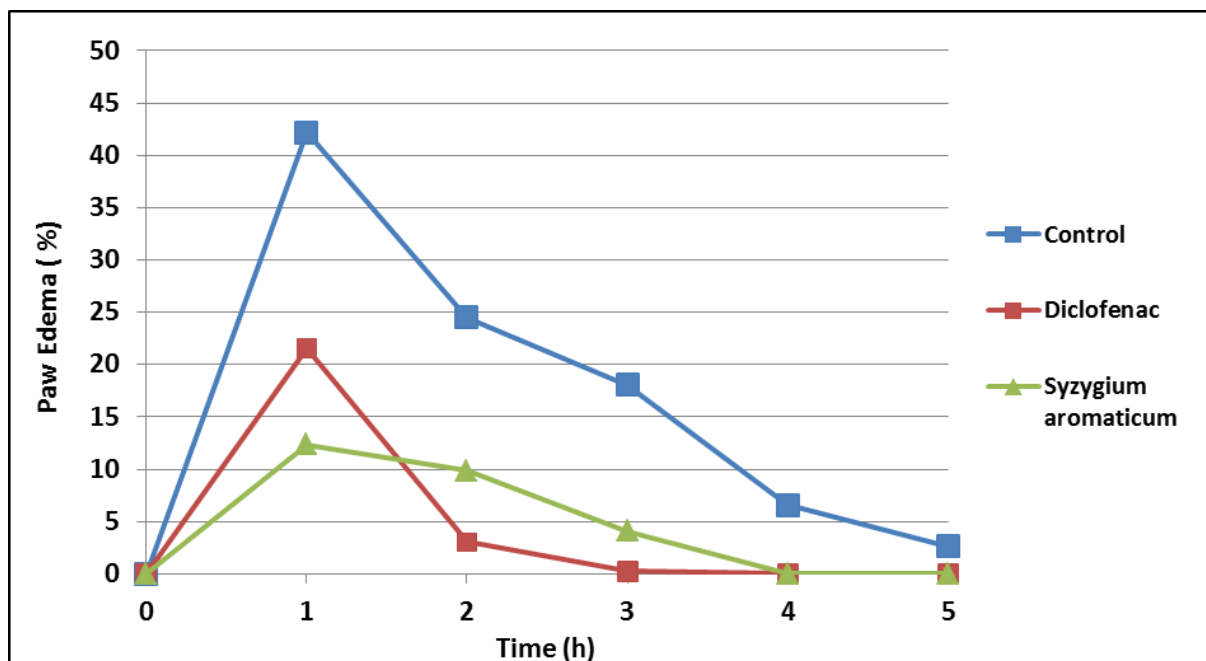


Figure 4: Anti-inflammatory effect of the fixed oil of *S. aromaticum* compared to that of Diclofenac.

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