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Original Research

Investigation of Phytochemical content, Antioxidant properties and Antibacterial Potential of Algerian *Bunium incrassatum*

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Abstract

The current study highlights the efficacy of tuber extracts from the Algerian medicinal plant *Bunium incrassatatum* for their phytochemical content, antioxidant and antimicrobial activities. Indeed, the ethanolic maceration method proved to be the most effective for phytochemical extraction, showing the highest yield extraction. Moreover, the ethanolic maceration displayed a higher polyphenol and flavonoid content compared to the methanolic extract. The antioxidant potentials of methanolic and ethanolic extracts of *Bunium incrassatum* were reported by the DPPH assay, revealing moderate efficacy of IC50 values of 380.53 µg/mL and 261.75 µg/mL, respectively, indicating that both of these extracts can be considered a good resource of natural antioxidants. In terms of antibacterial activity, the methanolic extract exhibited appreciable antimicrobial activities against different bacterial strains like *Staphylococcus aureus, Klebsiella pneumoniae, Bacillus cereus*, and *Escherichia coli*, with average zones of inhibition of 10.74 mm, 10.14 mm, 10.03 mm, and 9.45 mm, respectively. The present study underlined that the extracts from *Bunium incrassatum* tubers could be a rich raw material with an interesting profile in phytochemical content, antioxidant, and antimicrobial activities, deserving further studies for applications in drug medicine, therapy, and nutrition.

Keywords

Bunium incrassatum; tuber extracts; phytochemical content; antioxidant properties; antibacterial potential; ethnobotanical resources.

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Raziskava fitokemične vsebnosti, antioksidativnih lastnosti in antibakterijskega potenciala alžirske vrste *Bunium incrassatum*

Izvleček

Sedanja študija poudarja učinkovitost izvlečkov gomoljev iz alžirske zdravilne rastline *Bunium incrassatatum* zaradi njihove fitokemične vsebnosti, antioksidativnega in protimikrobnega delovanja. Dejansko se je izkazalo, da je metoda etanolne maceracije najučinkovitejša za fitokemično ekstrakcijo z največjim izkoristkom ekstrakcije. Poleg tega je etanolna maceracija pokazala večjo vsebnost polifenolov in flavonoidov v primerjavi z metanolnim ekstraktom. O antioksidativnem potencialu metanolnih in etanolnih izvlečkov *Bunium incrassatum* so poročali s testom DPPH, ki je pokazal zmerno učinkovitost vrednosti IC50 380,53 µg/mL oziroma 261,75 µg/mL, kar kaže, da se oba izvlečka lahko štejeta za dober vir naravnega antioksidanti. Kar zadeva protibakterijsko delovanje, je pokazal znatno protimikrobno delovanje proti različnim bakterijskim sevom, kot so *Staphylococcus aureus, Klebsiella pneumoniae, Bacillus cereus* in *Escherichia coli*, s povprečnimi conami inhibicije 10,74 mm, 10,14 mm, 10,03 mm oziroma 9,45 mm. Sedanja študija je poudarila, da bi lahko bili izvlečki iz gomoljev *Bunium incrassatum* bogata surovina z zanimivim profilom fitokemične vsebnosti, antioksidativnih in protimikrobnih aktivnosti, ki si zaslužijo nadaljnje študije za uporabo v medicini, terapiji in prehrani.

Ključne besede

Bunium incrassatum; izvlečki gomoljev; fitokemijska vsebnost, antioksidativne lastnosti; antibakterijski potencial; etnobotanični viri.

Introduction

Medicinal plants have been utilised for traditional medicine for many centuries. Their therapeutic value is presently more recognized, with many plant constituents being integrated into contemporary pharmacopoeia. Moreover, medicinal plants have consistently provided a rich source of bioactive compounds with significant therapeutic potential (Gurib-Fakim, 2006).

The use of plant extracts as antimicrobial drugs is becoming very popular and significant as microbes' resistance to the drugs increases rapidly (Banso et al., 2006; Khandal et al., 2012).

Singh and Kumar (2023) and Rasool et al. (2020) reported that ethnobotanical studies have hastened investigations into new natural compounds of vegetation that may answer great expectations in the development of novel therapies. However, safety and standardization with such medicinal herbs are still questioned; hence, the ability to integrate them into modern health systems is seriously challenged.

In addition, medicinal plants have played a very important active role in Algeria's traditional practices regarding the healing of several ailments. Ethnobotany has unravelled the richness and diversity of the plant species used by the local populations, underlining their importance in the health domain. Among them, Talghouda is considered synonymous with *B. incrassata* and *B. mauritanica*. *Bunium* is a medicinal plant belonging to *Apiaceae*, and it is widely distributed in several regions of Algeria (Quézel and Santa, 1963). It is found in several regions of Algeria, especially the northern part. Traditionally, Talghouda tubers are used to relieve bronchitis, thyroid disorders, inflammatory haemorrhoids, and antidiarrheals. Besides their usage in medicine, they are also consumed due to their nutritional value and as food (Bousetla et al., 2015). Interestingly, Talghouda (*B. incrassatum*) was one of the first medicinal plants documented in North Africa and Algeria. It has been used to treat asthma, cysts, thyroid disorders, and tonsillitis (Djahafi,2021).

The Talghouda plant, including varieties like *B. mauritanicum, B. incrassatum*, and *B. bulbocastanum*, has been reported on in earlier works on its chemistry (Bousetla et al., 2015; Bousetla et al., 2014; Dehimi et al., 2021). Further, Bousetla et al. 2015 reported that tubers of Talghouda in Algeria were consumed like potatoes. These dried and powdered medications serve as astringent and anti-diarrhoeal in action but anti-inflammatory in nature, especially in

conditions like haemorrhoids, besides bronchitis and cough.

According to Adoui et al. (2022), the chemical components of Talghouda are not yet fully described. In fact, only a limited number of studies have concerned the phytochemical characterization of Talghouda extracts and oils like Bousetla et al. (2014) on *B. incrassatum* fruits essential oil from Algeria and Bousetla et al. (2015) on *B. incrassatum* chloromethane methanol roots extract from Algeria; El Koli et al. (2017) on *B. incrassatum* aerial parts essential oils from Algeria; Karouche et al. (2020) reported on methanol and aqueous extracts of *B. mauritanicum* tubers; and Dehimi et al. (2021) reported on acetone and hexane extracts from *B. incrassatum* fruits. According to Adoui et al. (2022), the extracts of Talghouda, among other species of *Bunium*, possess remarkable biological activities. Therefore, the aim of the present study is to investigate the phytochemical composition, antioxidant activity, and antimicrobial properties of *B. incrassatum* tuber extracts, focusing on the quantification of key bioactive compounds and exploring their potential therapeutic application.

Materials and Methods

Plant extracts preparation

Bunium incrassatum, commonly called “Talghouda,” was collected in the region of Mila, in the east of Algeria. The tubers obtained were washed and homogenized with the help of a blender. Methanol and ethanol were employed to recover the phenolic compounds. Afterwards, 30 grams of the plant material was soaked with 300 ml of 80% methanol in a continuous stirring at room temperature, protected from light, together with 300 ml of 70% ethanol. After 72 h, the suspension was filtered, and the filtrate was collected before drying it in baking dishes at 40°C. The crude extract obtained was kept until use. Using the formula given by(Falleh et al., 2008), extraction yield was determined as follows:

Extractive yield = (Weight of extract obtained (g) / Weight of crude drug used for extraction (g)) × 100%.

Total Phenolic Content

The phenolic content was determined by using the Folin-Ciocalteu reagent assay (Singleton V et al., 1965). A standard calibration curve was plotted using gallic acid. Total phenolic content (TPC) was expressed as milligrams of gallic acid equivalents (mg GAE) per gram of extract.

Total Flavonoid Content

The content of flavonoids in the extracts of Bunium incrassatum was measured by an AlCl3 method, according to Lamaison and Carnet (1990). The amount of flavonoids was measured using a quercetin calibration curve and was presented as milligrams of quercetin equivalent per gram of dry weight of plant material- mg EQ/g Ps.

DPPH radical scavenging assay

The antioxidant activity was assayed by the method of DPPH radical scavenging (Kumarasamy et al., 2007). Briefly, 1 ml of the methanolic DPPH solution (0.2 mM) was added to 1 ml of different dilutions of plant extracts at concentrations ranging from 0 to 1 mg/ml. The mixture was kept at room temperature and in the dark for 30 minutes. Absorbance was measured against control at 517 nm of 1 ml DPPH solution and 1 ml methanol. The decrease in absorbance that occurred was monitored by a spectrophotometer, and the percentage inhibition PI was worked out using the following:

Percentage radical scavenged = [(A0 − A1/A0)] ∗ 100%

where A0 is the absorbance of the DPPH solution, and A1 is the absorbance of the sample.

The antibacterial activity

The antibacterial activity of the plant extracts was evaluated using the gel diffusion method (Treki et al., 2009), known as an antibiogram. The principle is based on the appearance of an inhibition zone in the culture medium (Chhetry et al., 2022). Mueller Hinton Agar (MHA) was prepared, sterilized, poured into Petri plates, and incubated at 37°C for 24 hours to check for contamination. Bacterial strains were streaked on the agar plates and incubated for 24 hours. Bacterial suspensions of *Escherichia coli* ATCC 11303, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Bacillus cereus* ATCC 10987, and *Klebsiella pneumoniae* ATCC 700603 were prepared in a nutrient medium and incubated for 24 hours at 37°C. Discs impregnated with different concentrations of the extract were placed on the agar. Gentamicin was used as

a positive control. Measurement of inhibition zones was performed after incubation - 24-48 hours at 37°C. Extracts were diluted in DMSO from 100 mg/mL stock to lower concentrations (T½, T¼, T⅛, etc.) for antibacterial testing.

Statistical Analysis

All the experiments were performed three times, and the data were presented as mean ± SD. Statistical significance of differences was calculated by one-way ANOVA and Tukey's test with significance set at $p < 0.05$.

Results

The extraction

Bunium incrassatum has been studied for its phytochemical composition and antioxidant properties. Research has shown that this plant contains significant amounts of polyphenols and flavonoids, which contribute to its antioxidant activities: total phenolics, flavonoids (Bousetla et al., 2015) using HPLC-DAD analysis; the presence of sterols, triterpenes, saponins, tannins, alkaloids and aglycone flavones using methanol and aqueous extract. The extraction yielded two types of extracts: ethanol extract (Et-OH) and methanol extract (Met-OH). Expressed as the percentage extract mass to the mass of the fresh plant, the highest yield was observed in the ethanol extract with a value of 12.266% compared to the methanolic extract. Results are represented in (Table 1).

Results of the Quantitative Study

Total phenolic content

The results obtained (Table 1) revealed that the ethanolic extracts contain a higher level of total polyphenols, with 19.25 mg Eq GA/g of extract, compared to the methanolic extracts, which registered 17.26 mg Eq GA/g. This suggests that ethanol is a more efficient solvent for extracting polyphenols from *B. incrassatum* tubers.

Total flavonoid content

The quantification of flavonoids was established through a calibration curve using quercetin as standard. Results are expressed in milligrams equivalent of quercetin per gram of extract (mg Eq Qu/g). Like the polyphenol content, flavonoid quantitative analysis revealed that the ethanolic tuber extracts of *B. incrassatum* showed higher flavonoid concentration compared to methanolic extracts, as shown in Table 1.

Biological Activity

Antioxidant Activity

The assessment of antioxidant activity using the DPPH assay revealed a progressive increase in free radical inhibition with increasing concentrations for both methanolic and ethanolic extracts of *B. incrassatum*.

The percentage of free radicals scavenged by ascorbic acid at different concentrations is shown in (Table 2). The methanolic extract achieved an inhibition rate of 20.76% at 100 µg/mL, while the ethanolic extract showed a slightly higher inhibition rate of 22.52% at the same concentration. These results indicate that both extracts exhibit moderate antioxidant activity, with similar performance at higher concentrations. However, ascorbic acid, used as a reference standard, demonstrated a clearly superior antioxidant activity, with an inhibition rate close to 89% at concentrations of 25 µg/mL and 50 µg/mL.

Our results also revealed that at low concentrations (1.5 µg/mL), ascorbic acid showed an inhibition of 24.39%, which is significantly higher than the methanolic and ethanolic extracts, which exhibited very low inhibition at this concentration.

Table 1. Results for the phytochemical screening of *B. incrassatum* extracted in different solvents.

Tabela 1. Rezultati fitokemičnega pregleda *B. incrassatum*, ekstrahiranega v različnih topilih.

Different samples	Yield %	TPC a mg Eq Qu/gE	TFCb Eq Qu/gE
Methanol (mg GAE/g)	9.29%	17.26 ±0.60	1.07 ±0.06
Ethanol (mg GAE/g)	12.26%	19.25 ±0.92	3.82 ±0.03

Note: Data were expressed as mean value ± standard deviation (n = 3). a: Total polyphenol content, b: Total flavonoid content.

These findings emphasize the potency of ascorbic acid as an antioxidant compared to *B. incrassatum* extracts, although the latter showed increasing activity with increasing concentrations, indicating a dose-dependent response.

Moreover, The IC50 values obtained from the DPPH analysis indicate a promising antioxidant activity for *B. incrassatum* extracts, with an IC50 of 261.75 µg/mL for the ethanolic extract and 380.53 µg/mL for the methanolic extract. Although these values are lower than those for ascorbic acid (IC50 of 3.41 µg/mL), they suggest that the plant extracts contain bioactive compounds capable of inhibiting free radicals.

Antibacterial Activity

The antimicrobial activity of the methanolic and ethanolic extracts was evaluated using the disc diffusion method, a qualitative technique based on measuring the diameter of the inhibition zones around the discs loaded with active substances. The table below (Table 3) presents the diameters of the inhibition zones obtained from extracts of *B. incrassatum* tubers.

The methanolic extract of *B. incrassatum* showed moderate antibacterial activity against *Staphylococcus aureus*, with an inhibition zone of 7.98 mm for the stock solution (100 mg/mL). Unexpectedly, a dilution to 50 mg/mL produced a larger zone of 10.74 mm, suggesting better diffusion of the bioactive compounds at a lower concentration. Although this inhibition is lower than that of the antibacterial standard (36.35 mm), it remains indicative of the presence of bioactive compounds capable of interacting with bacterial membranes. Further, the methanolic extract also moderately acted against the bacteria *Escherichia coli*, which was reflected as a 9.45 mm inhibition zone for a stock solution of 100 mg/mL. This activity is relatively constant, even at lower concentrations, but remains well below that of the antibacterial standard (30.8 mm). The extract appears to reach the highest level at a concentration of 12.5 mg/mL with an inhibition zone of 9.19 ± 0.25 mm. Such consistent activity, given the overall lower inhibition compared to the standard, emphasizes the potency of the extract for the target E. coli-a gram-negative bacterium known for its inherent resistance to various antibiotics.

The methanolic extract also exhibited moderate antibacterial activity against *Bacillus cereus*, with an inhibition zone of 10.30 mm for a stock solution of 100 mg/mL. In comparison, the antibacterial standard produced a much higher zone of inhibition, 33.58 mm. The observed activity with the extract could be considered a positive result. Knowing that *B. cereus* is known for its resilience and potential to cause foodborne illnesses, so even moderate inhibition from a natural extract like *B. incrassatum* highlights its potential as a valuable source of bioactive compounds.

Likewise, results also showed limited antibacterial activity against *Pseudomonas aeruginosa*, with an inhibition zone measuring 7.78 mm from a stock solution of 100 mg/mL of the extract. On the other hand, the antibacterial standard showed a rather larger inhibition zone measured at 37.77 mm. These findings signify that even though the crude extract had some activity against *P. aeruginosa*, its efficacy is much lower compared to the standard and needs further research to optimize its antibacterial potential. Finally, the methanolic extract presented very low activity against *Klebsiella pneumoniae*, with an inhibitory zone

Table 2. Percentage Inhibition of DPPH Free Radicals and IC50 Values by Methanolic and Ethanolic Extracts of *Bunium incrassatum*.

Tabela 2. Odstotna inhibicija prostih radikalov DPPH in vrednosti IC50 z metanolnimi in etanolnimi izvlečki *Bunium incrassatum*.

Concentration (µg/ml)	% Inhibition Methanolic Extract	% Inhibition Ethanolic Extract	% Inhibition ascorbic acid
0.3 µg/ml	4.81 ±0.08	0.12	4.16
1.5 µg/ml	6.14 ± 0.82	0.30 ±0.43	24.39
25 µg/ml	10.03 ± 0.41	6.24 ±0.17	89.09
50 µg/ml	13.28 ± 0.57	11.50 ±0.51	89.31
100 µg/ml	20.76	22.52 ±0.34	-
IC 50	380,53	261,75	3,41

Note: Values (mean of three replicates ± SD) represented in the same column by different letters are significantly different at $p<0.05$; IC50: concentration of the sample allowing 50% of the DPPH radical to be inhibited.

Table 3. The antibacterial activity of *B. incrassatum* tubers extracts was obtained by using different extraction solvents and four different concentrations.
Tabela 3. Antibakterijska aktivnost izvlečkov gomoljev *B. incrassatum*, pridobljenih z uporabo različnih ekstrakcijskih topil in štirih različnih koncentracij.

Bacterial strains	Concentration mg/mL					Standard
	Stock solution	1/2	1/4	1/16	1/18	
			Methanolic Extract			
S. aureus	7.98±0.027	10.74±0.28	-	-	-	36.35
P. aeruginasa	7.78±0.3	-	-	-	-	37.77
E. coli	9.45 ±0.05	8.59± 0.11	8.86±0.39	8.88±0.43	9.19±0.25	30.8
B. cereus	10.30±0.19	-	-		-	33.58
K. pneumonie	6.23±0.23	10.14±0.32	-	-	-	26.6
			Ethanolic Extract			
			N.S			

Note: Values are mean of inhibition in mm (mean ± SD) ; (-): No activity; NS: Not significative ; a : Gentamicin.

of 6.23 mm at stock solution concentration (100 mg/mL) improved to 10.14 mm at 50 mg/mL concentration. The antibacterial standard generated a larger diameter of inhibition of 26.6 mm. For the ethanolic extract's antibacterial activity, our results revealed that the zones of inhibition observed showed no statistically significant difference compared to the control, suggesting limited or insufficient antibacterial activity at these concentrations.

Discussion

As noted in previous literature, ethanol is an excellent solvent for plant maceration; it has been highly useful in many areas of plant research. Extensive studies were conducted on ethanol as an extraction solvent of bioactive compounds in various medicinal plants such as *Azadirachta indica* (Subapriya et al., 2005); *Moringa oleifera* (Sreelatha et al., 2011), and *Aloe vera* (Hamman, 2008) which was in agreement with our results which revealed that the extraction yielded higher ethanolic extracts from *B. incrassatum* tubers. Indeed, most research has proven that ethanol concentration is an important factor to consider during an extraction process to influence the yield of polyphenols, flavonoids, triterpenoids, and Vitamin C present in plants like *Clinacanthus nutans* (Qun et al., 2016).

Previous research on the same plant showed that the methanolic fraction contains the highest amount of total polyphenols extracted from the different parts: tubers of *Bunium mauritanicum*, seeds of *Bunium incrassatum*, and umbellules of *Bunium incrassatum* by Karouche et al. (2020), Toul et al. (2022), and Toul et al. (2023), respectively. These findings were not in agreement with our results, which showed oppositely higher total polyphenol concentrations from ethanolic maceration.

According to Dai et al. (2010), there is no universal extraction procedure suitable for the extraction of all plant phenolics. It is generally known that the solubility of polyphenols depends on the chemical nature of the plant sample, as well as on the polarity of used solvents (Toul and Djendar, 2023) and mostly on the extraction protocol conditions (extraction time, temperature, and pH) (Roselló-Soto et al., 2019).

Several studies reported the presence of flavonoids in extracts from different parts of *Bunium* species: flavonoids such as kaempferol and naringenin were identified from *Bunium incrassatum* Seeds (Toul et al., 2022); catechin, hesperetin, luteolin, and quercetin from *Bunium incrassatum* umbellules chloroform extract (Toul et al., 2023). Our results are in agreement with the findings of Karouche et al. (2020), who reported very low flavonoid contents in the tubers of *B. mauritanicum*. The results clearly show that the tubers of *Bunium* species are poor in flavonoids, which can be explained by the extraction method, concentration of solvent, environmental conditions, climate, collection period, genetic factor, and experiment conditions.

The quantitative study (extraction yield, polyphenol and flavonoids content) from *Bunium incrassatum* tubers

revealed that ethanolic maceration proved to be the most efficient extraction method, which is in accordance with the finding of (Sasidharan,2011), who reported that ethanol, a moderately polar solvent, is known to extract a wide range of compounds, including polyphenols, flavonoids, alkaloids, and saponins. Most plant phytochemical analyses include ethanol due to its ability to solubilize polar as well as weakly nonpolar compounds, thus offering more complete insight into the metabolic profile of plants.

In the current study, the methanolic extract of *B. incrassatum* exhibited moderate antioxidant; these results partially agree with El Kolli et al. (2017), who reported methanol extracts from *B. incrassatum* and *B. alpinum* to have the same activity, which is an indication that methanol is a suitable extracting solvent for these antioxidant compounds. However, our results disagree with Karouche et al. (2020), who obtained that water extracts of *B. mauritanicum* exhibited much stronger antioxidant activity (IC50 0.14 mg/mL), which supports that water can extract stronger antioxidants. Similarly, Dehimi et al. (2021) found that hexane and acetone fractions exhibited higher activities compared to their respective methanol fraction, further indicating that the possibility of bioactive compounds may be extracted with non-polar solvent extraction. Such findings suggest antioxidant extraction from *B. incrassatum* can be equally enhanced with other solvents like water or acetone. Further studies need to be undertaken on these solvents to optimize the plant's antioxidant potential.

In general, plant extracts tend to exhibit better inhibitory activity against Gram-positive bacteria compared to Gram-negative bacteria due to lipopolysaccharide composition in the multilayered cell wall of gram-negative strains (Chhetry et al.,2022; Essawi, 2000; Lin et al., 1999). The antibacterial quantitative study showed a positive response for both G-positive and G- G-negative bacterial strains used, which was in agreement with the findings of Karouche et al. (2020), who reported that tubers extract of *Bunium* reacted positively on the two reference strains: Gram-positive *Staphylococcus aureus* and Gram-negative *Pseudomonas aeruginosa*. Moreover, essential oils extracted from Algerian *B. incrassatum* and *Bunium alpinum* by El Koli et al. (2017) exhibited moderate antibacterial action against Gram-positive and Gram-negative bacteria, compared to gentamicin standard, which is in agreement with our results. Also, essential oils from *Bunium incrassatum* show promising antimicrobial activity, particularly against fungal strains, enhancing its value in natural medicine (Bousetla et al., 2014), which confirms the antibacterial properties of Talghouda tubers.

Since the present study underlines the benefits of *Bunium incrassatum*, further studies are necessary to explore its therapeutic application fully. Considering the antibacterial activity of *Bunium incrassatum* is promising, it remains relatively modest compared to conventional antibiotics. This points out the importance of continued research to enhance its efficacy and explore synergistic effects with other antimicrobial agents.

Conclusions

The present study revealed that the phytochemical composition of *Bunium incrassatum* tuber extracts indicated the presence of some bioactive compounds, such as polyphenols and flavonoids, which are likely responsible for their antibacterial activity. Also, the antioxidant activity of *Bunium incrassatum* extracts was evaluated through a DPPH assay, and it showed moderate efficacy. The tuber extract demonstrated significant activity against a wide range of pathogenic bacterial strains, suggesting its potential use in the treatment of microbial infections. These findings emphasise *B. Incrassatum* as a promising candidate in the development of plant-based antimicrobial agents, particularly in the context of Algerian medicinal plants. However, further in-depth investigations are necessary to identify the exact mechanisms of action involved and explore the full therapeutic potential of this plant, especially in its application in clinical practice.

Author Contributions

Conceptualization, S.B; H.B ; methodology, S.B; software, H.B.; N.B; Z.D; validation, H.B and S.B.; formal analysis, S.B investigation, N.B; Z.D; resources, H.B ; N.B and Z.D ; data curation, B.S.; writing—original draft preparation, S.B and H.B ; writing—review, editing, and paper communication, S.B; visualization, S.B; supervision, S.B and H.B ; All authors have read and agreed to the final version of the manuscript

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Conflicts of Interest

The authors declare no conflict of interest.

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